SEVENTH FRAMEWORK PROGRAMME
THEME [ENV.2012.6.3-1]
Innovative resource efficient technologies, processes and services

DUTH-WP3-D3.3-Final report on growth tests and biological validation

Project Acronym: **ZEPHYR**

Project Coordinator: **TUSCIA UNIVERSITY**

Proposal full title: **Zero-impact innovative technology in forest plant production**

Grant Agreement n°: **308313**

Document Title: **Final report on growth tests and biological validation**

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Summary: **This document provides data referring to growth test and biological validation conducted by UNITUS, AZORINA, DUTH, DU and UNINSUBRIA from month 18 to month 35 of work.**

Status: **Final**

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EXECUTIVE SUMMARY

The final report of this project for WP3 presents a full report of the results obtained by all Partners involved in the development of protocols when seedlings grow in an environment equipped with LED lamps. All 15 most important plant species indicated in the DOW for their importance for the European vegetation have been investigated beside a number of additional plant species which were not initially indicated. In regard of the biological validation of the protocols devised, it is possible to state that all the morphological, physiological, and biochemical parameters initially suggested as possible indicators of plant growth-kinetics have been measured and all showed to be a) reliable and b) effective independently from the differences the protocol adopted for each single plant species.

The considerable amount of data collected by all partners regarding both protocols and biological validation makes impossible to present them in single comparative table even when these are referred to the same plant species or to a single common parameter tested. For this reason the preference has been given to show in this final report all results by dividing them in chapters attributable each to a different partner. However, the comparative analysis of data presented by all partners in regard of biological validation suggests that the most effective spectrum is the one provided by the AP67 LED luminaire independently from the fact that the luminaire maintains a traditional form of a light bar or it has an innovative form of a light tube like the one presented by the commercially available fluorescent luminaire. One unexpected result of biological validations obtained by a number of partners is that some LED spectra were able to induce small but significant variations of the morphological parameters in plant organs. In fact, variations regarding leaves, shoots, and roots have been reported which seem to be correlatable with the LED spectra tested. This fact suggests that phenotypical variations could be voluntary induced in plant species by changing the LED spectrum used in the growth room and this approach could be used to influence the growing performance of the seedlings (resilience against environmental factors ?) at the time of their transplantation in the fields by the end of the pre-cultivation period in growth chamber. Another important conclusion which could be drawn after analyzing the biological validation presented by all Partners is that all the LED spectra tested enable a good level of growth even when they show a degree of growth-efficiency performance lower than the one showed by AP67 luminaire. This result suggests that in the case a particular plant species never tested before should not perform well in the growth chamber under the AP67 spectrum, then it would be possible to choose another spectrum among those tested in ZEPHYR project. In this way the biological validation shows that the LED light system tested in the ZEPHYR project seem to have a very high potential versatility to induce a near-natural growth with a wide number plant species.

The good performance of LED luminaire within the frame of each individual protocol devised for each plant species could be detected efficiently by means of a comparative analysis of images of the same plants acquired at regular time intervals by means of the optical system developed in the ZEPHYR project. In regard to this, the comparative image analysis made by a computer software developed during the ZEPHYR project, enables to produce step by step increasing values of parameters such as plant height or greenness level. This last is the number of colored pixel related to the leaves measured differentially in respect to the black background of the soil medium. The curve increase obtained by both parameters is significantly correlatable with the increase obtained when the same (or different) parameters were measured by traditional and destructive hand-methods such as for example the plant biomass and/or plant height.

1. INPUT OF UNITUS

1.1. Introduction

Indoor growth trials of many forest species under LED and fluorescent lights, according to protocols defined in the first 18 months of project. Outdoor trials in order to evaluate eventual transplant stress effects and growth performances of seedlings in open-field after 1 month of indoor growth.

1.2. Materials and methods

1.2.1. List of species

- *Fagus sylvatica*
- *Fraxinus excelsior*
- *Myrtus communis*
- *Punica granatum*
- *Quercus ilex*
- *Quercus suber*

1.2.2. Seed germination

Seeds of each species were rehydrated into tap water at room temperature for 48 hours. Afterthat empty floating seeds were eliminated and the remnants were surface sterilized with a 10% ipoclorite solution for 10 minutes than washed again with sterile water and subjected to germination protocols.

The species affected by dormancy (*Fagus sylvatica; Fraxinus excelsior; Myrtus communis; Punica granatum*) were subjected to variable periods of cold stratification, according to literature:

- *Fagus sylvatica:* wet perlite; 30 days at 4°C + 10 days at 22°C;
- *Fraxinus excelsior:* wet sand; outdoor temperature; 4 months (since December to April);
- *Myrtus communis:* wet perlite; 50 days at 4°C + 7 days at 22°C;
- *Punica granatum:* wet perlite; 50 days at 4°C + 7 days at 22°C.

All the seeds were germinated in constant darkness/under AP67 lights with a photoperiod equal to 12L 12D (according to their light needs) at 22°C and 60% of relative air humidity on wet sterile perlite then sown into the soil.

1.2.3. Indoor trials

As described in D 3.2, all the experiments were carried out in a climatically controlled room located into the University of Tuscia (Viterbo). Plants were cultivated on trolleys, equipped with a different light source per shelf (6 different light sources: 5 LEDs vs Fluorescence):

OSRAM L36W/77 FLUORA (tube, 4 lamps, length 120cm)

Valoya AP67 (bar, 2 lamps, length 120cm)

Valoya L20AP67 (tube, 4 lamps, length 120cm)

Valoya AP673L (bar, 2 lamps, length 120cm)

Valoya G2 (bar, 2 lamps, length 120cm)

Valoya NS1 (bar, 2 lamps, length 120cm)

All the species described in the following chapters were grown in constant conditions of temperature (22±2°C), relative air humidity (50±10%) and photoperiod (12L 12D).

The only variable parameter was the duration of the growth cycle (see table 1).

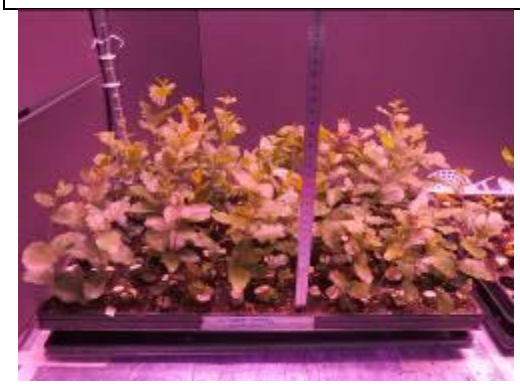
The distance between the trays and the lights was defined in order to ensure a light intensity equal to 120±20 PPFD per each shelf at the tray level.

2 HerkuPlast® QPD 104 VW trays (104 plants per tray) were employed per shelf per each species.

According to the ecological needs of the tested species, Jiffy soil, used in the previous tests (see D 3.2) was substituted by a mixed soil, containing peat, perlite and river sand (10:5:1).

TABLE 1. INDOOR GROWTH CYCLES

SPECIES	INDOOR GROWTH CYCLE
Fagus sylvatica	30 days
Fraxinus excelsior	30 days
Myrtus communis	52 days
Punica granatum	52 days
Quercus ilex	30 days
Quercus suber	30 days



1.2.4. Morphological, microscopic and biochemical analysis

List of Parameters

At the end of the indoor growth cycle, 24 randomly chosen seedlings per each light treatment were subjected to morphological non destructive and destructive analysis, in order to collect the below listed parameters:

1. Shoot length
2. Collar shoot diameter
3. Shoot fresh weight
4. Shoot dry weight
5. Root fresh weight
6. Root dry weight
7. Number of leaves
8. Average Leaf Area
9. Leaves fresh weight
10. Leaves dry weight
11. Growth curves
12. SPAD chlorophyll content

Three seedlings per light treatment were randomly selected for microscopic analysis. Three leaves were randomly selected per each seedling and analysed as follows:

1. Leaf area (LA)
2. Relative Stomata density (RSD)
3. Absolute Stomata number (RSD*LA)

Another sample of seedlings per each light treatment was randomly selected for biochemical analysis (the number of seedlings depends on the dimension and biomass of leaves. In order to carry on the first 4 tests

listed below, about 1 gr of leaf tissue is needed. For the 5th test 0.5 gr are needed). All the tests were done in triplicate.

1. Chlorophyll content
2. Protein content
3. Nitrate reductase activity
4. Glutammine synthetase activity
5. Lipid peroxidation level

Moreover, 24 randomly chosen seedlings per each light treatment for the construction of growth curves, measuring the shoot height twice a week during all the growth cycle, since the emergence of first leaves.

For more exhaustive description of the above mentioned analysis, please see deliv. 3.2.

1.2.5. Outdoor trials (greenhouse)

At the end of the indoor growth cycle, 40 seedlings per each light treatment were transplanted into bigger pots (Herkuplast® QP40T/18, 40 seedlings per tray) and transferred into a greenhouse for about a year (up to the beginning of autumn season) before the final open-field transplant into the forest nursery located into the Tuscia University farm “Nello Lupori” (Viterbo, Italy). The period of acclimation into the greenhouse is needed by some species in order to avoid some shocks due to the big differences, in terms of quality and quantity, between artificial and natural lights. In the case of oaks, this period may also be avoided thanks to the high natural adaptability of the species to different levels of lights.

1.2.5.1. Morphological analysis

The height of those 24 seedlings used for the construction of the indoor growth curves was still measured intot the greenhouse once a week up to July (corresponding to the natural period of growth interruption of Mediterranean species caused by the incoming summer).

At the end of 1-year-growth into the greenhouse, 10 randomly selected seedlings per each light treatment were subjected to morphological analysis, as described into the paragraph 1.2.4.

1.2.6. Outdoor trials (open-field)

After one year of acclimation into the greenhouse, at the beginning of summer (before the period of drought stress typical of Mediterranean area), three species (*Corylus avellana, Quercus suber* and *Quercus ilex*) were selected for a final step: the transplant into open-field.

At first, a mulching sheet was placed in the implant area directly onto the soil (to reduce the presence of weeds). Afterthat holes (~15 cm of diameter) have been practiced in the sheet at a distance of about 20 cm one from each other, forming lines distant from each other about 40 cm. In correspondence of each point, a hole into the soil was dug where to put a single seedling. An automatic irrigation system was installed and programmed to provide water to the plants twice a day. In addition, the implant of *Corylus avellana*, because of a higher susceptibility of the species to light stress during the transplant if compared to oaks, was covered with shade clothes placed at a height of about 1.90 m from the ground.

Per each species, 10 seedlings per each light treatment were transplanted and monitored measuring shoot height and survival percentage every two weeks up to the following autumn (after the drought stress period, in order to analyse the ability of seedlings in sustaining light and water stress at the same time).

1.3. Results

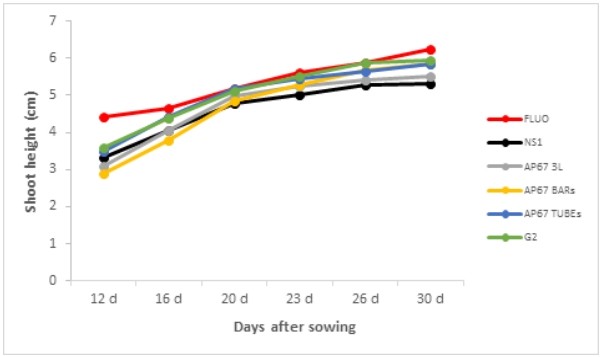
1.3.1. Indoor trials

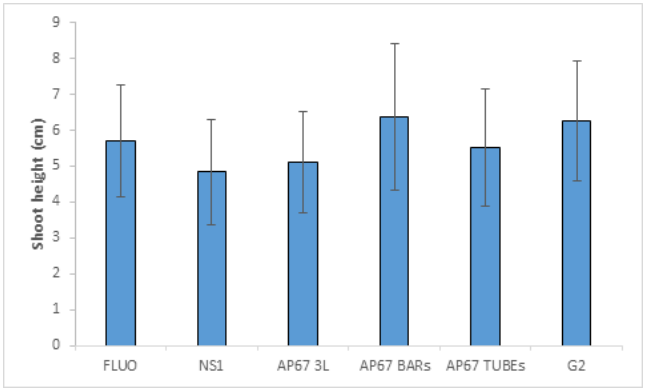
1.3.1.1. Morphological analysis

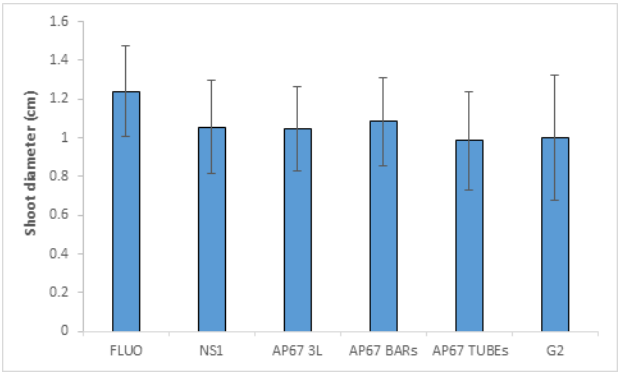
Results of morphological analysis of *Corylus avellana*, *Myrtus communis*, *Punica granatum* and *Quercus suber* have been already discussed into the deliverable 3.2.

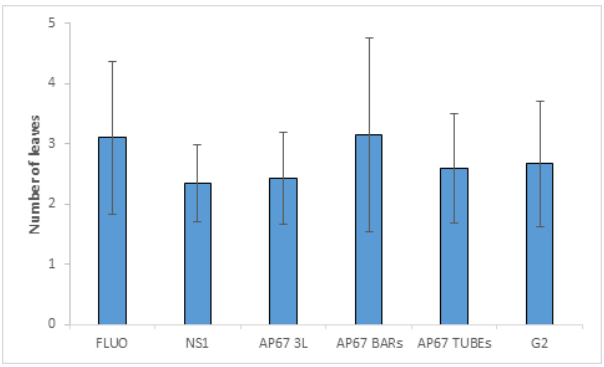
- ***Fagus sylvatica***

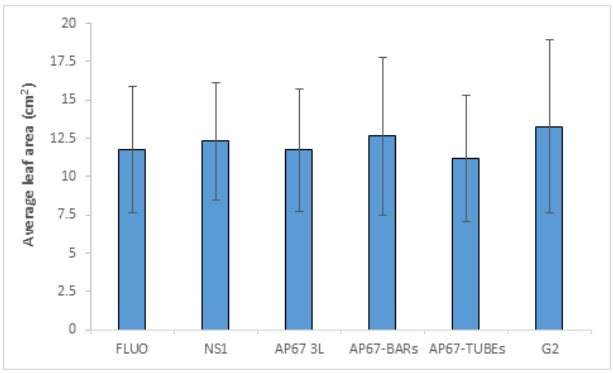
Growth curves

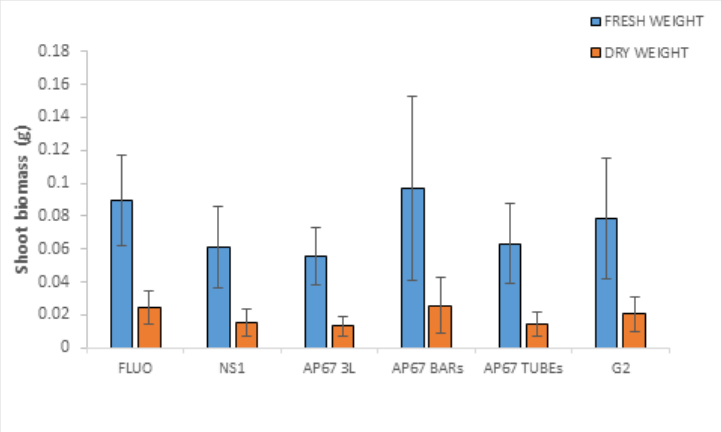


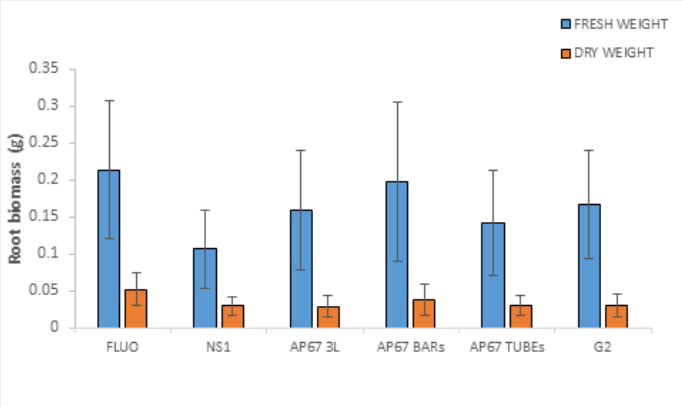

Shoot height (cm)
0
1
2
3
4
5
6
7
8
9
FLUO
NS1
AP67 3L
AP67 BARs
AP67 TUBEs
G2

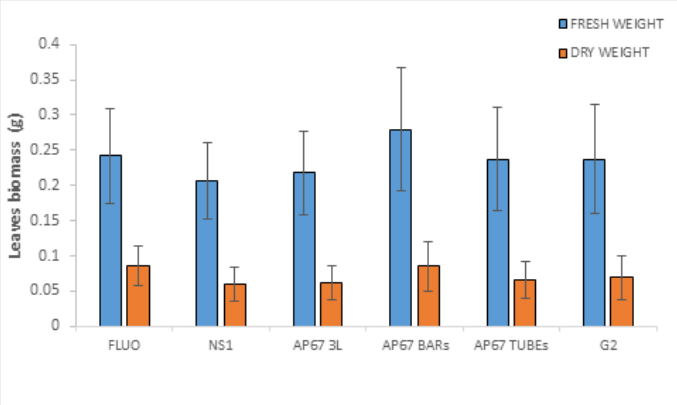

Shoot diameter (cm)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
FLUO
NS1
AP67 3L
AP67 BARs
AP67 TUBEs
G2


Number of leaves
0
1
2
3
4
5
FLUO
NS1
AP67 3L
AP67 BARs
AP67 TUBEs
G2


Average leaf area (cm²)
0
2.5
5
7.5
10
12.5
15
17.5
20
FLUO
NS1
AP67 3L
AP67-BARs
AP67-TUBEs
G2

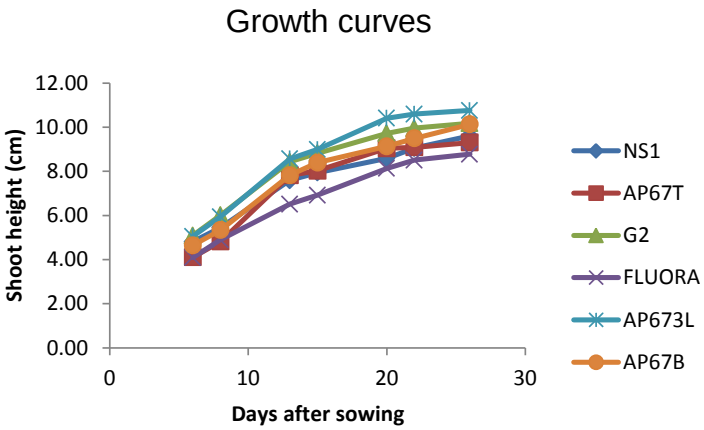


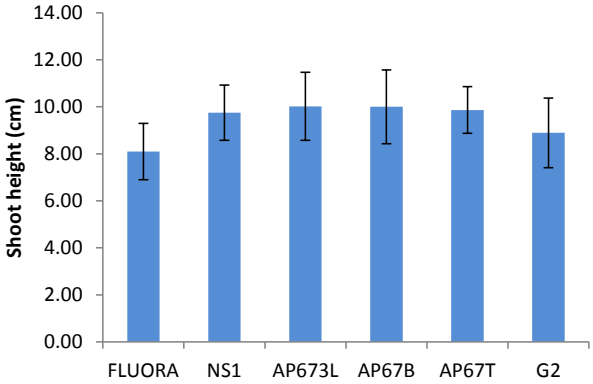


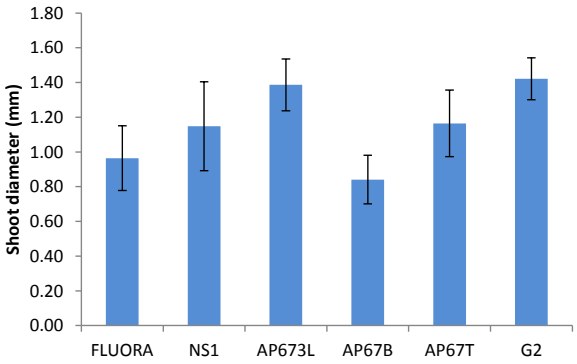


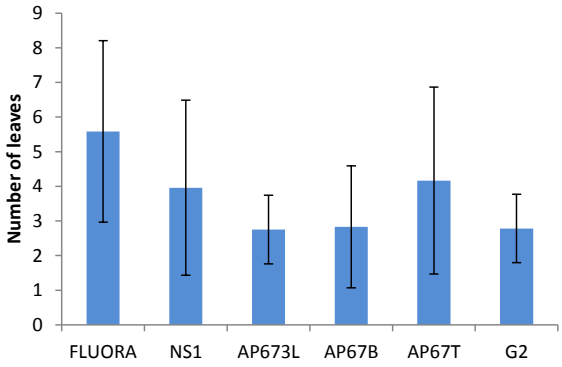
Morphological analysis of *Fagus sylvatica* showed that AP67 BARs lamp is better than fluorescent lights in terms of growth performances, in particular for shoot height, leaves and shoot biomass. In terms of average leaf area, FLUORA showed lower values than G2 and AP67B. On the other hand, fluorescent lights seem to increase the shoot diameter.

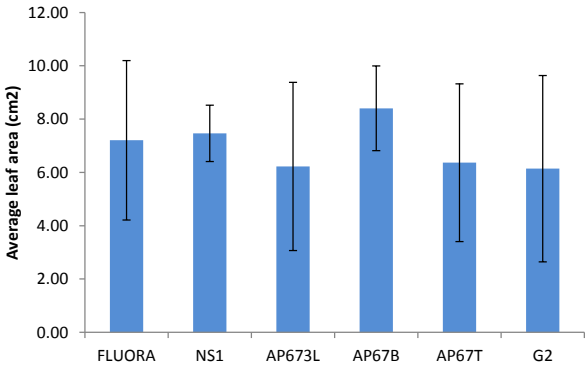
- ***Fraxinus excelsior***

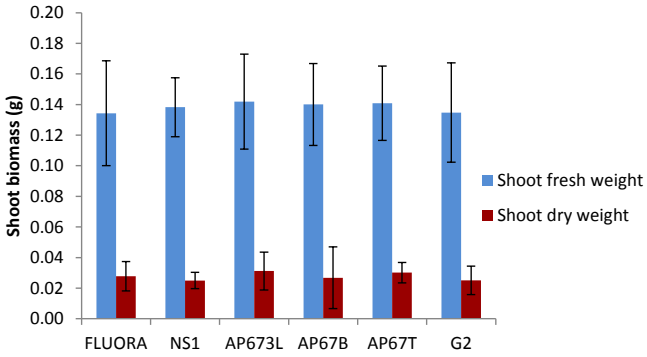


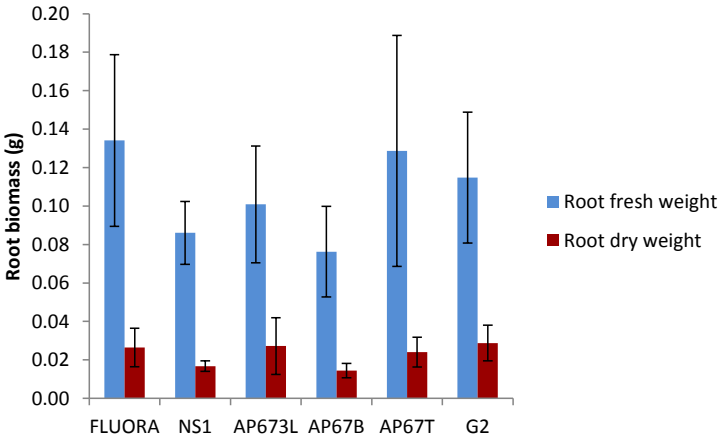


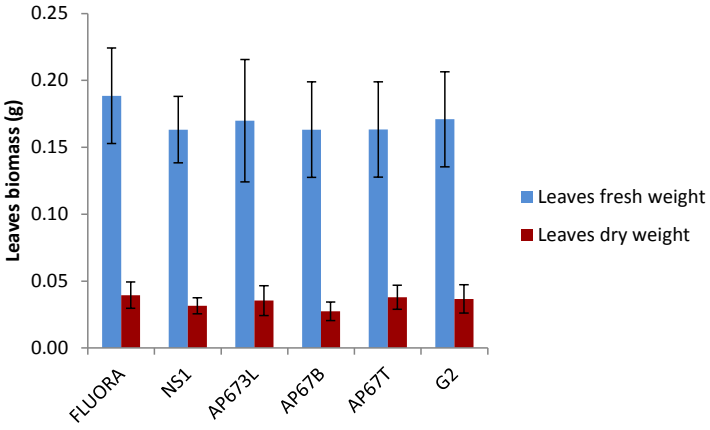



Number of leaves
0
1
2
3
4
5
6
7
8
9
FLUORA
NS1
AP673L
AP67B
AP67T
G2


Average leaf area (cm2)
0.00
2.00
4.00
6.00
8.00
10.00
12.00
FLUORA
NS1
AP673L
AP67B
AP67T
G2


Shoot biomass (g)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
FLUORA
NS1
AP673L
AP67B
AP67T
G2
Shoot fresh weight
Shoot dry weight

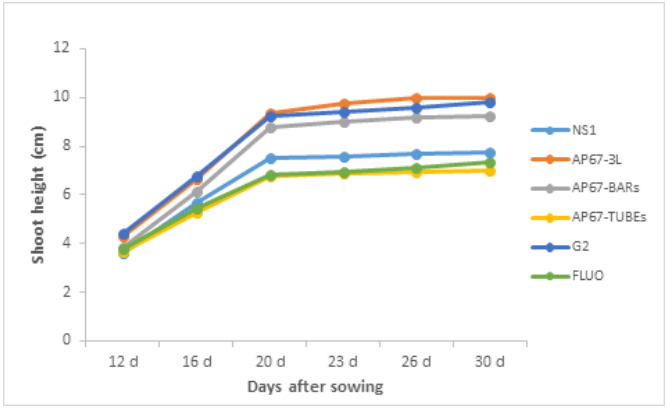

Root biomass (g)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
FLUORA
NS1
AP673L
AP67B
AP67T
G2
Root fresh weight
Root dry weight

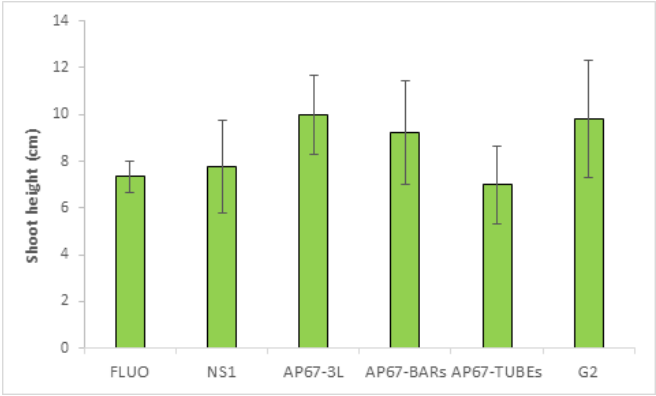


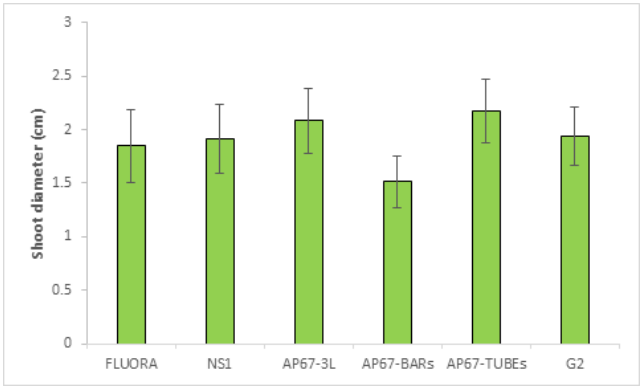
Morphological analysis of *Fraxinus excelsior* showed that LED lights are better than fluorescent lights in terms of growth performances, in particular FLUORA gave the lowest shoot height and shoot diameter values. The highest values were reached respectively under AP673L and G2. In terms of average leaf area, FLUORA showed lower values than NS1 and AP67B. On the other hand fluorescent lights seem to increase the number and biomass of leaves so as the biomass of roots.

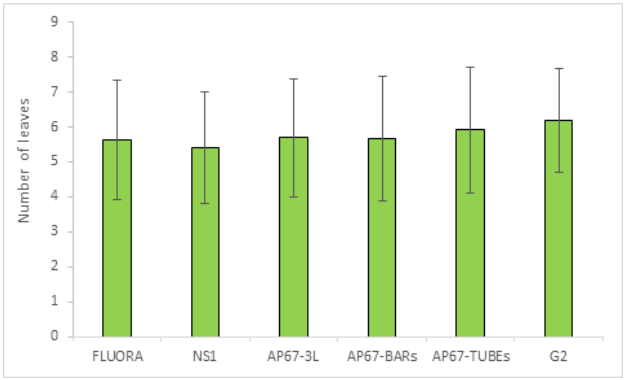
- ***Quercus ilex***

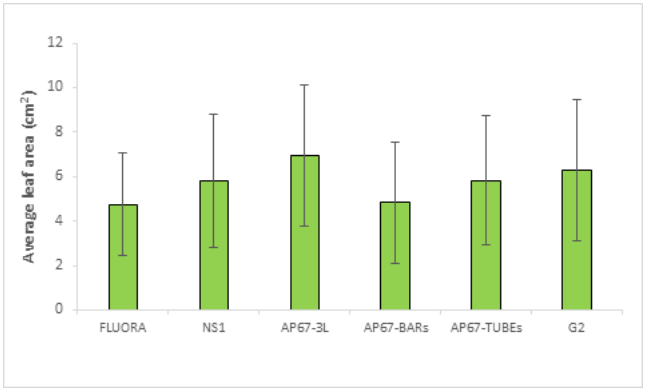
Growth curves

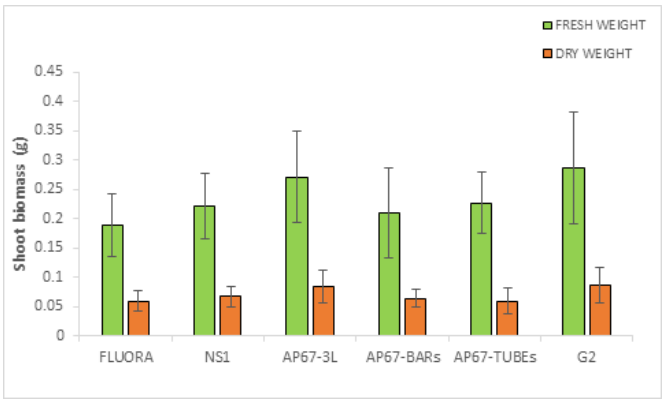


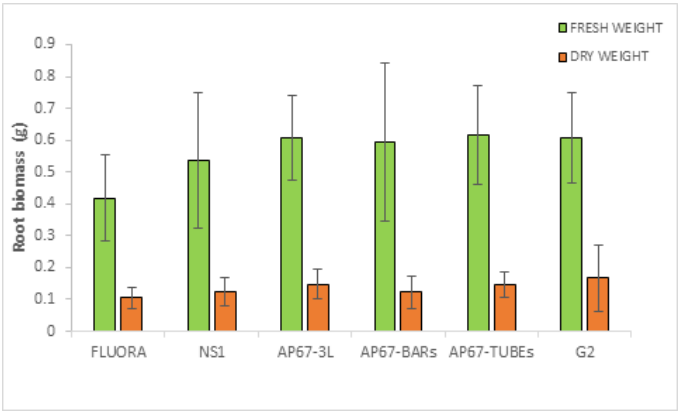

Shoot height (cm)
14
12
10
8
6
4
2
0
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

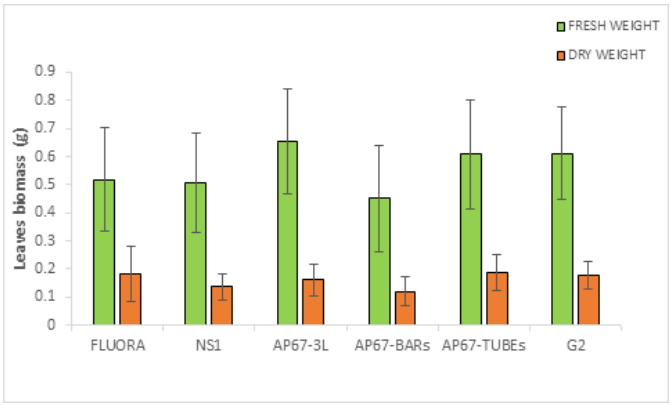

Shoot diameter (cm)
3
2.5
2
1.5
1
0.5
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2


Number of leaves
9
8
7
6
5
4
3
2
1
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2


Average leaf area (cm²)
12
10
8
6
4
2
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2



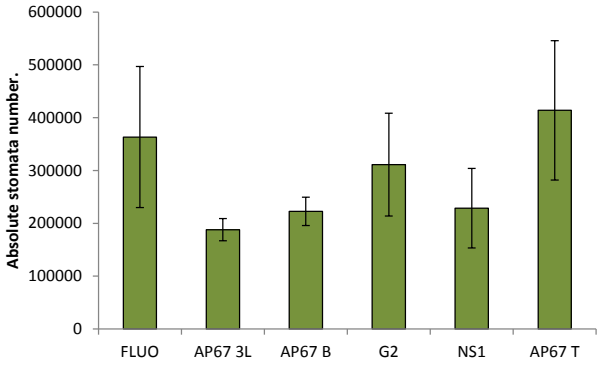




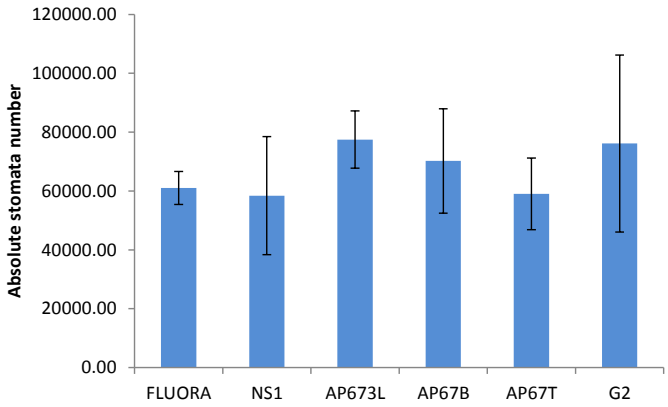
Morphological analysis of *Quercus ilex* showed that LED lights are better than fluorescent lights in terms of growth performances. In particular, AP673L showed the highest values for all the analysed parameters especially for root, shoot and leaves biomass. On the other hand, FLUORA gave the lowest root and shoot biomass. In terms of number of leaves, the LED lamps do not show differences if compared with fluorescent lights.

1.3.1.2. Microscopic analysis

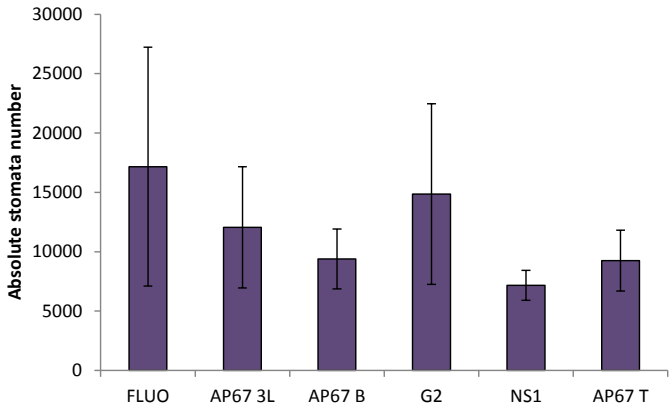
- ***Fagus sylvatica***



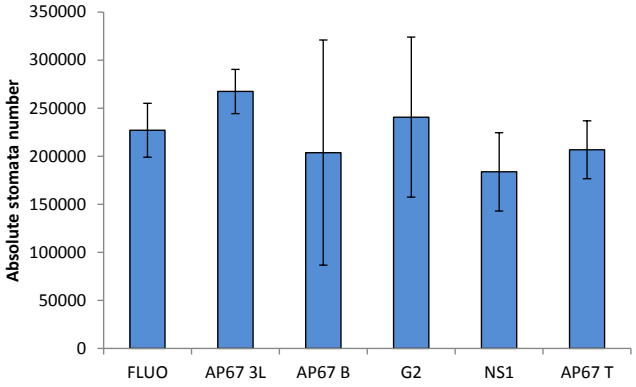
- ***Fraxinus excelsior***



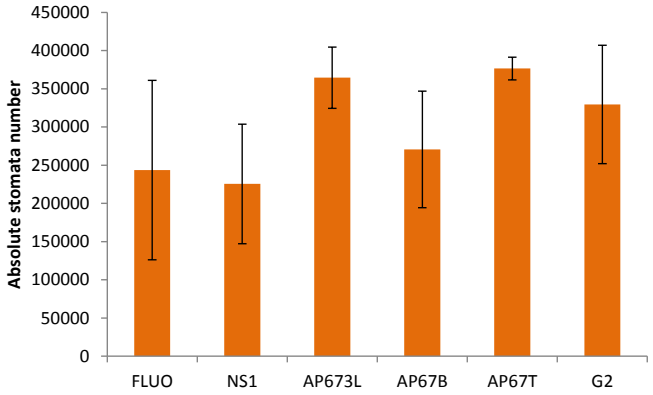
- ***Myrtus communis***



- ***Quercus ilex***



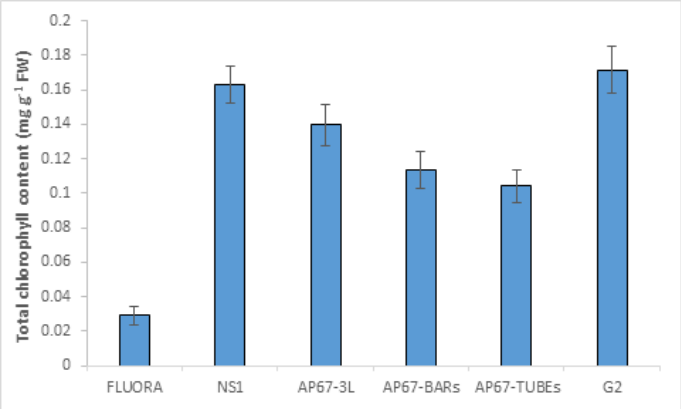
- ***Quercus suber***

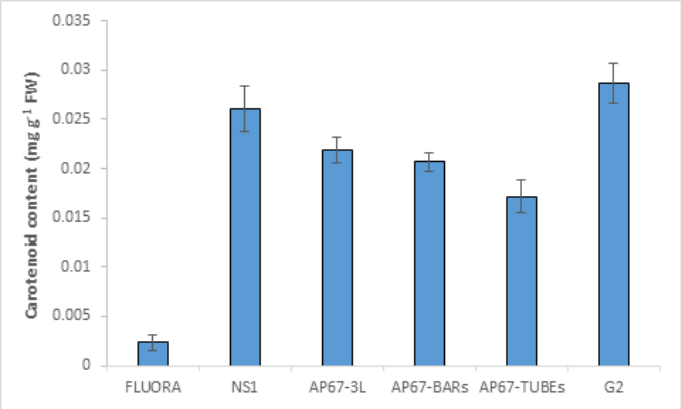


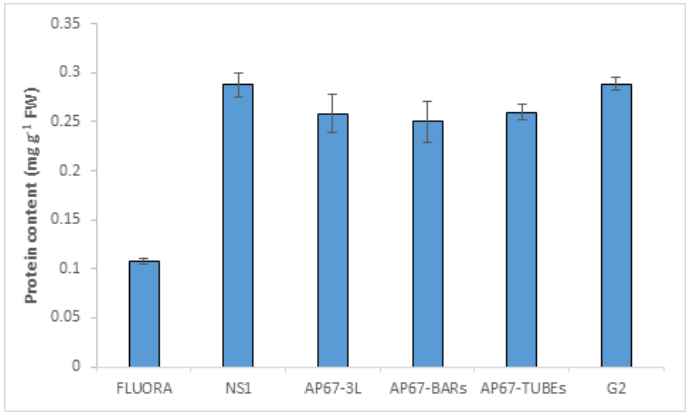
Fagus sylvatica and *Myrtus communis* showed higher or comparable results in terms of absolute stomata number under FLUORA than under LEDs instead *Fraxinus excelsior, Quercus ilex* and *Quercus suber* showed lower results under FLUORA than under LED lamps. In particular, AP673L and G2 resulted to be the best light for *Fraxinus excelsior* and *Quercus ilex*, while AP67T gave the best results with *Quercus suber*.

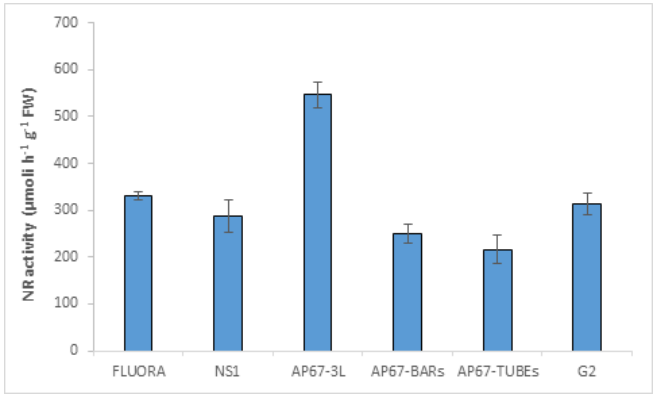
1.3.1.3. Biochemical analysis

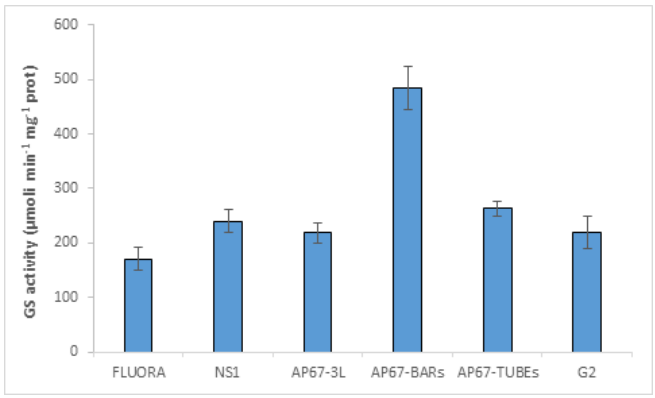
- ***Fagus sylvatica***

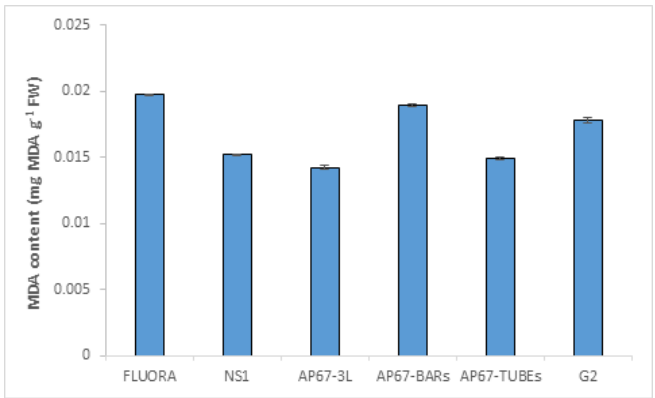





Protein content (mg g-1 FW)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

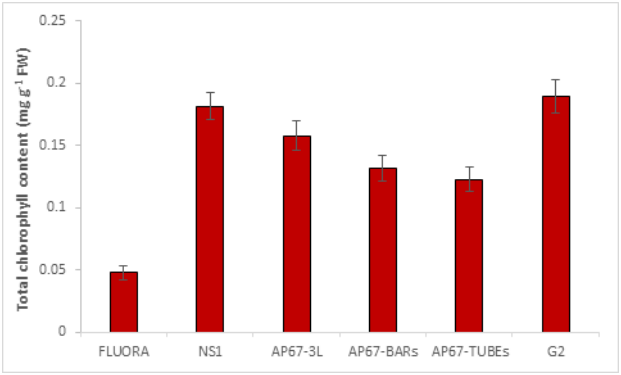

NR activity (µmoli h-1 g-1 FW)
700
600
500
400
300
200
100
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

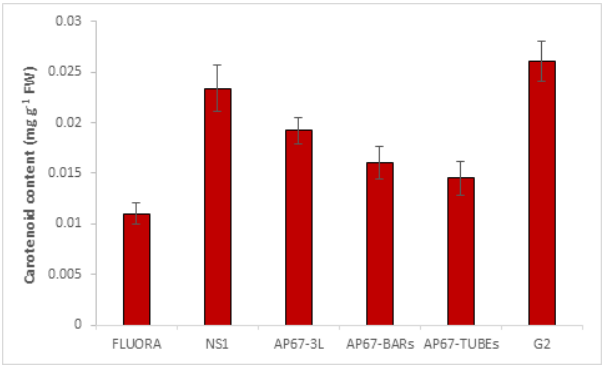

GS activity (µmoli min-1 mg-1 prot)
600
500
400
300
200
100
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

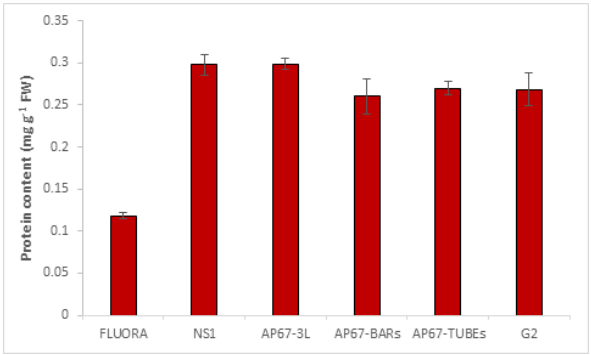

MDA content (mg MDA g-1 FW)
0.025
0.02
0.015
0.01
0.005
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

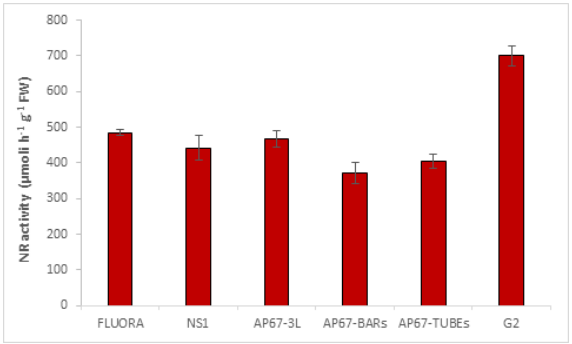
Biochemical analysis on *Fagus sylvatica* showed a lower chlorophyll, carotenoids and protein content in plants grown under traditional fluorescent lamps. The GS activity resulted to be higher for plants grown under A67B lamp, while the NR activity resulted to be higher for plants grown under AP673L lamp. The MDA content did not show particular differences among all the conditions but was lower under NS1, AP67-3L and AP67B lamps.

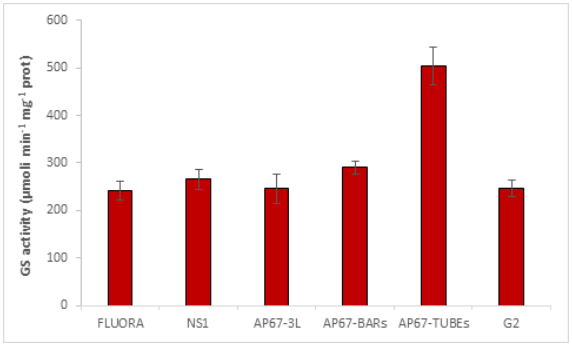
- ***Fraxinus excelsior***

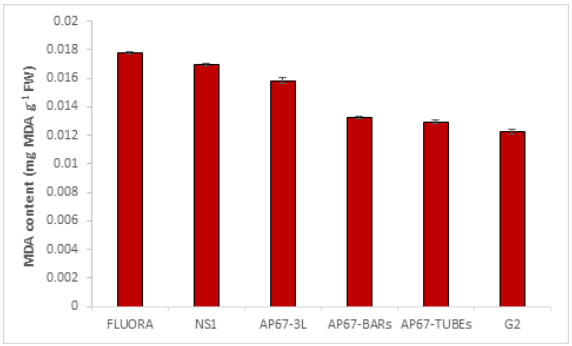






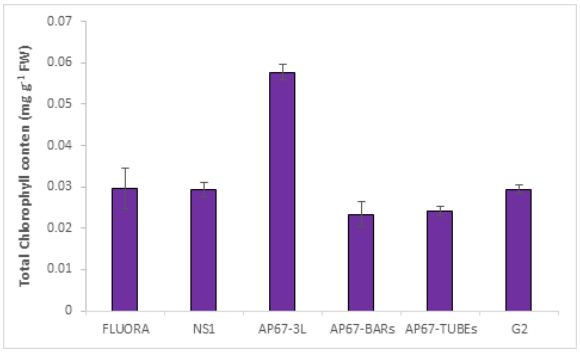


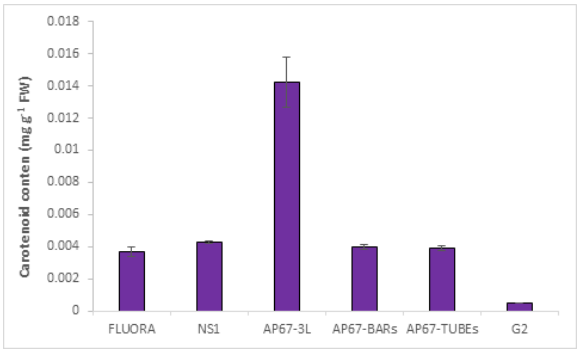


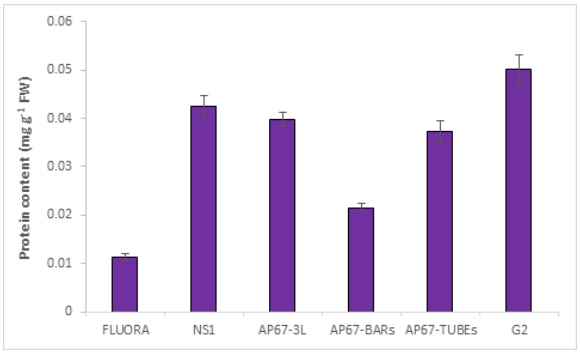


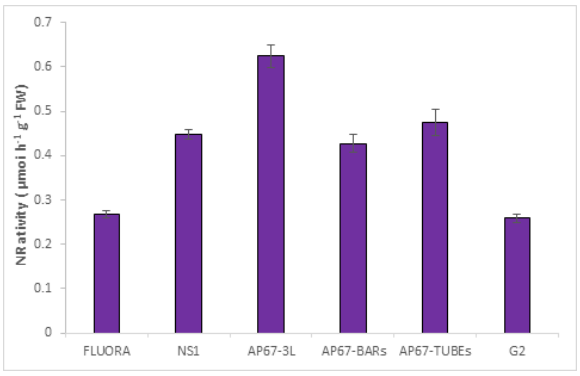
Biochemical analysis in *Fraxinus excelsior* showed a lower chlorophyll, carotenoids and protein content in plants grown under traditional fluorescent lamps. The GS activity resulted to be higher for plants grown under AP67T lamp, while the NR activity resulted to be higher for plants grown under G2 lamp. The MDA content was lower under AP67B, AP67T and G2 lamps.

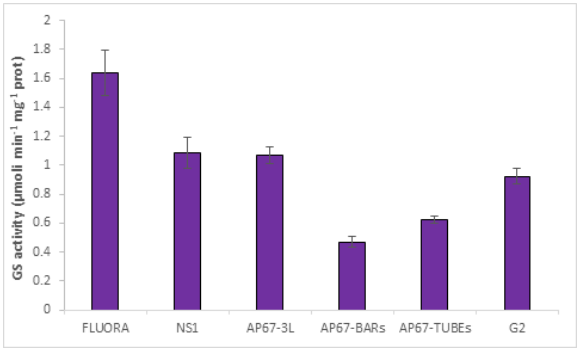
- ***Myrtus communis***

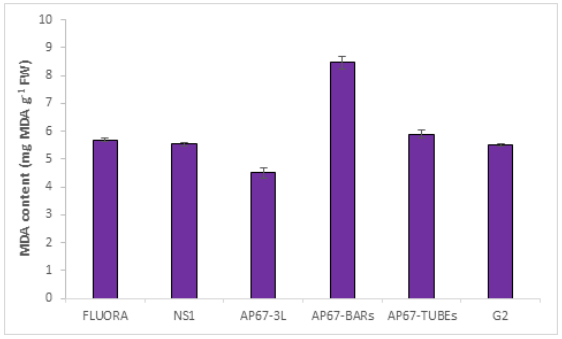






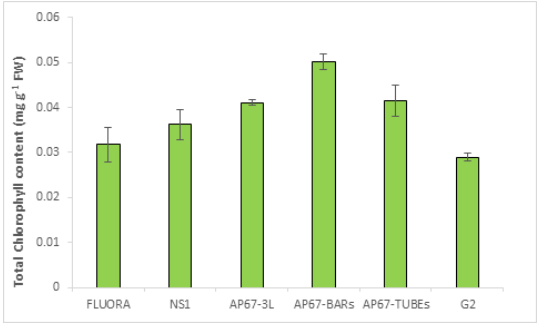


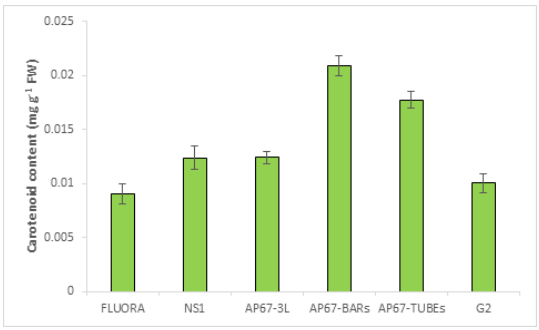


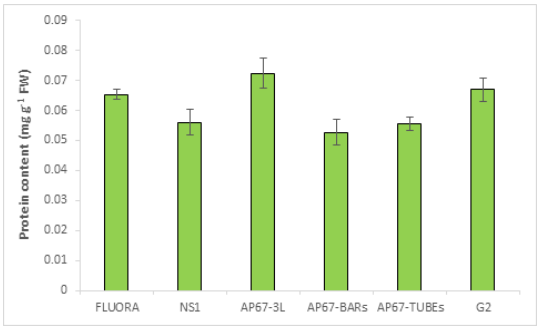


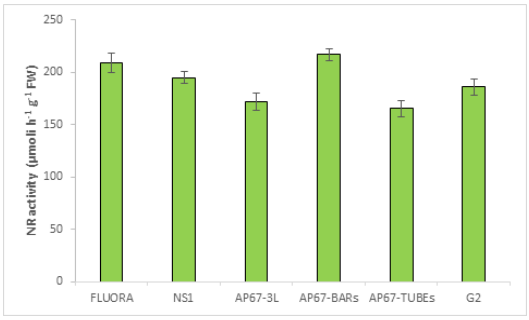
Biochemical analysis in *Myrtus communis* showed a higher chlorophyll and carotenoids content in plants grown under AP67 3L lamps. On the other hand, the GS activity resulted to be inhibited in plants grown under LED light. The AP67 3L lamp resulted to be the less stressful LED lamp (low MDA content).

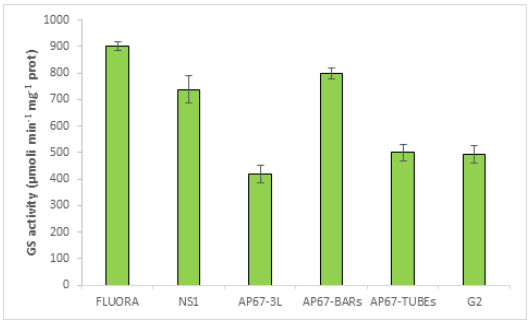
- ***Quercus ilex***

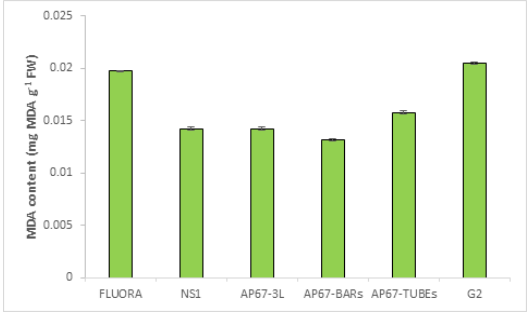



Carotenoid content (mg g-1 FW)
0.025
0.02
0.015
0.01
0.005
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2


Protein content (mg g-1 FW)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

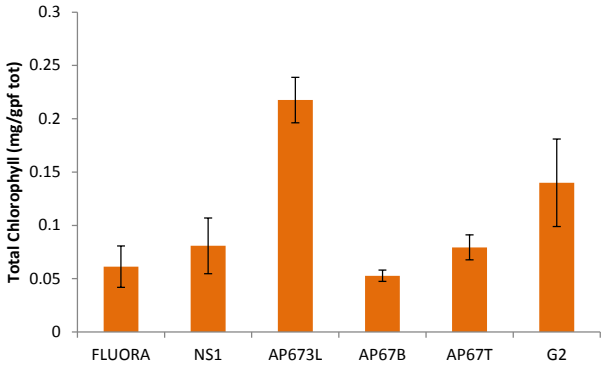

NR activity (µmoli h-1 g-1 FW)
250
200
150
100
50
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

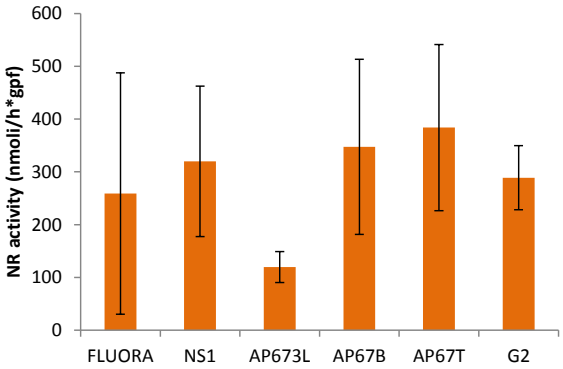

GS activity (µmoli min-1 mg-1 prot)
1000
900
800
700
600
500
400
300
200
100
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

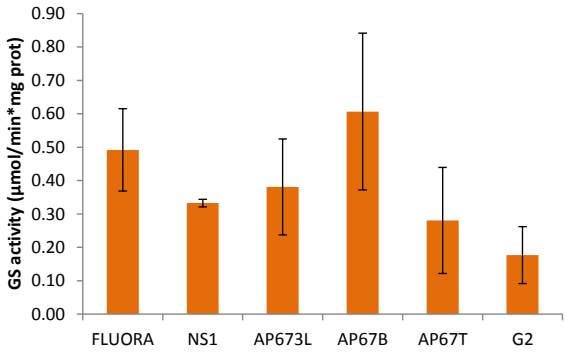

MDA content (mg MDA g-1 FW)
0.025
0.02
0.015
0.01
0.005
0
FLUORA
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

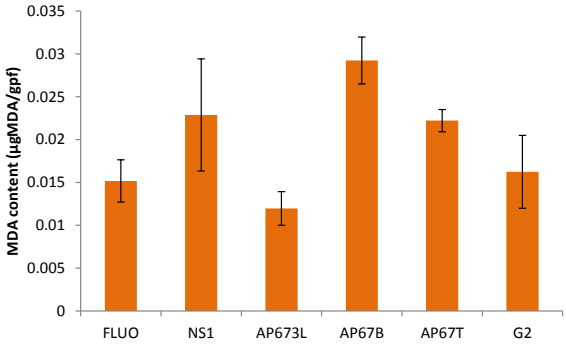
Biochemical analysis in *Quercus ilex* showed a lower chlorophyll and carotenoids content in plants grown under traditional fluorescent lamps. On the other hand, the GS activity resulted to be inhibited under AP673L light. The protein content resulted to be higher in plants grown under AP67 3L lamp. The high levels of MDA in plants grown under fluorescent light and under G2 lamp, suggest that both lamps are a source of stress for *Quercus ilex*.

- ***Quercus suber***









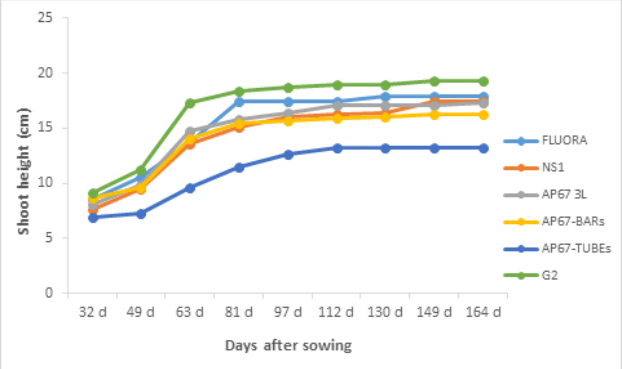
Fluorescent lights resulted to be better performant in terms of GS induction and protein production, respectively similar to AP67B and NS1. They resulted to be not too much stressful for platns thanks to a low lipid peroxidation so as G2 and AP673L. The most stressful lamp resulted to be AP67B. On the other hand FLUORA gave low results in terms of NR activity and chlorophyll content. AP67T gave the best results for NR activity and AP673L for chlorophyll content.

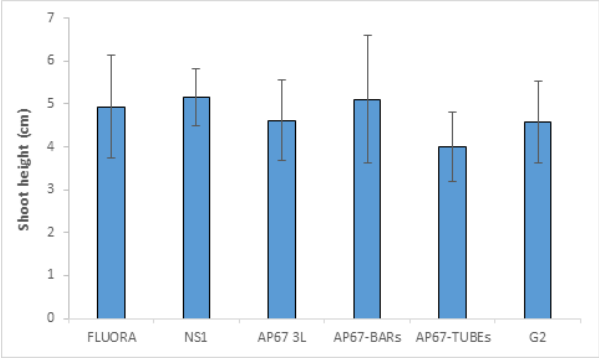
1.3.2. Outdoor trials (greenhouse)

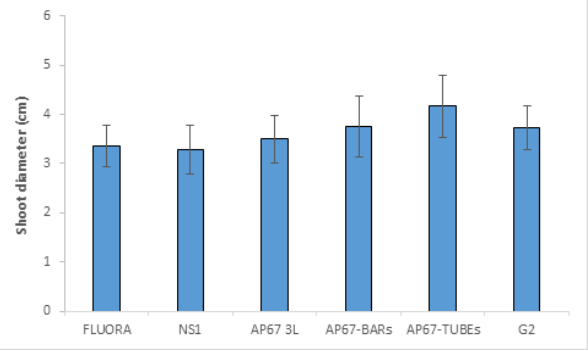
1.3.2.1. Morphological analysis

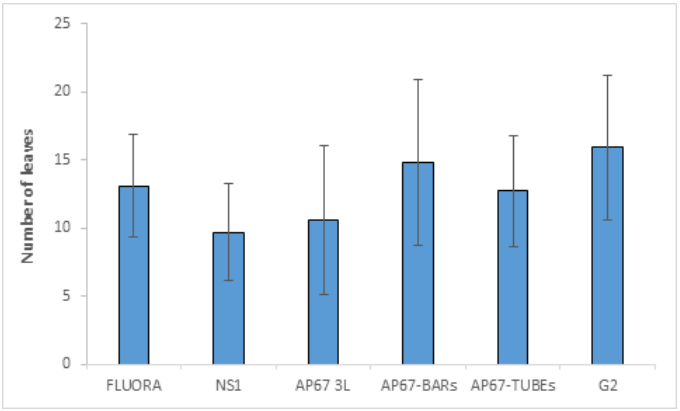
- ***Fagus sylvatica***

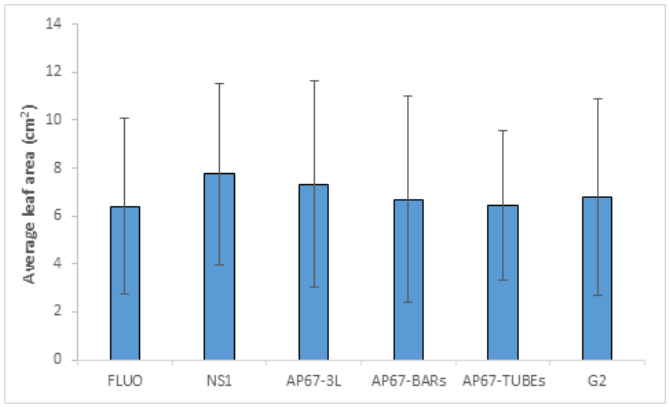
Growth curves

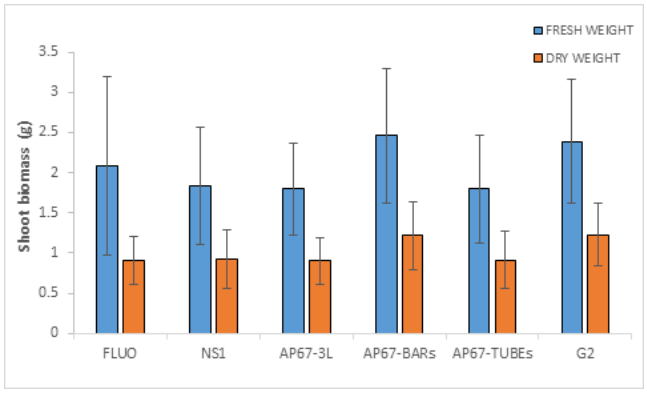


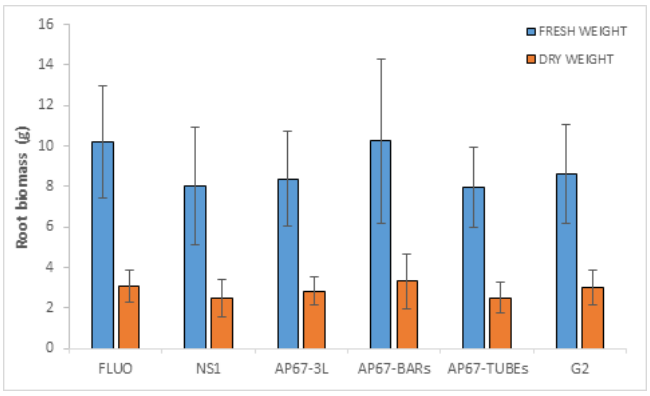


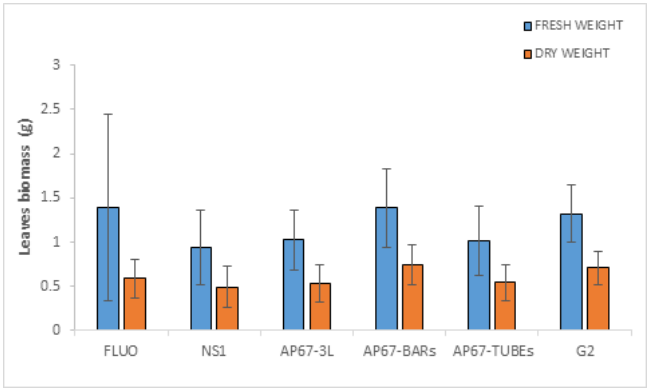



Number of leaves
25
20
15
10
5
0
FLUORA
NS1
AP67 3L
AP67-BARs
AP67-TUBEs
G2


Average leaf area (cm²)
14
12
10
8
6
4
2
0
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

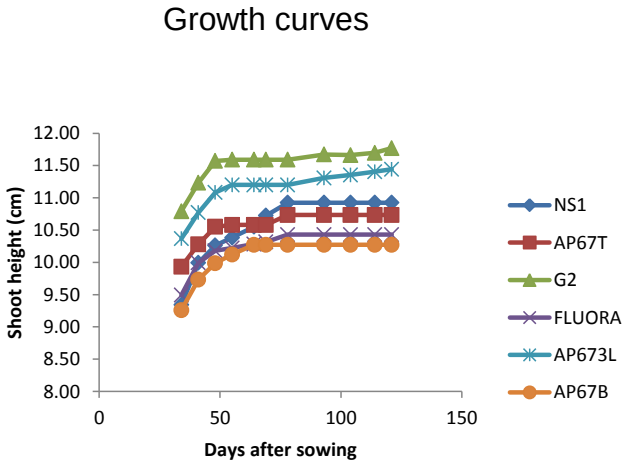

FRESH WEIGHT
DRY WEIGHT
Shoot biomass (g)
3.5
3
2.5
2
1.5
1
0.5
0
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

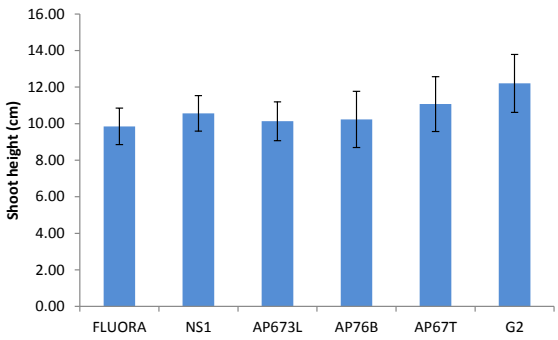

FRESH WEIGHT
DRY WEIGHT
Root biomass (g)
16
14
12
10
8
6
4
2
0
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

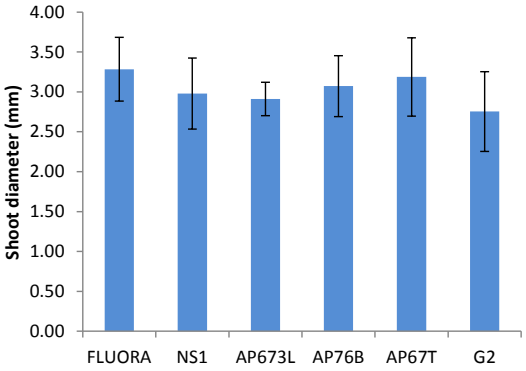


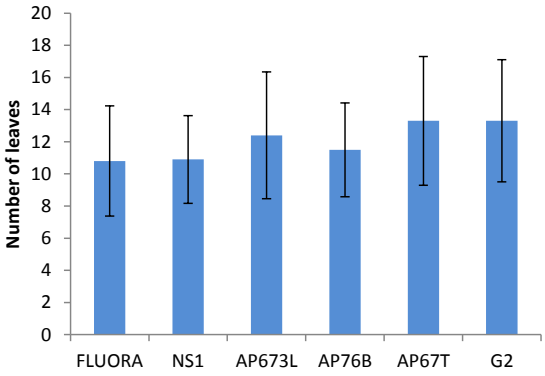
Morphological analysis in *Fagus sylvatica*, after the period in the greenhouse, showed no particular difference in plants grown under LED lights and under the traditional fluorescent lamp.

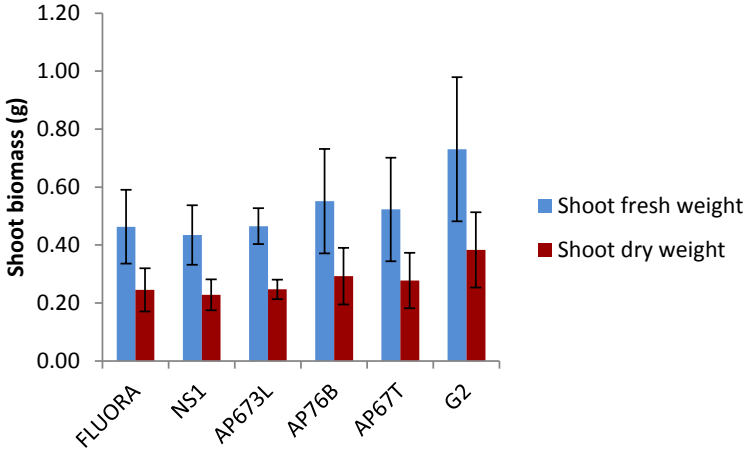
- ***Fraxinus excelsior***

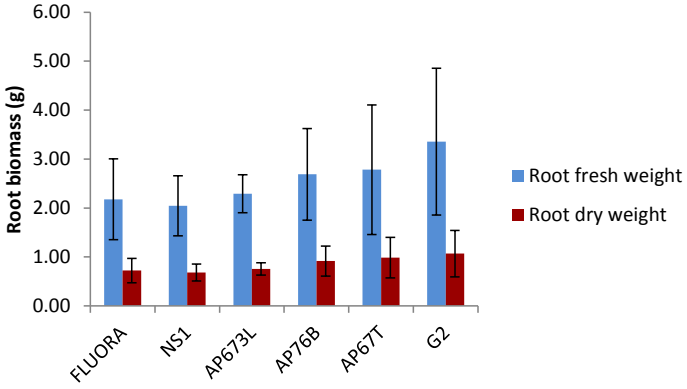


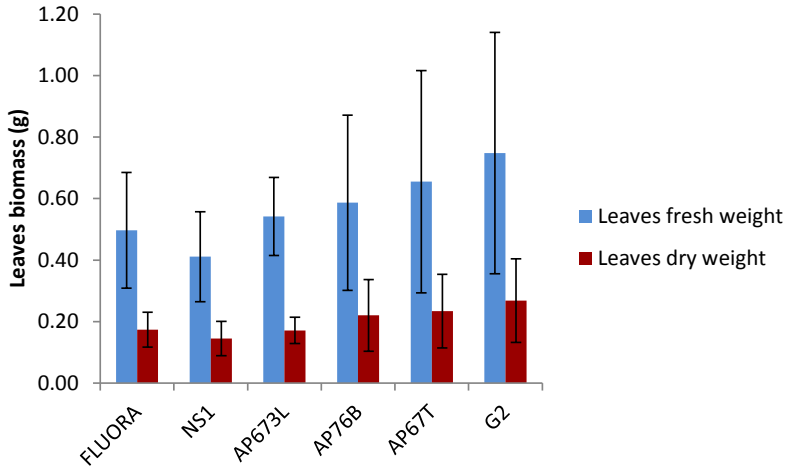


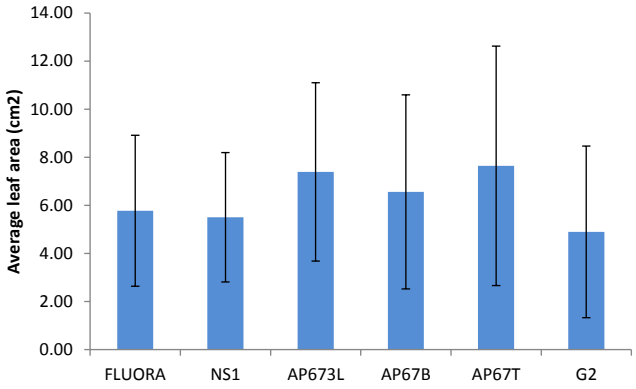

Shoot diameter (mm)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
FLUORA
NS1
AP673L
AP76B
AP67T
G2


Number of leaves
20
18
16
14
12
10
8
6
4
2
0
FLUORA
NS1
AP673L
AP76B
AP67T
G2


Shoot biomass (g)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
FLUORA
NS1
AP673L
AP76B
AP67T
G2
Shoot fresh weight
Shoot dry weight

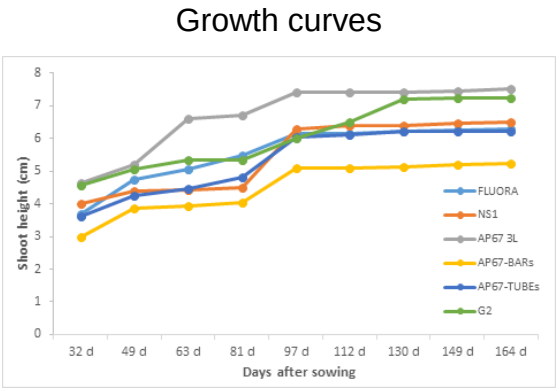

Root biomass (g)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
FLUORA
NS1
AP673L
AP76B
AP67T
G2
Root fresh weight
Root dry weight

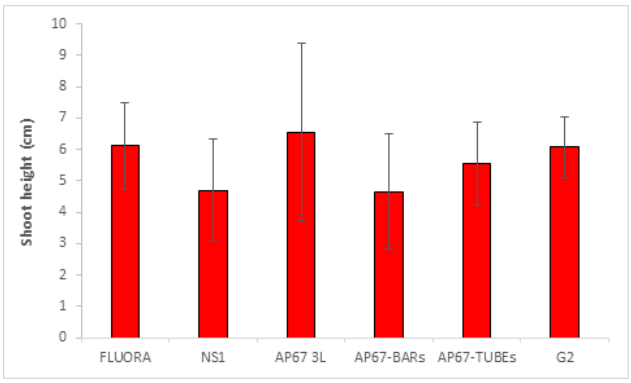


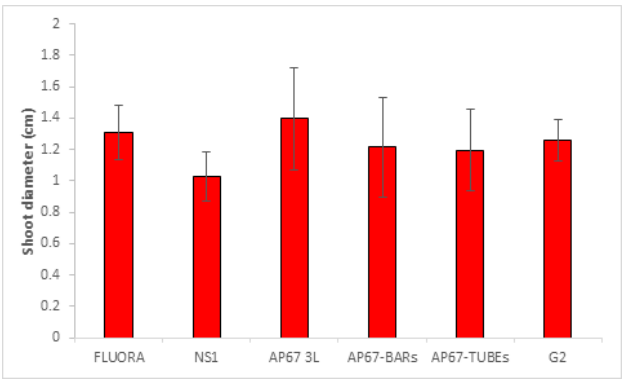


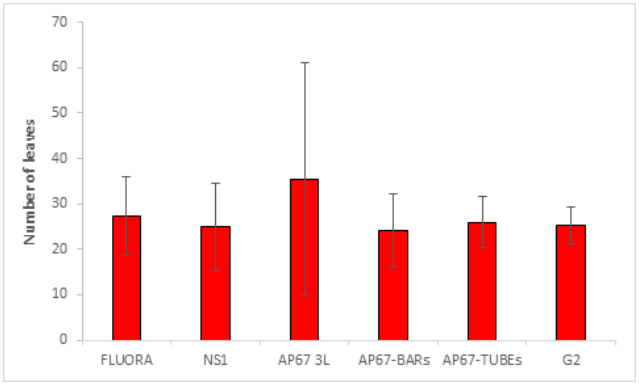
In the case of *Fraxinus excelsior*, LED lights gave best results than FLUORA in terms of shoot height, number of leaves, shoot and leaves biomass and leaf area. G2 resulted to be the most performant for all these parameters excepted for average leaf area, whose highest value was reached by AP673L and AP67B. On the other hand FLUORA gave the highest value for shoot diameter.

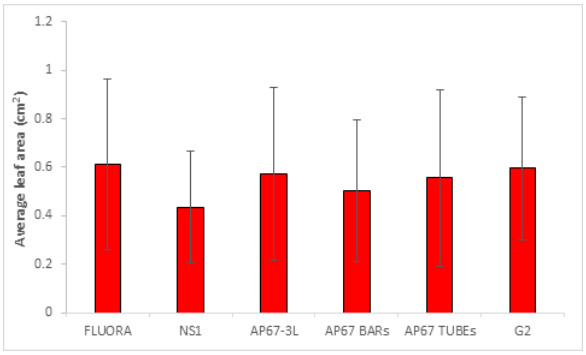
- ***Myrtus communis***

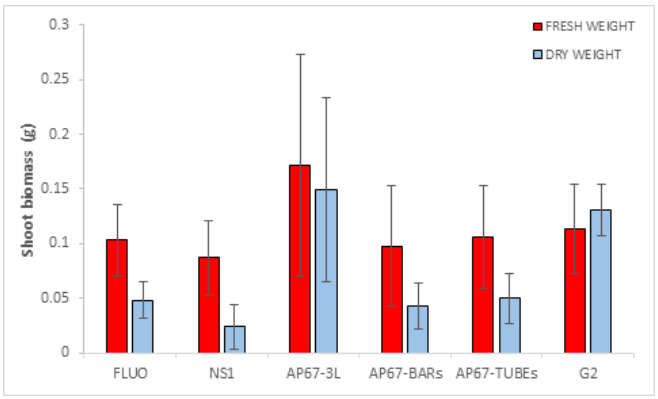


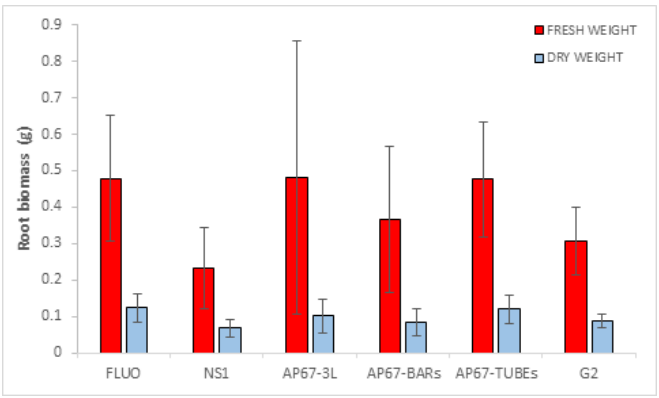


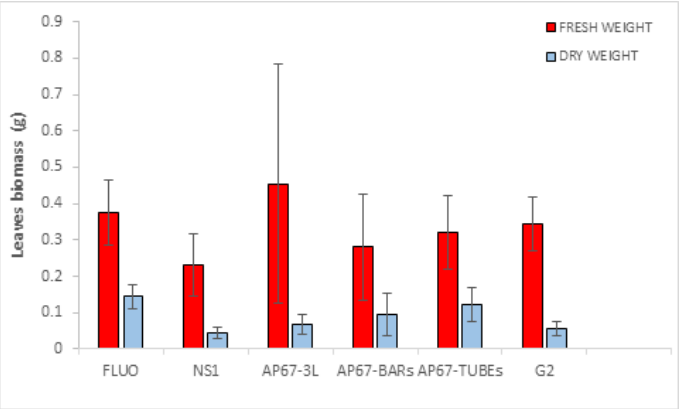



Number of leaves
70
60
50
40
30
20
10
0
FLUORA
NS1
AP67 3L
AP67-BARs
AP67-TUBEs
G2


Average leaf area (cm²)
1.2
1
0.8
0.6
0.4
0.2
0
FLUORA
NS1
AP67-3L
AP67 BARs
AP67 TUBEs
G2


Shoot biomass (g)
0.3
0.25
0.2
0.15
0.1
0.05
0
FRESH WEIGHT
DRY WEIGHT
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

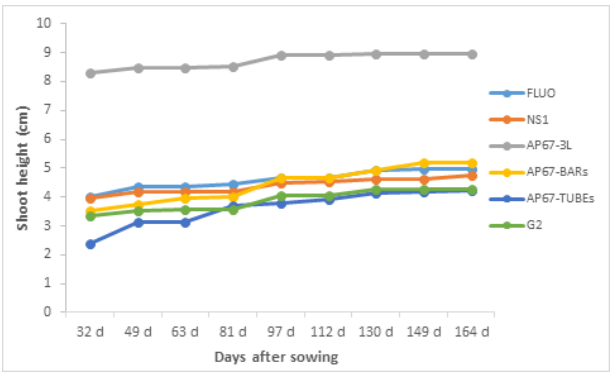

Root biomass (g)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
FRESH WEIGHT
DRY WEIGHT
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

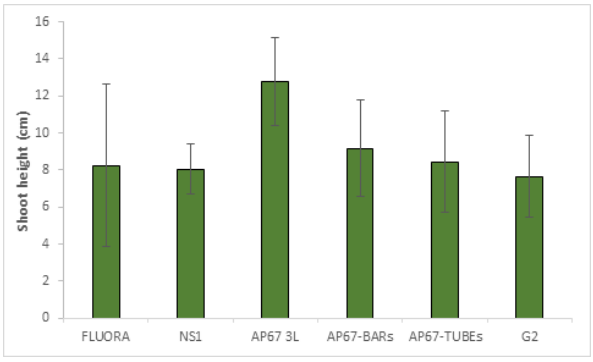


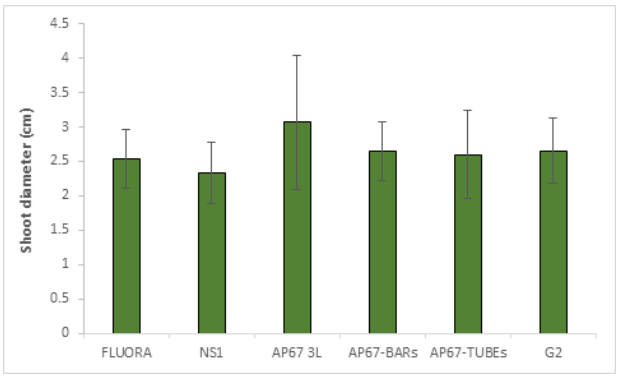
Morphological analysis in *Myrtus communis*, after the period in the greenhouse, showed no particular difference in plants grown under LED lights and under the traditional fluorescent lamp. Only AP67 3L showed highest leaves and stem biomass compared to all conditions tested.

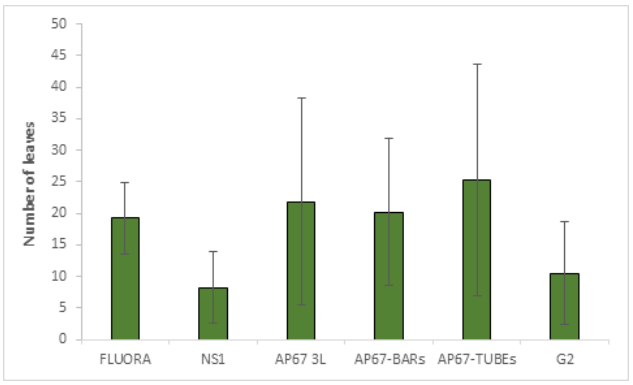
- ***Punica granatum***

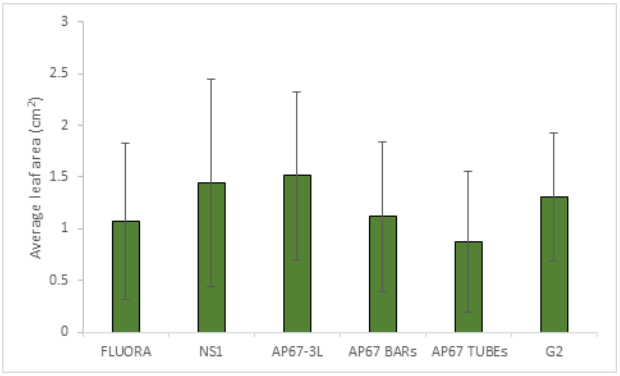
Growth curves

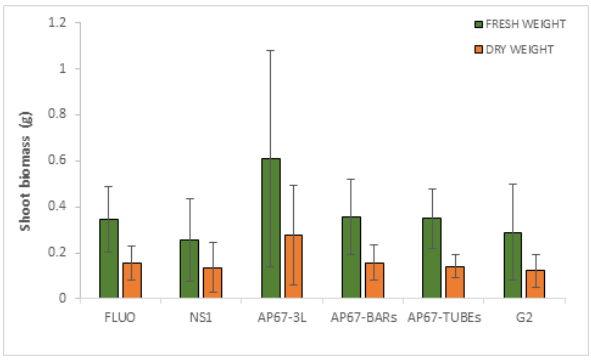


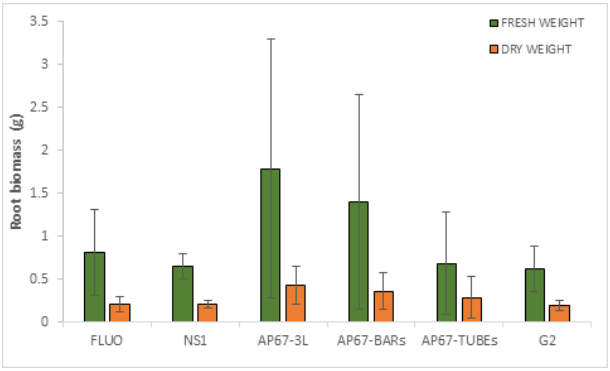


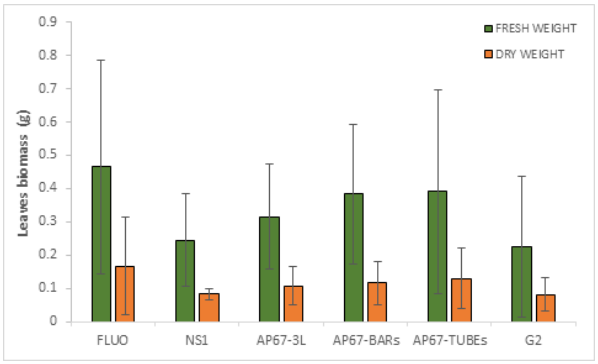

Shoot diameter (cm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
FLUORA
NS1
AP67 3L
AP67-BARs
AP67-TUBEs
G2


Number of leaves
50
45
40
35
30
25
20
15
10
5
0
FLUORA
NS1
AP67 3L
AP67-BARs
AP67-TUBEs
G2


Average leaf area (cm²)
3
2.5
2
1.5
1
0.5
0
FLUORA
NS1
AP67-3L
AP67 BARs
AP67 TUBEs
G2

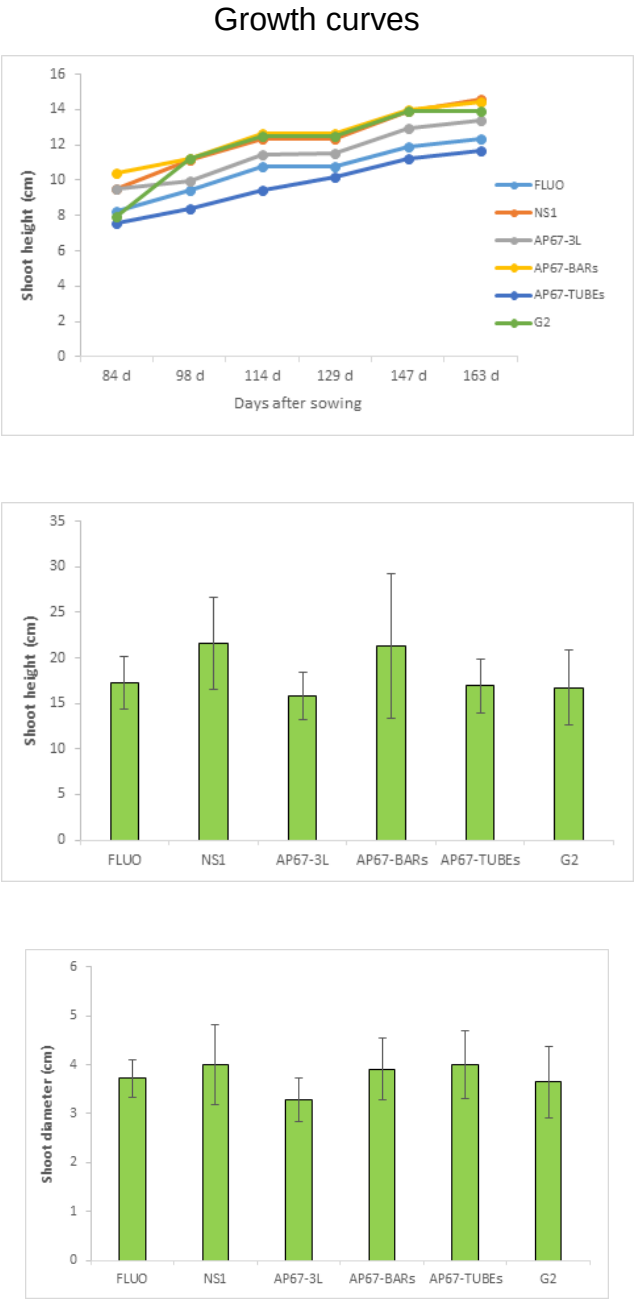

Shoot biomass (g)
1.2
1
0.8
0.6
0.4
0.2
0
FRESH WEIGHT
DRY WEIGHT
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

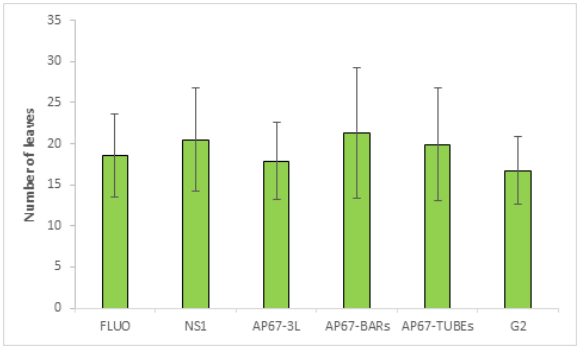


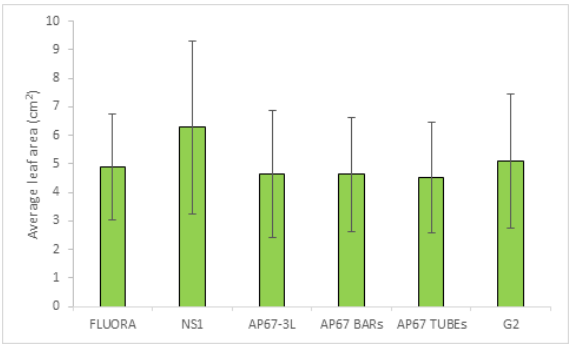


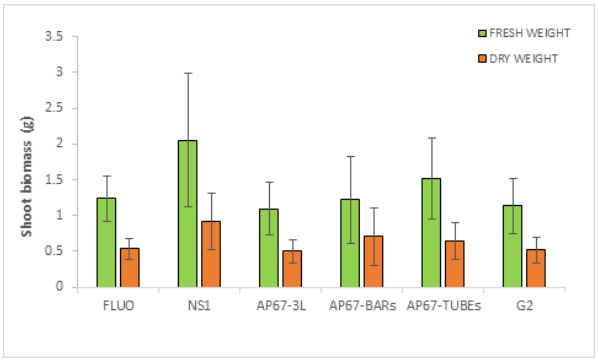
Morphological analysis in *Punica granatum*, after the period in the greenhouse, showed that the lamp AP67 3L provides the highest results in term of growth parameters. In particular, the plants grown under AP67 3L showed better results in terms of shoot height and stem diameter. On the other hand, the plants grown under FLUORA showed better results in terms of biomass of the leaves.

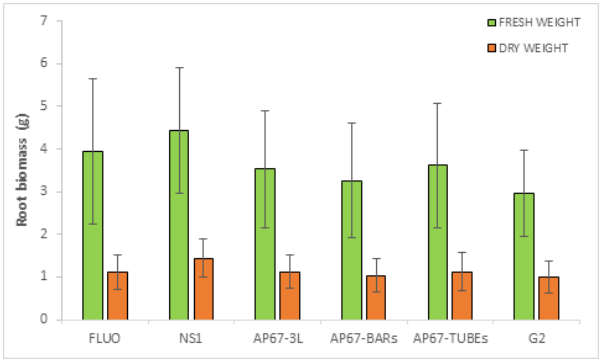
- ***Quercus ilex***

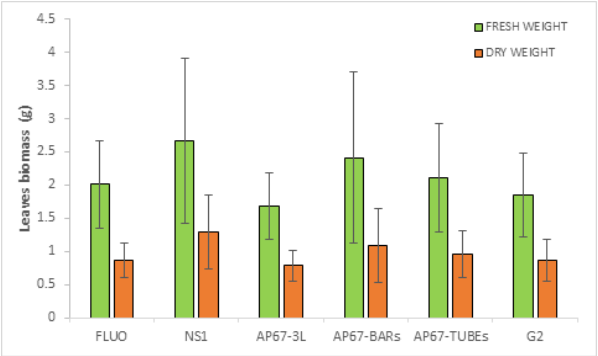



Number of leaves
35
30
25
20
15
10
5
0
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2


Average leaf area (cm²)
10
9
8
7
6
5
4
3
2
1
0
FLUORA
NS1
AP67-3L
AP67 BARs
AP67 TUBEs
G2

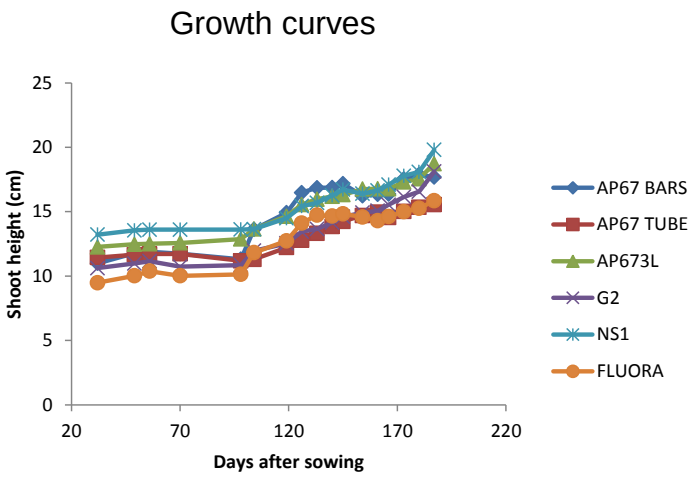

FRESH WEIGHT
DRY WEIGHT
Shoot biomass (g)
3.5
3
2.5
2
1.5
1
0.5
0
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

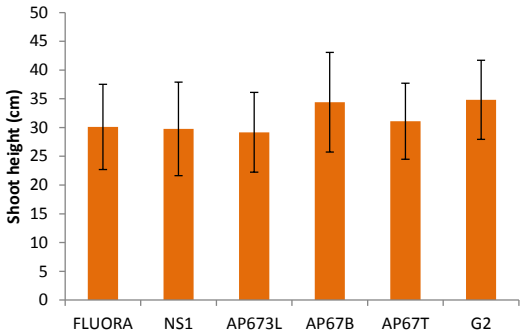

FRESH WEIGHT
DRY WEIGHT
Root biomass (g)
7
6
5
4
3
2
1
0
FLUO
NS1
AP67-3L
AP67-BARs
AP67-TUBEs
G2

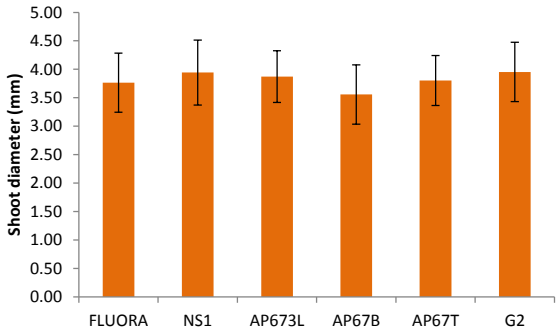


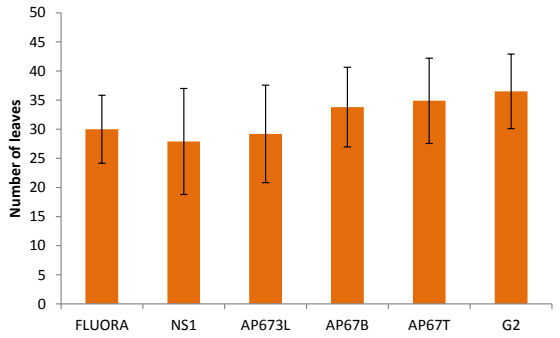
Morphological analysis in *Quercus ilex*, after the period in the greenhouse, showed no particular difference in plants grown under LED lights and under the traditional fluorescent lamp. Only NS1 showed highest leaves, roots and stem biomass compared to all conditions tested.

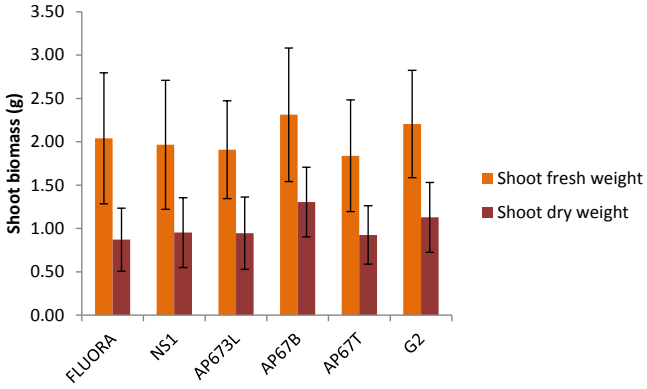
- ***Quercus suber***

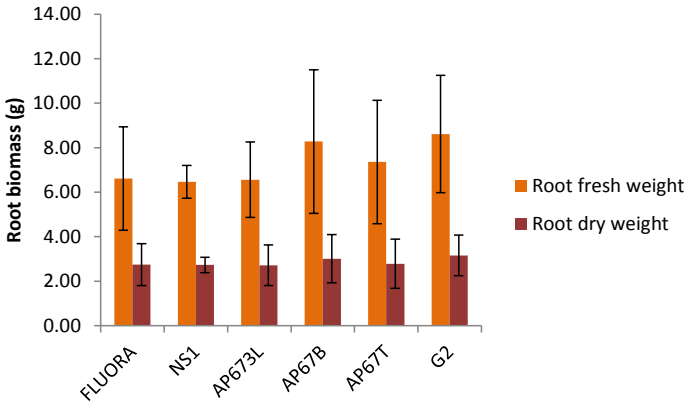


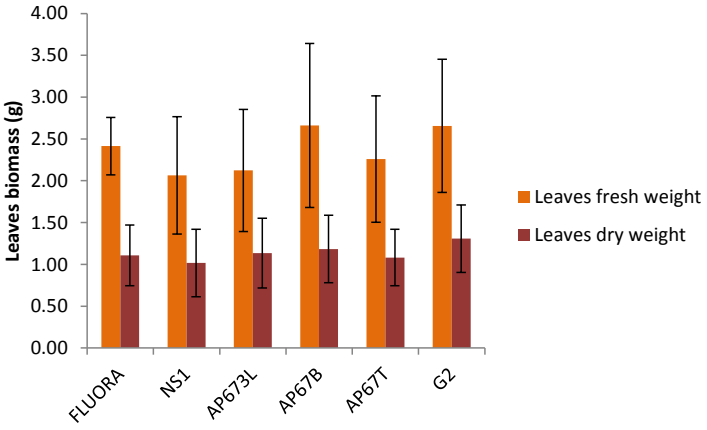


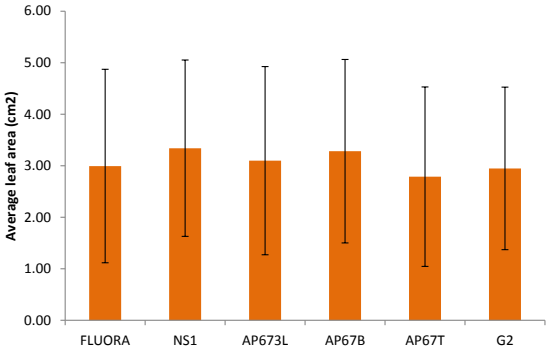

Shoot diameter (mm)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
FLUORA
NS1
AP673L
AP67B
AP67T
G2


Number of leaves
50
45
40
35
30
25
20
15
10
5
0
FLUORA
NS1
AP673L
AP67B
AP67T
G2


Shoot biomass (g)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
FLUORA
NS1
AP673L
AP67B
AP67T
G2
Shoot fresh weight
Shoot dry weight


Root biomass (g)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
FLUORA
NS1
AP673L
AP67B
AP67T
G2
Root fresh weight
Root dry weight



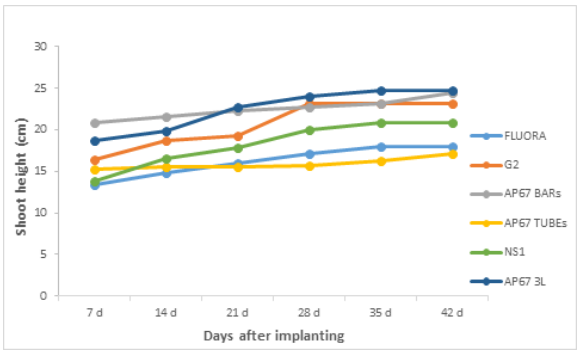


In the case of *Quercus suber*, fluorescent lights gave lower or comparable results to those obtained under LEDs for all the analysed parameters. AP67b gave the best results in terms of shoot height, shoot, leaves and root biomass and average leaf area. G2 gave the highest results in terms of number of leaves.

1.3.3. Outdoor trials (open-field)

- ***Corylus avellana***

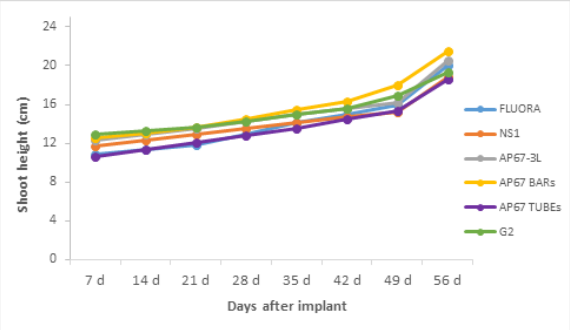
Growth curves



In plants of *Corylus avellana* all LEDs tested showed good results. In particular, the plants grown under AP673L showed the highest growth rate into open-field. Only AP67T showed a lower trend, in terms of growth, as well as for plants grown under fluorescent lights.

- ***Quercus ilex***

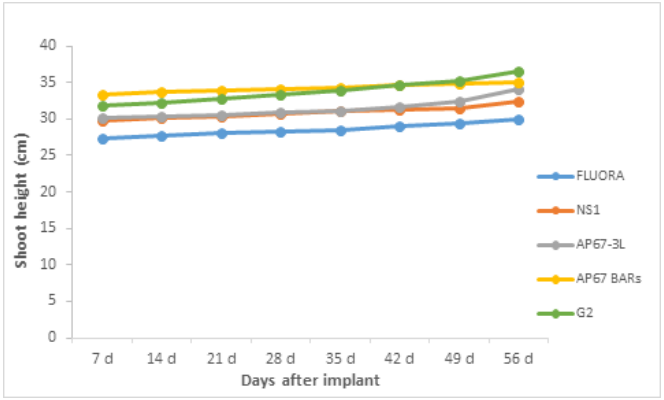
Growth curves



Plants of *Quercus ilex* did not show great increase in terms of growth rate at the beginning of the trial. But at day 42 there was an increase of growth especially for plants grown under AP67B. All other conditions tested showed no particular differences compared to plants grown under traditional fluorescent lamps.

- ***Quercus suber***

Growth curves



Quercus suber showed since the first day after the implant a better performance of plants grown under AP67T. On the other hand, FLUORA gave the lowest results.

1.4. Conclusions

- All the selected species showed good results in terms of growth rate and biomass allocation under innovative VALOYA LED lights, comparable or even better then those obtainable under conventional fluorescent lights.
- Each species reacts differently to each artificial light spectrum as a consequence of a different genetic background and ecology.
- Also biochemical analysis showed this characteristic. Each species showed to be more stressed by one or more than one light spectra but it is not possible to define an only one most stressful light source for all the tested species so as it is not possible to state what is the best light source for seedlings production, independently from the species.
- Anyway, when plants are transferred into a greenhouse for the acclimation period to the natural solar light, all the species show good survival rates, independently from the light source under which they have been grown before. Most of the losses are due to other factors like insects or probably simply to the biodiversity of the species which make some seedlings most performant than others. Also in this case it is not therefore possible to state that one light source is the best or the worst.
- Moreover, independently from the spectrum, seedlings show to react very well to the transplant into open field, without any relevant stress neither during the drought period typical of Mediterranean summer. Growth appears not to be stopped even if, as a consequence of the transplant shock from the pot to the ground, shoot height remains constant for a few weeks then growth rate restarts to speed up.

2. INPUT OF AZORINA

2.1. Azorean endemic species in Zephyr project

The Azorean forest for production is dominated by monospecific stands of exotic fast-growing species, such as *Cryptomeria japonica* and *Eucaplyptus globulus*.

There are none Azorean species used for production, although their timber has good value. Most of the island's natural forests were completely decimated for timber exploitation in the first centuries of colonization. Only recently the ecological awareness has risen and the society started to comprehend the need to protect and enhance the few remaining natural sites. As a result, ecological restoration efforts have started.

In addition to it, little is known about the native species germination, production and performance. Their seeds are difficult to obtain, and have different types of dormancy, in many cases they take months or even years to germinate. They haven't been genetically selected or bred, and there are no matured seed orchards so that the seeds could be easily and readily collected from, in large quantities. Fruiting individuals grow in natural populations, often in inaccessible places, in which they escaped from being harvested.

The importance of Zephyr project to the native *Laurissilva* forest is in a development of new growth protocols and trials for its endemic species. It is to enhance endemic species plant production, as the endemic *Laurissilva* forest occupies only a fraction of the islands surfaces, and there are not enough seeds available for further propagation. The three endemic species were initially chosen: *Prunus azorica, Frangula azorica and Juniperus brevifolia* to be tested, however the spectrum of trials was extended to other endemics as well.

The plants produced under the Zephyr trials are planted in the selected sites of the Furnas Lake Watershed Protected Landscape and they will serve as future seed production orchards.

2.2. Seed collection, treatment and storage

The seed collection was a significant part of the work performed in Work Package 3.

Endemic species seed collection required a licence issued by the Department of Nature Conservation in the Azores. The bibliographic research and study visits to the natural forest sites were accomplished as well as the logistics of seed collection was made in order to target the right period of fruit maturation and to be able to identify mother plants in production.

The collection was a lengthy and rewarding task, as the natural forest areas are scattered and they are found in remote places of the islands, having a rough hilly topography, at altitudes above 500 m.

Special measures were taken to sample as many plants as possible, however within the limits of not diminishing the natural regeneration capacity of the wild populations and not affecting the ecosystem. As an example there is an endangered endemic Azorean bullfinch (*Pyrrhula murina),* which needs fruits and seeds of several endemic species for their survival. Not enough seeds left for natural regeneration pose in danger the capacity of endemics competitiveness with invasive species, which rapidly invade forest clearings. That is why no more than 20 % of seeds were collected from each individual plant. There were different populations sampled to have a vast genetic variation, where fruits of at least 50 plants were collected within a single population, whenever it was possible.

The proceedings were different for fleshy and dry fruits. Fleshy fruits were soaked in water for 24 hours and then macerated, washed in water and air-dried for 2 weeks. If not subjected to treatments straight after drying, they were placed in a paper bags with silica sachets in a dark fridge at +4°C. They were subjected to several different treatments to test the possible methods of breaking the dormancy.

Dry frutifications (such as *Hypericum foliosum* or *Azorina vidalii*) were collected in paper bags and stored on open shelves, in a dark indoor room

with a good aeration, at the temperature of 18-20°C. The seeds collected in the humid days were spread on shelves together with moisture absorbents, such as silica gel.

2.3. Germination protocols

2.3.1. *Juniperus brevifolia*

This protected endemic species of great ecological importance, one of the few species that can survive in a high altitude, plays a role of water interceptor from mists, has associations with many endemic bryophytes and insects. It plays a role of a pioneer species in forest reconstruction in humid forests above 500 meters. What is more, it has a very valuable wood, the exploitation of which destroyed *Juniperus* forest on two of the Azorean islands, whereas on five islands the juniper forest is very fragmented and dispersed. It almost disappeared from low and medium altitude areas. That is why it is an important work in Zephyr project to promote seed germination methods and cultivation under different light spectra to find new ways of production for urgent ecological restoration of this species.

2.3.2. Seed collection

Juniperus brevifolia creates balanced ecosystem only on Terceira and Flores islands which is why those islands were targeted for seed collection:

- Within the month of October-December in a vast natural area on the island of Terceira, in Serra de Santa Barbara, at altitudes within the range of 810-950 meters above the sea level. The total of 515 fruits were gathered.
- In Flores island, at Ribeira de Ferreiro at altitudes above 500 meters. The total of 820 fruits were gathered.

Fig.1 Abundance of unripe fruits; the common two seeds per fruit.

Only the dark brown fruits were collected, as the *Juniperus brevifolia* fruits take about 18 months to mature on a tree. The *Juniperus* populations are fragmented, that is why the seed collection was extended.

The fruit collection was accomplished, with a total number of 1335 gathered, with an average of 2 seeds per fruit.

The berry-like seed cones were submerged in water for 24 hours to be subjected to fleshy scales removal. After that the seeds were extracted, being 2-3 per cone. The populations from Flores and Terceira were treated separately.

	No. of fruits	No. of seeds
Terceira island	515	1040
Flores island	820	1656
Total	1335	2696

Juniperus brevifolia plants accumulate a seed bank in the soil, the seeds are difficult to germinate, they show two types of dormancy, firstly because of the impermeable seed coat and secondly because of the embryo dormancy. In order to break the seed coat dormancy, 520 seeds were used per treatment and various treatments were performed, based on the successful germination case studies of juniper species. The treatments are presented below.

2.3.3. Treatments

Flores seed lot:

1) Control – untreated seeds sown under natural conditions and monitored during 120 days;
2) Mechanical scarification, followed by cold stratification in sand at +5ºC for 120 days.
3) Chemical stratification with H_2SO_4 for 45 minutes followed by cold stratification in sand at +5ºC for 120 days

Terceira seed lots:

4) Mechanical scarification followed by 150 days of cold stratification at +5ºC

5) Chemical scarification with H_2SO_4 for 45 minutes, followed by 150 days of cold stratification at +5ºC

All the seeds were treated with Azoxystrobin a preventive and curative systemic fungicide ($1gL^{-1}$ - active principle).

2.3.4. Results

The control didn´t show any germination activity, whereas the treatment number 2 and 3 showed 13% and 12 % germination rate respectively. It was already a good result, taking into account the fact that seeds sown with no pre-treatment show a germination rate that is inferior of 1% in the first year of cultivation. In the treatment 4 and 5 the stratification time was increased, and the results showed 14% and 15,5% of germination rates respectively.

The germination was spread in time, so that it was not possible to obtain a sufficient number of seedlings to perform LED light tests.

2.3.5. *Prunus azorica*

Over the past centuries, *Prunus azorica* exploitation was undergoing without allowing the populations to recover, which led to its present endangered status. Hence, it is extremely important to set growth protocols that allow improving the species status, but also to have excellent quality seedlings for afforestation promoting the diversification of Azores forest products. Main factors that inhibit the recuperation of the species are populations with very low density; subpopulations are very isolated and have a poor genetic diversity.

In São Miguel island it is occurring naturally only in a limited eastern part of the island, with a population of about 200 individuals, whereas in Flores island there is only 1 tree registered.

2.3.5.1. First seed collection

The seeds were collected from a small number of individuals, in São Miguel island, in total there were 430 mature seeds gathered. They were soaked for 24 hours and the fleshy mesocarp was removed. These seeds are affected by a deep dormancy, which can be removed by stratification in a moist medium (a mixture of peat and sand) (Suszka et al., 1996).

To break the seeds dormancy, two protocols were followed.

2.3.5.1.1. Treatment 1

Cold moist stratification at 4ºC for 90 days with the removal of the seed endocarp (Moreira et al., 2012).

Methodology

Germination tests were performed in chambers with automatic temperature control (error margin of ± 2ºC) and a light period of 12 h. The removal of the seed endocarp was labour intensive. The treatment was set with three replicates, of 40 seeds each.

Results

Despite the removal of endocarp, seed germination was very low, 18%, and not uniform. This protocol did not allow us to produce sufficient quantity of plants to proceed with growth tests under different light treatments.

2.3.5.1.2. Treatment 2

Alternating 25 °C (2 weeks), 3°C (2 weeks), 25 °C (2 weeks), 3 °C (12-16 weeks) until the start of the germination, without the removal of the seed endocarp.

Fig.2 P*runus azorica* fruits and seeds in treatment nr 2**.**

Methodology

The longer pre-treatment to break seeds dormancy, was set as follows: The total of 310 seeds was placed in plastic boxes with a mixture of peat and perlite, treated with Azoxystrobin a preventive and curative systemic fungicide ($1gL^{-1}$ - active principle methoxyacrylate) and kept at

25ºC for two weeks in a climatic chamber. To prevent fungi development, seeds were treated whenever necessary with the fungicide solution. Prior to start the cold treatment seeds were water bathed (tap water) for two hours, before applying the fungicide solution. Seeds were kept at 3ºC for two weeks, then submitted again to a heat treatment at 25ºC for two weeks and finally remained for seventh weeks in cold stratification.

Results

Prunus azorica dormancy was not removed, despite the pre-treatment applied. The Azorian cherry seed lot that was tested showed an extremely strong dormancy (12-24 weeks). Dormancy in *Prunus azorica*, might be as the one in *Prunus avium*, which is exceptionally difficult to remove, comprising a combination of physiological (hormonal control plus embryo immaturity) and physical mechanisms (impermeable seed coats).

Facing the fact of low seed germination rate it turns to be necessary to use a large amount of seeds to obtain a considerable number of seedlings to install the LED trials. Moreover, *P. azorica* is an endangered species, with very few adult trees in seed production; it is extremely difficult to obtain such large numbers of seeds. Hence, it was decided to use cuttings under the LED trials. If successful propagation protocol is established, it will allow to reduce the production time and hence, soon after, be able to set seed orchards.

2.3.5.2. Second seed collection

The seeds were collected at São Miguel island at the young forest stand of 5-year old plantations and in Terceira island from new roadside plantations. The seeds were collected over a period between 18 of September and 13 of October 2014. About 4000 seeds in total were collected. They were soaked for 24 hours and the fleshy mesocarp was removed, then they were air-dried and stored in a dark fridge at +4° C until 12 of December 2014.

2.3.5.2.1. Seeds pre-treatments

The following procedure was followed for the pre-treatments:

1. Rinse seeds with distilled water;

2. Hydrate them for 24 hours in distilled water and remove floating seeds;

3. Treat seeds with fungicide, ex. Teldor 3 ml/1liter of water for 10 minutes;

4. Leave seeds spread on the sheet for 3 hours;

5. Put the seeds into a moist but not excessively wet substrate (perlite), previously sterilized in the oven. Use only distilled water.

6. Place them in polythene bags in the fridge / chamber set for a certain temperature with the bag not completely closed for gas exchange;

7. After a set period of time test the seeds germination rate in the germination trials at the growth chamber at 20 C on moist filter paper placed inside Petri dishes, and submitted to 12 hours of light of fluorescent tubes. To avoid fungal contamination seeds were washed every 7 days with commercial bleach (NaClO) diluted at 5% and after with distilled water. The filter paper was moistened with distilled water and after 15 days was replaced by new filter paper.

There were 4 replicates per treatment and 30 seeds per replicate.

For all treatments: endocarp was removed, just seeds were pre-treated. The treatments for breaking the dormancy where the following:

Treatment 1: cold moist stratification at 4ºC for 30 days;
Treatment 2: cold moist stratification at 4ºC for 60 days;
Treatment 3: cold moist stratification at 4ºC for 90 days;

2.3.5.2.2. Results

It was not possible to obtain conclusive results due to fungal contamination during the cold moist stratification period, and the germination test under fluorescent light. For this reason it is suggested to conduct a new break dormancy test, this time using a different protocol to prevent fungal contamination.

2.3.6. *Frangula azorica*

2.3.6.1. First seed collection

The seed were collected in September from a young stand of trees reintroduced to Furnas Protected Landscape, in the island of São Miguel, in plantation actions that had taken place 6 years ago. In total 480 seeds were gathered. The fruits collected were soaked for 24 hours, their pulp was then removed and seeds were washed with water; according to the protocol developed by LIFE priolo project. After that, without drying they were placed in perlite substrate at +18°C and natural light.

The germination was slow and uneven, starting only after 16 weeks, with a germination rate of about only 10%. This number didn´t allowed for comparing the growth in between the different spectra; and greater seed number to conduct further germination tests was not available.

2.3.6.2. Second seed collection

The seeds were collected at São Miguel island at the young forest stand at 5-year old plantations. The seeds were collected over a period between 18 of September till 12 of November. This was a first abundant production of this new forest stand, and about 6000 seeds were collected.

The seeds were cleaned off the pulp by soaking in the water for 24 hours and then macerating them manually on a sieve. After that they were spread on a plastic tray and left in a naturally ventilated room for 2 weeks. After being dried they were placed in paper bags with silicone sachets in a dark fridge at +4C, till the pre-germination treatments began.

Treatments

Seeds pre-treatment protocol was followed as in chapter 2.3.3.

Results

During this germination test the germination chamber broke down and the test had to be repeated, since there were not enough seeds available for conducting a new trial it was necessary to harvest new seeds. This time the seeds were harvested in the Botanical Garden of Faial, during the month of July 2015.

Germination tests are still on going, however, it has been observed that the germination is occurring very slowly and unevenly. The longer test with an average of 8% of germination (after 45 days on the germination chamber).

2.3.7. *Morella faya*

2.3.7.1. Seed collection

The fruits were collected in August and September at São Miguel island from 3 different populations. They were soaked in water for 24 hours and then macerated and air-dried. Then they were placed in paper bags with silicone sachets in a dark fridge at +4°C, till the pre-germination treatments began.

2.3.7.2. Treatments

Seeds pre-treatment protocol was followed as in chapter 3.2.2.1.

2.3.7.3. Results

The germination tests are still on going, however, it is possible to verify some differences: treatment 2 (60 days of cold moist stratification at 4° C) seems to have better results than treatment 1 (30 days of cold moist stratification at 4° C), and treatment 3 is not occurring long enough for comparison. After 88 days, with treatment 1 there was a germination percentage of 67.5 ± 5. After 56 days, with treatment there was a 2 germination percentage of 85 ± 6:38. After 26 days, with treatment 3 there was a germination percentage of 90.8 ± 5.7.

The effect of the different treatment of cold moist stratification was compared after 56 days of germination (Table 1).

Table 1. The effect of days in cold moist stratification on the germination characteristics of *Morella faya*.

Treatment	N	Days to first radicle emergence	Mean time to germination (days)	Percent of germination at 56th day
1	4x30	7	15,7 ± 2,6	64,2 ± 3,2
2	4x30	0	4,9 ± 1,1	86 ± 6,4

2.4. LED trials

In LED trials by Azorina, there were 2 inox trolleys put up in a dark room which is one of the laboratories of the University of the Azores. On each shelf there two tubes of the following light sources installed:

1) OSRAM L36W/77 FLUORA (Fluorescent)
2) 2 bar lamps Valoya AP67 (120cm)
3) 2 bar lamps Valoya AP673L (120cm)
4) 2 bar lamps Valoya G2 (120cm)
5) 2 bar lamps Valoya NS1 (120cm)

Each shelf had dimensions to provide for two trays Quickpot HerkuPlast QPD 104 VW with Pre-forma Jiffy substrate.

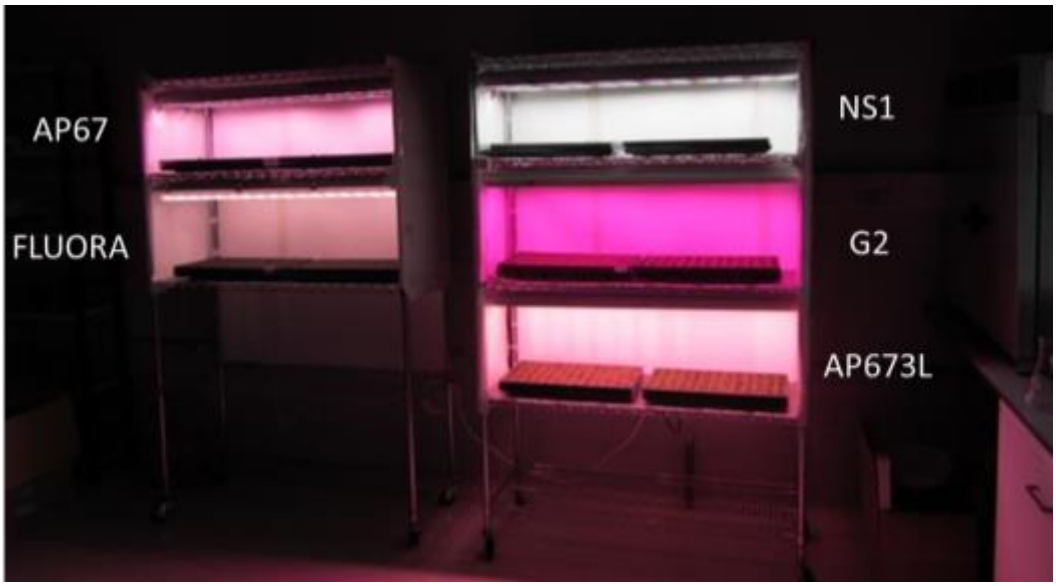


Fig.3 Disposition of Led lights in the laboratory

2.4.1. *Prunus azorica*

2.4.1.1. Pre-cultivation of cuttings

Cuttings were obtained from the year grown branches and transported in plastic bags to the laboratory, to avoid moisture loss in December (1st trial) and again in April (2nd undergoing trial). The leaves were removed and fresh cuts were made in the laboratory obtaining 5-10 cm long cuttings, including 3-4 nodes from the apex. Each cutting received a basal quick-dip in a solution of Indole-3-Butyric Acid (IBA). For the first trial, the IBA solution concentration was 5mg.ml^{-1}, whereas on the second trial it was 10mg.ml^{-1}.

The first trial had only 10% of rooting success that is why the IBA concentration was raised in the second trial. In the second trial the cuttings did not developed any roots in 95 % of the cases, which is very uncommon for that species. Initially the cuttings were looking promising and started sprouting new leaves; however they did not root and as soon as their energy reserves have finished, after 2 months of cultivation under different lights as well as in a greenhouse with natural light (control) , they started dying off.

Fig.4. *Prunus azorica* cuttings subjected to LED trials

2.4.2. *Frangula azorica*

2.4.2.1. Materials and methods

Due to the problems encountered with seed availability and their germination, micropropagated plants were produced for growth tests. After the multiplication stage the in vitro apical shoots 2–3 cm in length were rooted on 200-cm^3 glass

vessels containing 25 cm^3 perlite and 20 cm^3 of liquid rooting and four shoots per vessel. The cultures were maintained in a growth chamber at 22 ± 1 °C under cool white fluorescent lamps (55 µmol $m^{-2}s^{-1}$) with a 16-h photoperiod for 7-8 weeks. After that, the micropropagated plants were put in Quickpot HercuPlast QPD 104 VW trays with preforma Jiffy substrate. They were placed in a dark room on shelves of two inox trolleys, each shelf equipped with a certain light type, photoperiod set for 10 hours of light. In the first 17 days, the trays with the plants were inside a plastic bag to maintain a high humidity environment. The room temperature was about 22ºC.Ten days after the transplant of the micropropagated plants of *F. azorica* the measurements started and were taken once a week, during five weeks. For that, seven plants growing under each light type were taken off the trays at random and their roots were washed over a mesh screen to take out the soil particles and immediately dried in paper towel. After that the height of the 35 plants were obtained (aerial part), the plants were separated in the aerial and root parts. Each fraction was than weighed, in fresh and after drying for two days at 55ºC, in a high precision weighing scale that measured in mg.Data was analysed using one way ANOVA of the StatPlus:mac, Copyright 2009 AnalystSoft Inc. The means were compared with the test of Tukey-Kramer at P≤0.05

Fig. 5. Weekly sampling and analyzing of root and shoot growth

2.4.2.2. Results

The differences in the dry weight of stems and leaves of *Frangula azorica* under different light types were statistically different only in the last harvest although AP67 and NS1 had higher weights than the other plants in the 4th harvest but not yet

significantly different. In the 5th harvest the weights of the plants under the lights AP67 were significantly higher than the weights of the plants under the other light types, except NS1 (Table 2).The differences in the dry weight of the roots of *Frangula azorica* under different light types were statistically different only in the last harvest although AP67 and NS1 had higher weights than the others in the 4th harvest but not yet significantly different. In the 5th harvest the weights of the roots under the lights AP67 were significantly higher than the weights of the roots under the other light types, except NS1 (Table 3).

The dry matter content (DM) of the plants under Fluora light, since the first harvest, was significantly lower than the DM of the plants under AP67 (Table 4). Although the plants in the Fluora treatment were as tall as the plants under AP67 and NS1, their dry weights were lower and it was possible to see that the plants were less lignified that the plants under the other light sources, especially under AP67, AP 673 L and NS1. In average, over all harvests, NS1 had more leaves (14) and all the other treatments had 11. For each light type, after the second harvest, the number of leaves was almost constant, since as new leaves were formed some old ones died.

Fig. 6. The 5-week grown plants under different LED light spectra: AP673L, AP67, G2, NS1.

Table 2. Dry weights (g) of stems and leaves of *Frangula azorica* in the five harvests.

LIGHT TYPE	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	0.0236 [a]	0.0257 [a]	0.0374 [a]	0.0423 [a]	0.0645 [b]	0.0387
AP 673 L	0.0135 [a]	0.0252 [a]	0.0338 [a]	0.0309 [a]	0.0436 [b]	0.0294
AP 67	0.0225 [a]	0.0477 [a]	0.0369 [a]	0.0595 [a]	0.1194 [a]	0.0572
NS1	0.0215 [a]	0.0273 [a]	0.0335 [a]	0.0564 [a]	0.0699 [ab]	0.0417
G2	0.0182 [a]	0.0197 [a]	0.0194 [a]	0.0251[a]	0.0299 [b]	0.0225
AVERAGE	0.0199	0.0291	0.0322	0.0428	0.0655	

Note 1: The average dry weight of stems and leaves at start was 0.0084 ± 0.0041 g

Note 2: In each harvest column the means with the same letters were similar at $P \leq 0.05$

Table 3. Dry weight (g) of roots of *Frangula azorica* in the five harvests.

LIGHT TYPE	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	0.0045[a]	0.0047 [a]	0.0085 [a]	0.0106 [a]	0.0159 [c]	0.0088
AP 673 L	0.0034[a]	0.0062 [a]	0.0110 [a]	0.0115 [a]	0.0220 [c]	0.0108
AP 67	0.0050[a]	0.0090 [a]	0.0117 [a]	0.0161 [a]	0.0490 [a]	0.0182
NS1	0.0038[a]	0.0062 [a]	0.0057 [a]	0.0183 [a]	0.0267 [ab]	0.0121
G2	0.0045[a]	0.0036 [a]	0.0055 [a]	0.0089 [a]	0.0140 [bc]	0.0073
AVERAGE	0.0042	0.0059	0.0085	0.0131	0.0255	

Note1: The average dry weight of roots at start was 0.0025 g

Note 2: In each harvest column the means with the same letters were similar at $P \leq 0.05$

Table 4. The dry matter content (%) of the aerial part of the plants in each harvest

LIGHT TYPE	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	21.29 c	26.76 b	29.72 b	32.80 b	27.64
AP 673 L	27.90 ab	32.56 a	34.50 ab	36.54 ab	32.88
AP 67	25.11 b	34.79 a	35.10 a	40.49 a	33.87
NS1	28.81 ab	32.18 a	32.36 ab	36.45 ab	32.45
G2	28.82 a	33.43 a	31.03 b	31.84 b	31.28
AVERAGE	26.39	31.94	32.54	35.62	

Note: In each harvest column the means with the same letters were similar at $P \leq 0.05$

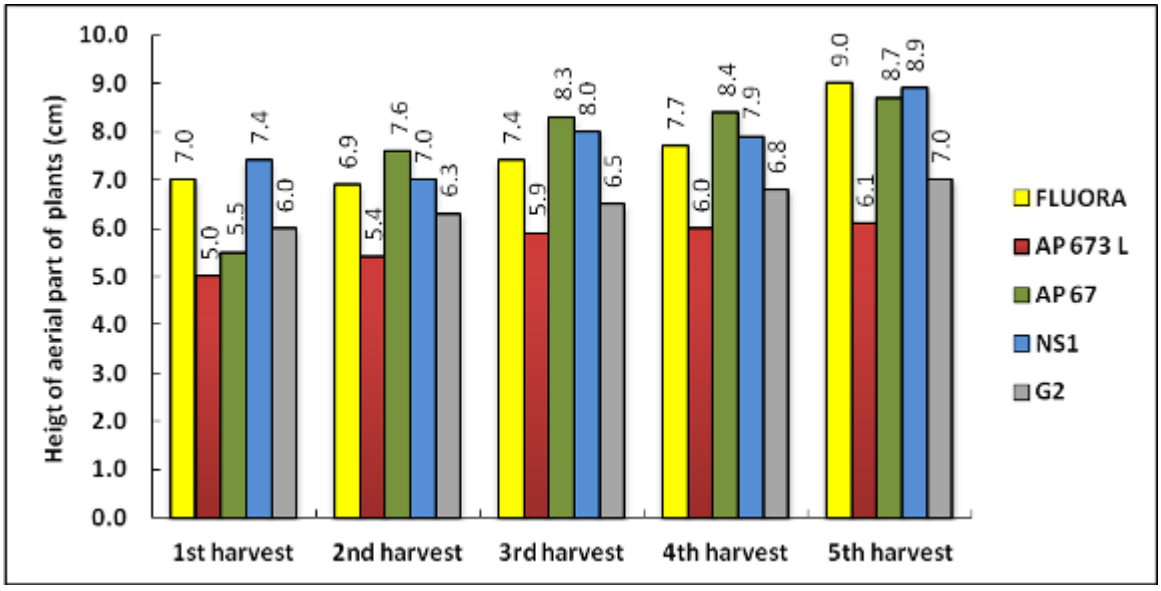


Fig. 7. Height of aerial part of plants (cm) in the five harvests

2.4.3. *Juniperus brevifolia*

2.4.3.1. Materials and methods

Due to the problems encountered with seed availability and germination the micropropagated plants were produced for growth tests. For materials and methods the same steps were followed as in chapter 4.2.1.

2.4.3.2. Results

The differences in the dry weight of stems and leaves and roots of *Juniperus brevifolia* under different light types were not statistically different but AP67 and AP673L had generally lower weights than the others in the last three harvests (Table 5). For each light source the aerial part of the plants and the roots always had the same pattern of growth (Fig 10).In the 5th harvest the plants of *J. brevifolia* had 5.1 cm in the NS1 light, 4.6 cm in the G2 and 4.2 cm in the other lights. The micropropagated plants had 2 or 3 branches and the plants continue to ramificate. Since the 2nd harvest the plants under Fluora light had significantly lower dry matter content (Table 7) than the plants under the other lights.

Fig. 8. The micropropagated plants, Produced at the Azores Biotechnology Centre

Fig. 9. Weighing of aerial part

Fig. 10. The 5-week grown plants under different light spectra: AP673L, AP67, Fluora, G2 and NS1.

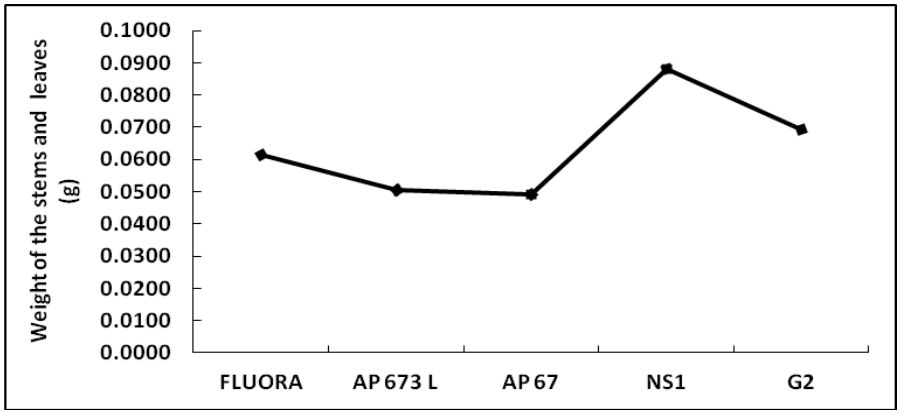


Fig 11. Dry weights of the stems and leaves (g) of the *Juniperus brevifolia* plants (4th harvest)

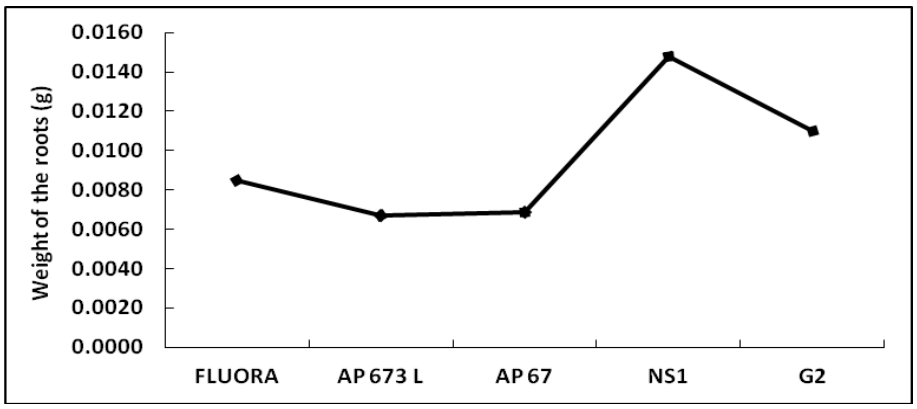


Fig. 12. Dry weights of the roots (g) of *Juniperus brevifolia* plants (4th harvest)

Table 5. Dry weights of stems and leaves of *Juniperus brevifolia* in the five harvests.

LIGHT TYPE	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	0.0526 a	0.1020 a	0.0796 a	0.0614 a	0.0588 a	0.0709
AP 673 L	0.0401 a	0.0551 a	0.0459 a	0.0505 a	0.0602 a	0.0504
AP 67	0.0529 a	0.0435 a	0.0564 a	0.0492 a	0.0625 a	0.0529
NS1	0.0825 a	0.0689 a	0.0708 a	0.0881 a	0.0915 a	0.0804
G2	0.0701 a	0.0571 a	0.0467a	0.0694 a	0.1027 a	0.0692
AVERAGE	0.0596	0.0653	0.0599	0.0637	0.0751	

Note 1: The average dry weight of the aerial part of plants at start was 0.0467 ± 0.0193 g

Note 2: In each harvest column the means with the same letters were similar at $P \leq 0.05$

Table 6. Dry weights of the roots of *Juniperus brevifolia* in the five harvests.

LIGHT TYPE	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	0.0073	0.0157	0.0089	0.0085 a	0.0103 a	0.0101
AP 673 L	0.0054	0.0088	0.0042	0.0067	0.0085 a	0.0067
AP 67	0.0077	0.0058	0.0075	0.0069	0.0082 a	0.0072
NS1	0.0097	0.0083	0.0098	0.0148	0.0136 a	0.0112
G2	0.0090	0.0071	0.0059	0.0110	0.0175 a	0.0101
AVERAGE	0.0078	0.0091	0.0073	0.0096	0.0116	

Note 1: The average dry weight of the roots at start was 0.0076 ± 0.0038 g

Note 2: In each harvest column the means with the same letters were similar at $P \leq 0.05$

Table 7. Stems and leaves dry matter content in the five harvests (%)

LIGHT TYPE	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	AVERAGE
FLUORA	32.22 a	29.02 b	29.04 b	29.24 b	28.57 b	28.97
AP 673 L	34.68 a	29.66 b	32.34 ab	30.88 ab	29.13 ab	30.50
AP 67	30.26 ab	27.17 b	33.51 ab	30.51 ab	29.56 ab	30.19
NS1	31.65 ab	36.92 a	35.35 a	32.94 a	32.71 a	34.48
G2	24.79 b	29.33 b	34.41 a	31.27 ab	30.45 ab	31.37
AVERAGE	30.72	30.42	32.93	30.97	30.08	

Note: In each harvest column the means with the same letters were similar at $P \leq 0.05$

2.4.4. Additional species

Azorina vidalli and *Hypericum foliosum* were added to the originally proposed species list, to complement germination trials of *Prunus azorica and Juniperus brevifolia;* their low germination rates combined with low seeds availability made it very difficult to perform trials under the LED lights. The new species were chosen, owing to the absence of physiological dormancy, and to its importance in the Azorean endemic flora. *A. vidalli* is one of the top 100 endangered flora species and it belongs to the only endemic plant Genus of this archipelago, whereas *H. foliosum* is important endemic in Azores bullfinch diet.

2.4.4.1. *Hypericum foliosum*

The seeds were collected in Terceira island at Serra de Santa Barbara and at Furnas hydrographic basin in São Miguel Island. At each cavity of Jiffy 2-3 seeds were placed for direct germination tests under LED lights, as the species doesn't present any dormancy. At the initial tests there was no success with germination, because of the use of immature seeds, collected prematurely. Within the next lot of seeds there has been a very good germination rate in all the spectra; however the plants suffered a big mortality, as there was a fungal disease, probably infected during the seed handling and storage.

Having in mind the difficulties mentioned above, pre-germination was conducted on Petri dishes filled with a thin layer of sand and a filter paper, and placed at +18°C. The species has shown a very good germination rate of 96%, however it had tiny seeds, very difficult to handle so during transplanting into Jiffy about 40% didn't survive and they had to be repeatedly replanted.

What is more the slow growth and microscopic size in the first 5 weeks of pre-cultivation didn't allow for the measurements to take place.

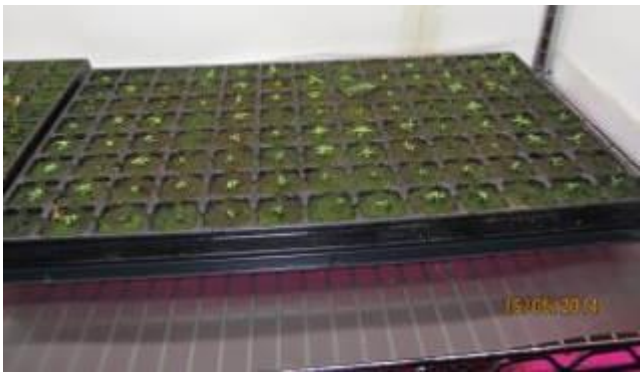


Fig 13. Hypericum foliosum after 5 weeks of cultivation under Fluora light.

2.4.4.2. *Azorina vidalii*

The seeds were collected in October in Terceira Island in 3 large populations in Monte Brasil, Porto Martins and Quatro Ribeiras, as well as in late November in São Miguel island, in Mosteiros.

This species germinates readily on Petri dishes at the temperature of +18°C within 12 days, having a germination rate above 90%. The transplanted pre-germinated seedlings were very small, of 1-2 mm, the initial growth under test lights was very insignificant, and the weekly measurements were not feasible. The prolonged light tests, over 10 weeks led to problems with algae and fungal growth, while growth was not accelerated.

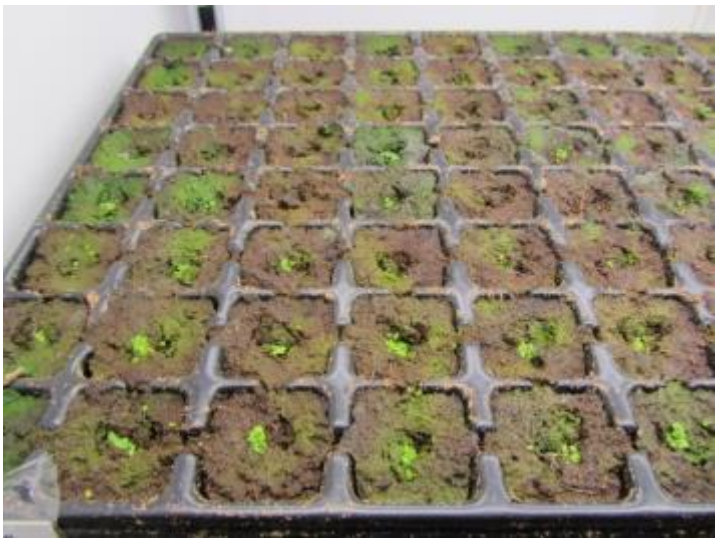


Fig.14. Led trails with *Azorina vidalii* placed in Jiffy substrate in Quickpot Herculplast trays

2.4.5. *Vaccinium cylindraceum*

2.4.5.1. Materials and methods

The micropropagated plants were produced for growth tests. For materials and methods the same steps were followed as in point 4.2.1.

Fig 15. Preparation of *Vaccinium* for morphological measurement.

2.4.5.2. Results

For Vaccinium the correlation between root dry weight and shoot dry weight was very positive, ranging from 98% for Fluora and AP673L, 92% for Control, 91% for G2, 90% for AP67, and 81% for NS1. The correlation between the shoot height and its dry weight was 53%, whereas the correlation between root length and its dry weight was low, of only 31%. While comparing the shoot and root length, the roots grew

more than shoots however they are much lighter, with lower values of dry weight than shoots.

Table 8. The Vaccinium plants shoot and root dry weight and length values for each light spectrum

LIGHT TYPE	AERIAL PART DRY WEIGHT (g)	ROOTS DRY WEIGHT (g)	AERIAL PART LENGHT (cm)	ROOTS LENGHT (cm)
CONTROL	0.1042	0.0756	6.43	14.00
FLUORA	0.2960	0.1236	8.44	13.39
AP673 L	0.2255	0.1399	7.17	14.94
AP 67	0.2749	0.1535	7.72	12.89
NS1	0.1735	0.0966	6.28	11.67
G2	0.1819	0.0950	6.83	12.67
AVERAGE	**0.2093**	**0.1140**	**7.15**	**13.26**

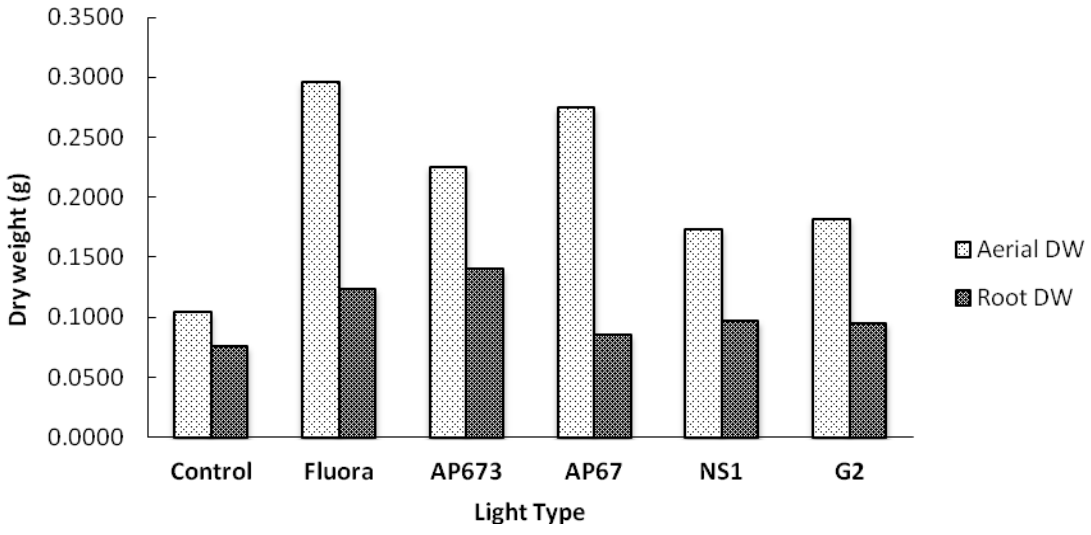


Fig. 16. Comparison of *Vaccinium cylindraceum* root and shoot dry weight after 7 weeks of pre-cultivation under different light spectra.

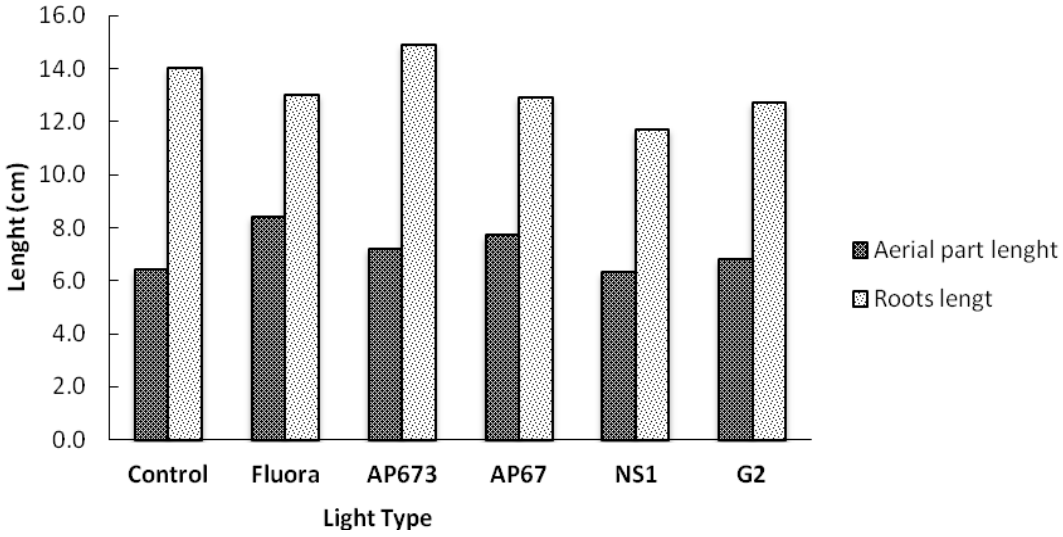


Fig 17. Comparison of *Vaccinium cylindraceum* root and shoot length after 7 weeks of pre-cultivation under different light spectra.

Fig 18. *Vaccinium cylindraceum* plants after 7 weeks of pre-cultivation under different light spectra: AP673L, AP67, Fluora, G2, NS1 and natural light.

2.4.6. *Morella faya*

2.4.6.1. Materials and methods

The seedlings produced from point 3.4 were used for growth tests. For materials and methods the same steps were followed as with micropropagated plants in point 4.2.1. But the measurements were made only after 7 weeks, with 10 plants.

Data was analyzed using SPSS Statistics, Copyright IBM 2014. A Levene test was used to test the homogeneity of the variables. When homogeneity was encountered, data were statistically compared using one–way analysis of variance (ANOVA), and, when the null hypothesis was rejected, the Tukey multiple comparison test was used. When homoscedasticity was not verifiable, data were statistically compared using the Kruskal-Wallis test for nonparametric analysis of variance; and, when the null hypothesis was rejected, a non-parametric Tukey-type test for multiple comparisons was used.

2.4.6.2. Results

The differences in the plant height of *Morella faya* under different light types were statistically significant. The heights of the plants under the lights FLUORA were significantly higher than the heights of the plants under the other light types, except for the plants under G2 (Table 9).

The plant diameter 1cm above de substrate surface of *Morella faya* under different light types were statistically different. The diameters of the plants under the lights FLUORA were significantly higher than the heights of the plants under the lights AP673L and NS1 (Table 9).

The number of leaves of *Morella faya* under different light types were statistically different. The number of leaves of the plants under the natural light were significantly higher than the number of leaves of the plants under the other light types, except for the plants under FLUORA and G2 (Table 9).

The plant fresh weight of *Morella faya* under natural light was statistically higher than the weight of the plants under the other light types (Table 9).

The plant dry weight of *Morella faya* under different light types were statistically different. The dry weights of the plants under the natural light were significantly higher than the heights of the plants under the lights AP673L and NS1 (Table 9).

There were no statistically differences between root dry weights of *Morella faya* under different light types (Table 9).

Table 9. The *Morella faya* plants measurements values for each light spectrum, after 7 week of growing. In measurement column the means with the same letters were similar at P≤0.05

LIGHT TYPE	PLANT HEIGHT (cm)	PLANT DIAMETER	NUMBER OF LEAVES	PLANT FRESH WEIGHT (g)	PLANT DRY WEIGHT (g)	ROOT DRY WEIGHT (g)
Control	2,51 ^{ab}	1,84 ^{ab}	5,9 ^{b}	0,0499 ^{b}	0,0164 ^{b}	0,0025 ^{a}
FLUORA	3,37 ^{c}	2,14 ^{b}	4,9 ^{ab}	0,0246 ^{a}	0,0118 ^{ab}	0,0019 ^{a}
AP673L	2,17 ^{a}	1,69 ^{a}	4,4 ^{a}	0,025 ^{a}	0,0092 ^{a}	0,0054 ^{a}
AP67	2,58 ^{abc}	1,96 ^{ab}	4,5 ^{a}	0,0236 ^{a}	0,0131 ^{ab}	0,0038 ^{a}
NS1	2,10 ^{a}	1,68 ^{a}	4,4 ^{a}	0,017 ^{a}	0,0092 ^{a}	0,002 ^{a}
G2	3,19 ^{bc}	2,04 ^{ab}	5,20 ^{ab}	0,0283 ^{a}	0,0140 ^{ab}	0,0023 ^{a}
AVERAGE	**2,653**	**1,892**	**4,68**	**0,02807**	**0,01228**	**0,00308**

Note: In each column the means with the same letters were similar at *P*≤0.05

2.5. Acclimation and field trials

2.5.1. Preparation of sites for acclimation

In order to test the field performance of the LED produced plants an area had been prepared during several months, to be planted with both LED produced and traditional produced seedlings (Fig. 19).

The area where these trial plots were established is located at *Furnas Protected Landscape*, a public area that hosts *Furnas Landscape Laboratory (FurnasLandLab)*, the *Project for the Ecological and Landscape Restoration of Furnas Lake Watershed*, managed by Azorina, S.A.

The concept on the basis of *Furnas LandLab,* is that the public area (300 ha) acquired for the restoration of Furnas Lake Watershed is made available for the needs of science and research projects mostly regarding natural science studies.

2.5.2. Methods

The sites to be planted at 505 m above the sea level, were very degraded by the previous occupations, and a large percentage of the area was invaded by many exotic species.

At the Furnas Lake watershed, invasive flora predominated in the abandoned pastures, the streams and erosion gullies, being represented by species from different parts of the word: *Hedychium gardnerianum*, *Hydrangea macrophylla*, *Rubus ulmifolius*, *Leycesteria formosa*, *Pteridium aquilinum*, *Gunnera tinctoria*, *Solanum mauritianum*, *Clethra arborea, Pittosporum undulatum* and *Acacia melanoxylon* among others.

Since the beginning of the Zephyr an area of about 5000 m^2 has been targeted for invasive species control, in order to install these plots with seedlings.

Fig. 19. Localization of the field trails (37º46´02.19´´N 25º20´20.14´´O, elevation: 505 m; source: Google maps

The eradication of the invasive plants has been a time consuming process, requiring repeated treatments due to abundant seedbank in the soil and high dispersion rate by birds. Without this intervention, the plant invaders would continue to thrive and subjugate the native plants to be installed under the Zephyr project, considerably reducing the survival chances of both LED and traditional produced plants. By properly removing the invasive species before planting, the costs of future maintenance of the planted area shall also be reduced, when in comparison with a poorly controlled site.

Regarding the eradication there were different methods and herbicides used to eliminate different target species. In this case the most common and effective has

been glyphosate, with the exception of the Kahili ginger (*Hedychium gardneranum*) that demands a different herbicide for its control.
Below there is a table (Table 10) with a list of the previously existing species and the herbicides and methods used for their eradication on the site.

Table 10. List of the previously existing species, and herbicides and methods used for their eradication.

Species Latin name	**Used Herbicide**	**Development stage**	**Method**
Hedychium gardneranum	Metsulfuron -Methyl		Rhizomes fresh cut - manual spray
			Foliar application - tractor hand spray
Rubus ulmifolius	Glyphosate	all	Foliar application - tractor hand spray
Leycesteria formosa		all	Foliar application - tractor hand spray
Pteridium aquilinum		Mature	Foliar application - tractor hand spray
Hydrangea macrophylla		Early Spring sprouts	Foliar application - tractor hand spray
Gunnera tinctoria		all	Rhizomes fresh cut - manual spray
			Foliar application - tractor hand spray
Solanum mauritianum,		Seedling	Foliar application - tractor hand spray
		Mature tree	Stump application -manual spray to the fresh cut
Clethra arborea,		Mature tree	Stump application -manual spray to the fresh cut
Pittosporum undulatum		Mature tree	Stump application -manual spray to the fresh cut
Acacia melanoxylon		Mature tree	Stump application -manual spray to the fresh cut

The biomass accumulated was, to some extent, burned through a prescribed fire. This also helped to kill the seed bank present in the soil, although in this case that

result was not obtained due to the small intensity of the fire. The remaining biomass was then shredded with moto-manual machines, as most of the terrain did not allow access for a tractor to reach.

Regarding the biomass of trees, to reduce expenses and assure sustainability a partnership was established with a local fruit cooperative of pineapple production. The trees were pulled away from the site. The timber was then used for firewood, and the canopies biomass (branches and foliage) was shredded by a machine to be used as green fertilizer for the pineapple production greenhouses.

After this large clean-up, the site had been under close monitoring, so that the germinating seed bank was eradicated before the planting of the produced seedlings. To have a proper invasive eradication from a site, the cleaning operations need to impact the area for a period of at least 12-18 months.

Due to the large abundance of rabbits a fence was erected around the planted site, to guarantee the seedlings are not predated.

2.5.3. Field trials

The plants grown in Jiffy plugs under different light treatments after 7 weeks were transplanted into bigger plastic containers of 125 cm3, filled with a local garden soil (2/3), mixed with local pumice (1/3).

The acclimation took place as follows:

- 2 weeks under a 75 % light filtering shade cloth,
- Followed by 4 weeks under the 50% light filtering shade cloth.

The plants were watered regularly, according to the weather conditions, usually every 2 days and after 6 weeks the cloth was removed and plants were subjected to natural light. This type of treatment was appropriate for acclimation to UV light spectrum of natural light, not resulting in any kind of leaf burns.

The planting took place in September. The plants were put into lines on slopes, with 2 repetitions. The species for field testing included: *Hypericum foliosum and Juniperus brevifolia*.

For *Hypericum foliosum* each spectrum consisted of 30 plants in the field, whereas for *Juniperus brevifolia*, as a difficult species to be propagated, it was possible to produce 10-15 plants per spectrum.

Each plant was individually protected with netting and stakes due to the plague of rodents (rabbits and rats) as well as the irrigation tubes were installed to secure the successful establishment of the plants. The maintenance in the first two years is being performed every second month, consists of the clearing off the site with streamers and applying herbicides wherever the invasive species reappear.

Fig. 20. Field acclimation trials in different sites for *Hypericum foliosum* and *Juniperus brevifolia*.

2.5.4. Results

The results of the morphological measurements of the aerial growth are presented below. Monitoring of the specimens after 6 months of the plantation showed a very good survival results in the field planting, as well as very good performance of all the plants (green leaves, no signs of phytopathological problems). The plantation of *Hypericum foliosum* was very successful,

counting with 99,4% of plants alive; only 1 plant died in spectrum NS1 out of 165 planted.

When it comes to *Juniperus brevifolia*, in total 90% of the plants survived in the field planting; survival of plants that had been pre-cultivated in different light spectra is presented in table X.

The monitoring is being performed twice a year. It is an added-value to the knowledge of the endemic species performance, as so far there have not been any growth rates records executed and published.

Table 11. Number and percentage of *Juniperus brevifolia* plants in the field

Light type	G2	Fluora	AP673L	AP67	Natural	NS1
dead plants	3	0	1	2	1	1
total planted	14	15	15	10	15	10
mortality rate %	21,4	0,0	6,7	20,0	6,7	10,0

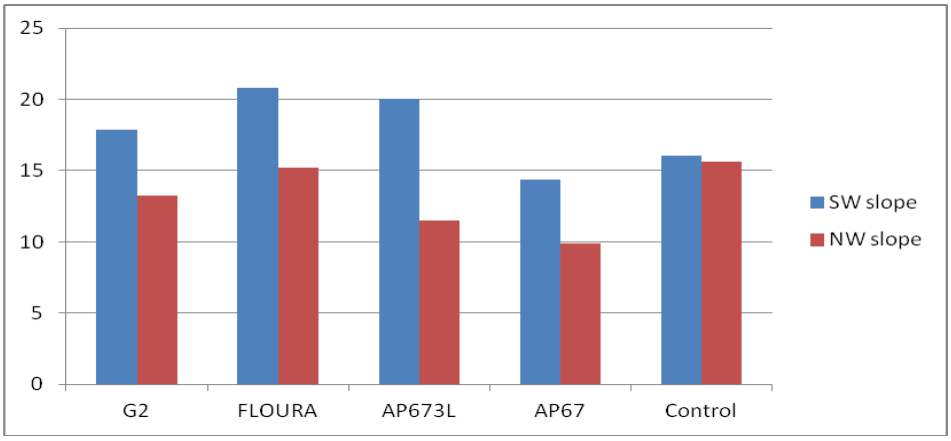


Fig. 21. The heights of plants (cm) measured in 2 trials for field acclimation of *Hypericum foliosum*

3. INPUT OF DUTH

Data analysis regarding newly selected growth protocols and their relative efficiency

3.1. Introduction

Plant growth and development is a product of the genetic potential of the plant and how it responds to stimuli from the ambient environment. This interaction is conducted by specialized photosensory receptor proteins that adapted to signals of the incident light spectrum due to best utilization. In the case of artificial production of plants, light quality, quantity, and duration inform them of the current conditions that ultimately contribute to their productivity and quality (Singh *et al*., 2011; Barrero *et al*., 2012).

Light is not only the energy source for photosynthesis but also the regulator of numerous processes such as seed germination, leaf development, stomatal development, and membrane transport of guard cells (Walters *et al.,* 2003). Failure to dissipate or avoid excessive light leads to oxidative damage to the photosynthetic apparatus, bleaching, chlorosis and bronzing of leaves (Karpinski *et al.,* 1999; Mullineaux and Karpinski, 2002). Therefore, acclimation to excessive light conditions requires protective strategies such as decrease in the number of photosynthetic reaction centers (Walters *et al.,* 1999), alternation of stomatal behaviour (Willmer and Fricker, 1996), and changes in leaf and whole-plant morphology (Horton *et al.,* 1996). Stomata are microscopic structures formed by two guard cells flanking a central pore in plants. Stomata optimise the uptake of CO_2 and minimise the loss of water to suit the prevailing environmental conditions (Araïjo *et al.,* 2011). Many factors affect stomatal behaviour, including hormones, light quality and irradiance, humidity, CO_2 concentration, and biotic and abiotic stresses (Shimazaki *et al.,* 2007; Mott *et al.,* 2008; Chen *et al.,* 2012). Therefore, the fine regulation of opening and closure of stomata in response to light is crucial to crop production (Lee et al. 2007).

Since today, a wide variety of artificial lights have been used in horticulture including incandescent, fluorescent and high intensity discharge lights (Sager *et al.,* 1982). Incandescent lighting is typically high in the red and infrared wavelengths. Fluorescent lights produce more white light but the fixtures must be located very close to the plants. High intensity discharge (HID) lights, such as high pressure sodium and metal halide, have been used in growth chambers to supplement natural sunlight and

increase photosynthetic rates (Seelye and Mullan 2010). Because of the large amount of electrical energy required, adding lights to increase photosynthesis is, for most reforestation services and native plant nurseries, economically impractical (Downs, 1977; Warrington *et al.,* 1976)

Light emitting diodes (LEDs) are the newest light source being used in controlled environments and greenhouse plant culture, which are solid-state, durable, lightweight, extremely long-lived, and come in selectable narrow-waveband emissions such as red and blue that can be matched to the absorption spectra of plant pigments by eliminate other wavelengths found within normal white light, thus reducing the amount of energy required for power (Goins *et al.,*1997; Kim *et al.,* 2005; Landis *et al.,* 2013).

The effects of different light qualities, quantities and duration are reported by several authors considering that the percentage absorption of blue or red light by plant leaves is about 90% and that of green light is about 70–80%. Thus, plant development and physiology are strongly influenced by blue or red light (Terashima *et al.,* 2009). Blue light suppresses hypocotyl elongation and induces biomass production, and red light induces hypocotyl elongation and expansion in leaf area (McNellis and Deng, 1995; Johkan *et al.,* 2010).

Plant growth under the combination of blue and red light has been studied in lettuce, spinach, komatsuna (Japanese mustard spinach) and radish (Yorio *et al.,* 2001; Hanyu and Shoji, 2002; Ohasi-Kaneko *et al.,* 2007). The combination of red and blue light was an effective lighting source to produce plant biomass, and the addition of green light with blue and red light was also effective (Kim *et al.,* 2004; Pardo *et al.,* 2013).

Additionally it has been shown in cucumber plants, which were grown under different combinations of red and blue light supplied by light-emitting diodes (LEDs), that light quality by itself can induce photosynthetic and morphological properties in leaves that normally occur at high light intensity, although the plants were grown under low light intensity (Hogewoning *et al.,* 2010). On the other hand, numbers of stomata, rate of photosynthesis and transpiration, and stomatal conductance increased progressively with increasing photosynthetic photon flux density (PPFD) in plantlets of Withania somnifera L. (Lee *et al.,* 2007). Also raising the PPFD from 25 to 200 μmol $^{m-2\ s-1}$ increased the average number of stomata and stomatal length and reduced stomatal frequency in barley (Kubinova, 1991). Stomatal pore length, stomatal density and

index were all influenced by the irradiance signal and were reversible upon changing irradiance (Thomas *et al.,* 2003).

Nevertheless, photosynthetically inefficient light qualities also convey important environmental information to a developing plant. For example, far-red light reverses the effect of phytochromes, leading to changes in gene expression, plant architecture, and reproductive responses (Yeh and Chung, 2009). Moreover UV-A induction of anthocyanins accumulation was observed in grape (Kataoka *et al.,* 2003) and lettuce (Tsormpatsidis *et al.,* 2008). As previously mentioned, light quality could positively affect phytochemical accumulation in plants but the effects are more complex. Anthocyanins are members of a class of water-soluble pigments that can be classified as flavonoid and biophenolic compounds. These, together with carotenoids and chlorophylls, are responsible for color in many plants (Llorach *et al.,* 2008; Zhang and Folta, 2012). Effects of light quality on photochemical characteristics such as anthocyanin synthesis and antioxidant properties have been reported for several different fruits and leafy vegetables. For example, blue light increased levels of anthocyanins in tomato (Giliberto *et al.,* 2005), carotenoids in coffea (Ramalho *et al.,* 2002) and ascorbic acid in lettuce and komatsuna, but not in spinach (Ohashi-Kaneko *et al.,* 2007). In contrast, in cranberry fruits, red light seems to be most effective in anthocyanin production (Zhou and Singh, 2002). Similarly, lower red/far-red ratio (R/FR), or more FR relative to R, was shown to decrease anthocyanins concentration in many species (Yanovsky *et al.,* 1998; Ramalho *et al.,* 2002; Alokam *et al.,* 2002).

Another morphological parameter that is under consideration is the root development of seedlings that grown in a controlled environment under the effect of artificial light sources, especially for those produced for regeneration material. Root regeneration is of critical importance to establishment of planted seedlings. New root growth enables the seedling to establish a functional connection with the soil and thereby overcome the moisture stress imposed by transplanting (Burdett 1990; Krasowski 2003; Grossnickle 2005). For this reason, a great deal of seedling quality research has been undertaken on root morphology and related physiological processes (Ritchie and Dunlap 1980; Davis and Jacobs 2005).

However most of the studies with LED lighting were performed in the controlled environment growth chambers, where the main environmental parameters, as temperature, humidity, CO2 concentration and photosynthetic flux daily integral can

be controlled independently of external influences. Unfortunately well-succeed lighting strategies in phytotrons not necessarily produce the same results in greenhouse conditions (Pinho *et al*., 2007) especially when variable daylight effect in the complex exposure is involved. Therefore individual experiments should evaluate the background growth conditions in conjunction with the natural effect lighting.

3.1.1. Tested species- Seed/acorn/fruit collection - Removal of dormancy

***Pinus sylvestris* L.**

Scots pine is an evergreen coniferous tree growing up to 35 m in height and 1 m trunk diameter when mature. The bark is thick, scaly dark, grey to brown on the lower trunk, and thin, flaky and orange on the upper trunk and branches (Rushforth, 1999).

This species has the largest geographical distribution of any pine species, is one of the most abundant trees in Europe and one of the most widespread conifer species on earth. It has a wide distribution that extends among almost all the width of Eurasia (Carlisle and Brown, 1968). At an altitudinal scale, *P. sylvestris* occurs from sea level to 1000 m a.s.l. in the north of its range, whereas it occurs from 1200 to 2600 m a.s.l. in the south This wide distribution range encompasses the broad range of climatic conditions that Scots pine is able to tolerate, from the severe cold winters of northern Siberia to the Mediterranean climate of southern Spain; and from the wet, oceanic climate of the west coast of Scotland to the dry continental climate of central Europe and Asia (Steven and Carlisle, 1959).

Scots pine is a very valuable species both from ecological and economic perspectives (Kuper, 1994) From an ecological point of view, it is the only native pine in northern Europe and a keystone species for many ecosystems such as the Caledonian forest, taiga or Mediterranean mountain forests and supports many species of lichens, mosses, fungus and insects (Archibold, 1995). From an economic point of view, this species is found in all member states of the EU, where it constitutes approximately 20% of the commercial forest area, and it is of considerable importance as a timber producing species, particularly in Nordic countries (Mason and Alia, 2000).

The seeds of *P. sylvestris* were provided by the Ministry of Rural Development and Food (Section of Forest Nurseries and Seed Production, Athens). The seeds were

collected from Laylia, Serres in central Greece (23^0 34′ 00″ N, 41^0 17′ 00″ E) in 2012. The seed germination ability, determined according to the policies of ISTA (2008). *P. sylvestris* seeds were hydrated for 24 h and after one week in the phytotron chamber set at 20^0C for 16 hours with light and at 15^0C for 8 hours without light, while light was provided by cool-white fluorescent lamps on both side walls of the chamber, we have 75% germination success.

***Picea abies* Karst.**

Norway Spruce is the most important forest tree species throughout Central Europe, including Germany (Schelhaas *et al*., 2003). It was one of the comparably few tree species to survive the last glaciations in Europe, and spread from only four refugia: the Apennines, the Carparthians, the Dinaric Alps, and the area north of Moscow, Kostroma (Vendramin *et al.,* 2000; Ravazzi, 2002). Favored by modern forestry as a fast-growing tree species with high growing stocks and valuable wood, spruce has been cultivated in large-scale plantations at lower elevations instead of the natural beech and oak trees, even far from its cooler montane and subalpine natural ranges (Walentowski *et al*., 2004). The importance of Norway Spruce in European forests is reflected in the several fields that deal with its role as a host tree for arthropods. One field, pest management (Wermelinger, 2004), focuses on species living on Norway Spruce, particularly with regard to risk assessment (Schelhaas *et al*., 2003). Another field, modern forestry, aims not only at maximum timber production, but also at biodiversity (Brockerhoff *et al.,* 2008). A third field deals with climate change and its consequences on community composition, and this field touches on both pest management and conservation (Jönsson *et al*., 2009; Muller *et al*., 2009). Seeds were hydrated for 24 hours in room temperature and placed for one week in a phytotron chamber set at 20^0C for 16 hours with light and at 15^0C for 8 hours without light, while light was provided by cool-white fluorescent lamps on both side walls of the chamber. It was succeed 80% germination.

***Pinus nigra* Arn.**

Black pine (*Pinus nigra* Arn.) is widely distributed along the Mediterranean basin. In the western part, black pine forests are commonly found in an intermediate altitudinal belt between the more thermophilic pines at lower altitudes (*Pinus halepensis* Mill., *P. pinea* L., *P. pinaster* Ait.), and the more mesic pines at higher altitudes (*P. sylvestris* L.), and as mixed forests in the transitions zones (Barbéro *et al.,* 1998). In the Iberian

Peninsula, black pine is distributed along the eastern mountain ranges and covers a wide latitudinal gradient. These forests have been greatly affected by climate change during the second half of 20th century (Andreu *et al.,* 2007). These facts place black pine in an intermediate position between boreal locations, in which mainly positive growth trends have been detected (Martínez-Vilalta *et al*., 2008), and more xeric pines, where negative trends dominate (Sarris *et al*., 2007). Therefore black pine is an interesting species to study the effects of climate change on tree growth. Seeds of *P. nigra* were collected from the Samarina village, located in Grevena in Noerthwestern Greece (40°6' 20" N, 21°1' 8"E) and were provided by the Ministry of Rural Development and Food (Section of Forest Nurseries and Seed Production, Athens). Seeds were hydrated for 24 hours and placed in a phytotron chamber (20^0C/15^0C for 16/8 hours phototperiod, light provided by cool-white fluorescent lamps on both side walls of the chamber) due to succeed full germination; indeed after one week we had 70-75% germination success.

***Quercus ithaburensis* var. macrolepis**

Quercus ithaburensis is an East-Mediterranean deciduous oak easily distinguished from the other species by its semicircular crown and sized acorns. Sub-species of this oak are reported in southeast Italy (Pignatti, 1982), south Albania (Villaeys, 1990), Greece (Athanasiadis, 1986b; Christensen, 1997), Turkey (Davis, 1982), Syria and Lebanon (Mouterde, 1966), Israel (Kaplan and Gutman, 1999), northwestern Jordan (Zohary, 1973) and west Anatolia (Lattif and Younis, 1984). According to Christensen (1997), the sub-species *Q. ithaburensis* ssp. *macrolepis* (Kotschy) Hedge & Yalt., is present in Greece. Up to the last century, the main uses of the species were for wood and acorn production (Giannakopoulou, 2002). Furthermore, tannins extracted from *Q. ithaburensis* ssp. *macrolepis* acorn cups supported a great tannery and dye industry (Ioannidis, 2002). It is of great importance the fact that it forms some of the few deciduous oak forests in the eastern Mediterranean zone (*Quercetalia ilicis* zone) (Dafis, 1973). Acorns of *Q. ithaburensis* were collected from plantations placed in the Forest Research Institute located in Thermi, Vasilika, Thessaloniki, Greece **(**40°32' 54.67**"** N, 23°1' 10.72"E). In order to succeed higher germination of *Quercus* acorns the pericarp was totally removed. Also the 1/3 of their cotyledons was cut off.

***Castanea sativa* Mill.**

Sweet chestnut (Castanea sativa Mill.), the only native species of Castanea genus in Europe, is distributed in majority of the Mediterranean countries, extending from Caucasus to Italy, France, Spain, Portugal and south England. Thus, this wide-range distribution throughout southern Europe highlights the ability of the species to adapt to varying environmental conditions (Lauteri *et al*. 1998; Martín *et al.* 2010). Chestnut is one of the multipurpose species of major economic importance in the Mediterranean basin, valued not only for fruit and timber but also for its contribution to the landscape and environment. Because of the multipurpose characteristics of the species, chestnut populations have been affected by clonal propagation, silvicultural practices, etc. This along with the changes in land use and the accelerating dynamics of global and climatic changes have resulted in a fragmentation of habitats, a reduction of population size and probably a loss in biodiversity (Conedera *et al.*, 2004). *Castanea* fruits were collected from natural stands located in Zagliveri-Petrokerasa, Thessaloniki, Greece (40°32' 40.86" N, 23°15' 26.31"E). In order to succeed higher germination of *Castanea* fruits were stratified in the refrigerator at 2-5^{0}C into plastic bags fulfill with moist vermiculite for four months.

***Myrtus communis* L.**

Myrtle is a member of the *Myrtaceae* plant family which is botanically related to eucalyptus. Myrtle is a native plant of North Africa and is commonly found growing in the southern Mediterranean region including France, Spain, Corsica, Tunisia and Italy. Myrtle is a small tree or large bush (of up to 0.9m in height) with pointed leaves, white flowers, and black berries; with aromatic leaves and flowers. Mostly the fresh or dried leaves are used and the dried berry fruits are also aromatic. The leaves have an aromatic and refreshing smell somewhat reminiscent to myrrh or eucalypt; the taste is very intensive, quite disagreeable and strongly bitter (Travese, 2001; Aronne, 2004). In medicine practices the decoction of leaves and fruits are used for stomachache, hypoglycemia, cough, constipation and externally for wound healing (Serce *et al*., 2010). In our case *Myrtus communis* seeds were provided from Skioni, Chalkidiki, Greece (39°56' 51.51" N, 23°31' 49.56"E) of the year 2013. Seed coating was carefully cracked and removed, they were allowed to dry and stored at 4^0 C Generally the Greek variety of myrtle shows no dormancy so seeds were only hydrated with distilled water for 24 hours at room temperature and were placed in a phytotron chamber set at 20^{0}C for 16 hours with light and at 15^{0}C for 8 hours without

light, while light was provided by cool-white fluorescent lamps on both side walls of the chamber. Good germination results were succeeded around 75-80 %. Further we tried another pretreatment, a 30 days cold stratification at 2-5 ^{0}C that showed higher germination percentage around 80-90%.

***Ocimum basilicum* L. & RR hybrid**

Basil is a common name for the culinary herb *Ocimum basilicum* L. of the family Lamiaceae and comes from the Greek word basileus, meaning "king". Basil is originally native to Asia such as India and other countries. It has such different scents due to its composition of several essential oils that are existed in various proportions for each of the breed types. Basil is very sensitive to cold and to water stress, although it prefers more hot and dry conditions and it could be sown in soil once frost is gone. Lettuce leaf basil is a large-leaf variety of *Ocimum basilicum*. The large, crinkled leaves, which are grown on the short, wide plant, are sweet in taste, but not as intense as other sweet basils. Red Rubin basil has lavender-like flowers and is grown for its ornamental foliage as well as for culinary purposes (Makri and Kintzios, 2007). Seeds of Lettuce Leaf (LL) and Red Rubin (RR) varieties were provided from a nursery (Geniki Fytotechniki of Athens) in 2013. The LL seeds were hydrated for 24 hours and the germination success was 85%. Additionally, seeds were collected from mountain Athos in 2012. The RR and Athos varieties were hybridized and the seeds (RR hybrid) were hydrated for 24 hours with 55% germination success.

***Cornus sanguinea* L.**

The common dogwood (*Cornus sanguinea* L.) belongs to the family *Cornaceae*. It is a medium to large deciduous shrub, growing 5 m tall, with dark greenish-brown branches and twigs. The leaves are opposite, 4–10 cm long and 3-6 cm broad. The hermaphrodite flowers are small, with four creamy white petals. The fruit is a globose black berry containing a single seed. Its natural range covers most of Europe and western Asia and it grows in the margins of forests or unforested areas (Stankovic and Topuzovic, 2012). Seeds of *Cornus sanguinea* were collected from Kydonies (43°04’56’’ N, 45°29’236’’ E) in the provenance of Thessaloniki. The seeds were collected in the year 2012. The fruits weighed from 300 to 1,000 g and the collection point elevation was at 489 m. For breaking the dormancy the seeds were hydrated for 24 hours and cold stratified for a period of 9 months at 3-5 ^{0}C with 90% germination success.

***Prunus avium* L.**

Wild Cherry is the common name of *Prunus avium* L. Cherries are members of the *Rosaceae* family and are native to many European and Asian regions. *Prunus avium* is a deciduous tree growing from 15 to 32 m tall, with a trunk up to 1.5 m in diameter. The hermaphroditic flowers are produced in early spring and are bee-pollinated. It is mainly cultivated for its fruit (drupe), as well as for its timber and as an ornamental plant. *Prunus avium* seeds were collected from the Vermio mountain (32°58'80'' N, 45°06'042'' E), that located in the provenance of Edessa here in Greece during 2011. In order to break the dormancy seeds were hydrated for 24 hours and placed for 4 weeks in a phytotron chamber for warm stratification with conditions set at 20 °C, 8/16 hours day/night, 70% RH; following by cold stratification for 20 weeks at 3-5 °C.

***Punica granatum* L.**

Pomegranate (*Punica granatum* L.) is a deciduous shrub or small tree that is a member of the family Punicaceae. It grows between 5 and 8 m tall and the fruit collection is held from September to February. The most popular cultivar is 'Wonderful' as it is large-fruited and fruits are well-colored. Pomegranate is considered to be a native of Iran and Afghanistan. It is also found growing wild in the warm valleys and outer hills of the Himalayas (Satyavati *et al*., 1978). The pomegranate fruit consists of the peel, seeds, and the arils. The peel makes up about 50% of the fruit, whereas the arils and seeds make up 40%and 10%, respectively. The peel is rich in many compounds such as phenolics, flavonoids, ellagitannins and pro-anthocyanidin compounds, complex polysaccharides, and many minerals including potassium, nitrogen, calcium, magnesium, phosphorus, and sodium (Viuda-Martos *et al*., 2010). It is one of the most potent fruit-bearing medicinal herbs widely distributed throughout the Mediterranean region of southern Europe, northern Africa and tropical Africa, Indian subcontinent, Central Asia and the drier parts of South-East Asia (Siddiqui and Arshad, 2014). *Punica granatum* var. Wonderful ripe fruits were collected from Riza, Chalkidiki provenance (40°30'214''N, 23°26'612''E), in October 2013. The fruits were quartered and crushed in order to lift out the clusters of juice arils that maintain the seeds. After removing the juice arils, seeds were dried and stored at 4°C in polyethylene bags. Pomegranate seeds were hydrated for 24 hours and placed in Petri dishes on top of moist sand at 4°C for 2 months. Then the

seeds were transferred in phytotron chambers (20°C, 8/16 day/night, 70% RH). The germination rate was 46% and 78% after 3 and 5 weeks, respectively.

3.2. Materials and methods

3.2.1. Soil substrates – Mini-plug sizes & Environmental chamber conditions

We used different soil substrates and mini-plug sizes for each of the experiment held. Also in specific circumstances due to reconstructions into the growth chambers, different light intensities applied for the tested species. Combined effects of the tested light treatments due to different percentages covering the specific light spectrum areas were shown in Table 1. Specifically:

Testing coniferous species such as *P. sylvestris* (both provenances), *P. abies* (Swedish provenance) and *P. nigra*:

- Pre-germinated seed material was sown in **stabilized peat soil substrate** (Preforma PP01, Jiffy Products International AS, Stange, Norway) being the world leading producer of growing substrate to forest and horticultural nurseries. The substrate had a pH of 5.0. With a technology based on pre-cultivation and transplanting. It is very favorable in regard of reducing the risk of losing part of the substrate around the roots or compression of the root system during the automatic transplanting operation. **Mini-plug size** used was the **QPD 104 VW** (HerkuPak, Germany) (tray dimension 310x530; cell size 38.5 mm; plant centre 43/43 mm; depth 50 mm; volume 50 cc; 510 plant/m^2).
- **Tested light treatments**: L20AP67(4 tubes), FL (4 tubes), AP673L, G2, AP67, NS1 (Photo.1)
- **Environmental conditions**: in both chambers were set at a 17-h photoperiod of a photosynthetic photon flux density (PPFD) of 140 µmol m^{-2} s^{-1}, with air relative humidity (RH) of 80 +/- 10%, and a diurnal cycle of 20/15 °C of day/night. Watering was applied twice a day by automatic sprinkles at 9.00 a.m. and 24.00 a.m. for duration of 20 sec. In order to ensure uniform growth conditions full rotations were applied in each tray on a regular basis.

3.2.2. Testing the broad-leaved species of *Q. ithaburensis var.macrolepis* and *Castanea sativa* Mill.

- Acorns of *Q. ithaburensis* were sown in **enriched peat** soil substrate (Klassmann TS1, Klassmann-Deilmann GmbH, Geeste, Germany) mixed with perlite on the surface. **Mini-plug** containers **size** of **DL48 R** (tray dimension 315x550; cell size 45X 53; plant centre; depth 5 mm; volume 80 cc; 273 plant/m^2)**.**
- Germinated *Castanea* fruits were sown in **enriched peat** soil substrate (Klassmann TS1, Klassmann-Deilmann GmbH, Geeste, Germany) mixed with perlite on the surface. **Mini-plug** containers **size** of **QP60/7R** (tray dimension 310x530; cell size 46X 75 mm; volume 95 cc; 363 plant/m^2)
- **Tested light treatments**: L20AP67(4 tubes), FL (4 tubes), AP673L, G2, AP67, NS1 (Photo.1)
- **Environmental conditions**: in both chambers were set at a 17-h photoperiod of a photosynthetic photon flux density (PPFD) of 150 µmol m^{-2} s^{-1}, with air relative humidity (RH) of 80 +/- 10%, and a diurnal cycle of 20/15 °C of day/night. Watering was applied twice a day by automatic sprinkles at 9.00 a.m. and 24.00 a.m. for duration of 20 sec. In order to ensure uniform growth conditions full rotations were applied in each tray on a regular basis. It should be mentioned that we kept the mini-plus of both species in the middle self distance from the light sources of L20AP67 and FL.
- Also another set of germinated fruits of *Castanea* were sown into the **same mini-plug size** but into a **mixture of peat and perlite** (3:2) (**substrate used in Greek nurseries**) also added 5-10% of the total volume of loose clay sandy soil **enhanced with fertilizers** such as 1.3 kg albatros (15-30-15 and minerals), 0.6 kg (SOP)(0-0-50),1.0 kg (G-SSP) (0-20-0) and 0.4 kg ($MgSO_4$). Those trays were kept under **sodium lamps and fluorescent tubes** (as another reference light) (Root Growth Potential light environment) (Photo 3) with a PPFD at plant level of 274 µmol m^{-2} s^{-1}, an air temperature of 21±2 °C, RH of 60±10%, and 16-h photoperiod.

3.2.3. Testing *Myrtus communis* L.

- Pre-germinated seeds of common myrtle were sown in **enriched peat**. **Mini-plug size** used was the **QPD 104 VW** (tray dimension 310x530; cell size 38.5 mm; plant centre 43/43 mm; depth 50 mm; volume 50 cc; 510 plant/m^2).
- **Tested light treatments**: L20AP67(**6 tubes**), FL (**6 tubes**), AP673L, G2, AP67, NS1 (Photo.2)
- **Environmental conditions**: in both chambers were set at a 17-h photoperiod of a photosynthetic photon flux density (PPFD) of 150 µmol m^{-2} s^{-1}, with air relative humidity (RH) of 80 +/- 10%, and a diurnal cycle of 20/15 °C of day/night. Watering was applied twice a day by automatic sprinkles at 9.00 a.m. and 24.00 a.m. for duration of 20 sec. In order to ensure uniform growth conditions full rotations were applied in each tray on a regular basis.
- Also another set of germinated seeds of common myrtle were sown into the **same soil substrate and mini-plug size**. Those trays were kept under **sodium lamps and fluorescent tubes** (as another reference light) (Root Growth Potential light environment) (Photo 3) with a PPFD at plant level of 274 µmol m^{-2} s^{-1}, an air temperature of 21±2 °C, RH of 60±10%, and 16-h photoperiod.

3.2.4. Testing the basil varieties such as *Ocimum basilicum* L. and *Ocimum basilium* RR hybrid and *Cornus sanguinea* L.

- Pre-germinated seed material of basil varieties and *C. sanguinea* was sown in **stabilized peat soil** substrate and in the **mini-plug size** of **QPD 104 VW** (tray dimension 310x530; cell size 38.5 mm; plant centre 43/43 mm; depth 50 mm; volume 50 cc; 510 plant/m^2).
- **Tested light treatments**: L20AP67(4 tubes), FL (4 tubes), AP673L, G2, AP67, NS1 (Photo.1)
- **Environmental conditions**: in both chambers were set at a 17-h photoperiod of a photosynthetic photon flux density (PPFD) of 140 µmol m^{-2} s^{-1}, with air relative humidity (RH) of 80 +/- 10%, and a diurnal cycle of 20/15 °C of day/night. Watering was applied twice a day by automatic sprinkles at 9.00 a.m. and 24.00 a.m. for duration of 20 sec. In order to ensure uniform growth conditions full rotations were applied in each tray on a regular basis.
-

3.2.5. Testing *Prunus avium* L.

- Pre-germinated seeds of wild cherry were sown in **stabilized peat soil** substrate and in the **mini-plug size** of **QPD 104 VW** (tray dimension 310x530; cell size 38.5 mm; plant centre 43/43 mm; depth 50 mm; volume 50 cc; 510 plant/m^2).
- **Tested light treatments**: L20AP67(4 tubes), FL (4 tubes), AP673L, G2, AP67, NS1 (Photo.1)
- **Environmental conditions**: in both chambers were set at a 17-h photoperiod of a photosynthetic photon flux density (PPFD) of 150 µmol m^{-2} s^{-1}, with air relative humidity (RH) of 80 +/- 10%, and a diurnal cycle of 20/15 °C of day/night. Watering was applied twice a day by automatic sprinkles at 9.00 a.m. and 24.00 a.m. for duration of 20 sec. In order to ensure uniform growth conditions full rotations were applied in each tray on a regular basis. It should be mentioned that we kept the mini-plus of both species in the middle self distance from the light sources of L20AP67 and FL.

3.2.6. Testing *Punica granatum* L.

- Pre-germinated seeds of pomegranate were sown in **enriched peat**. **Mini-plug size** used was the **QPD 104 VW** (tray dimension 310x530; cell size 38.5 mm; plant centre 43/43 mm; depth 50 mm; volume 50 cc; 510 plant/m^2).
- **Tested light treatments**: L20AP67(**6 tubes**), FL (**6 tubes**), AP673L, G2, AP67, NS1 (Photo.2)
- **Environmental conditions**: in both chambers were set at a 17-h photoperiod of a photosynthetic photon flux density (PPFD) of 150 µmol m^{-2} s^{-1}, with air relative humidity (RH) of 80 +/- 10%, and a diurnal cycle of 20/15 °C of day/night. Watering was applied twice a day by automatic sprinkles at 9.00 a.m. and 24.00 a.m. for duration of 20 sec. In order to ensure uniform growth conditions full rotations were applied in each tray on a regular basis.

Light treatments / color bands in light spectrum	400-500 nm	500-600 nm	600-700 nm	700-800 nm	R:FR ratio
FL	**34.8%**	**24.1%**	**36.7%**	**4.4%**	**5.74**
L20AP67	**10.5%**	**26.2%**	**48.9%**	**14.4%**	**2.91**
AP673L	**11.9%**	**19.3%**	**60.5%**	**8.3%**	**5.56**
G2	**7.7%**	**2.4%**	**64.4%**	**25.5%**	**2.51**
AP67	**13.8%**	**15.1%**	**53%**	**18.1%**	**2.77**
NS1	**20.2%**	**38.9%**	**35.7%**	**5.2%**	**8.16**

Tabl.1. *Light treatments used in the experiments such as Fluorescent (FL), L20AP67, AP673L, G2, AP67, NS1 and the different percentages covering specific colour bands in the light spectrum.*

It should be mentioned that for the High Pressure Sodium Lamps that found accompined with the fluorescent tubes in the Root Growth Potential facilities that emit light by passing an electric arc through a chamber containing sodium and small amounts of neon and argon. The strongest emission line is at 819 nm. There are secondary lines at 569, 564,595,598, 582, 585, 584 and 616 nm. In addition to the 819 nm line, there are infrared emission lines at 767, 1139, 1269, 1846, 2207, and 2339 nm (Elvidge et al., 2007 a,b).

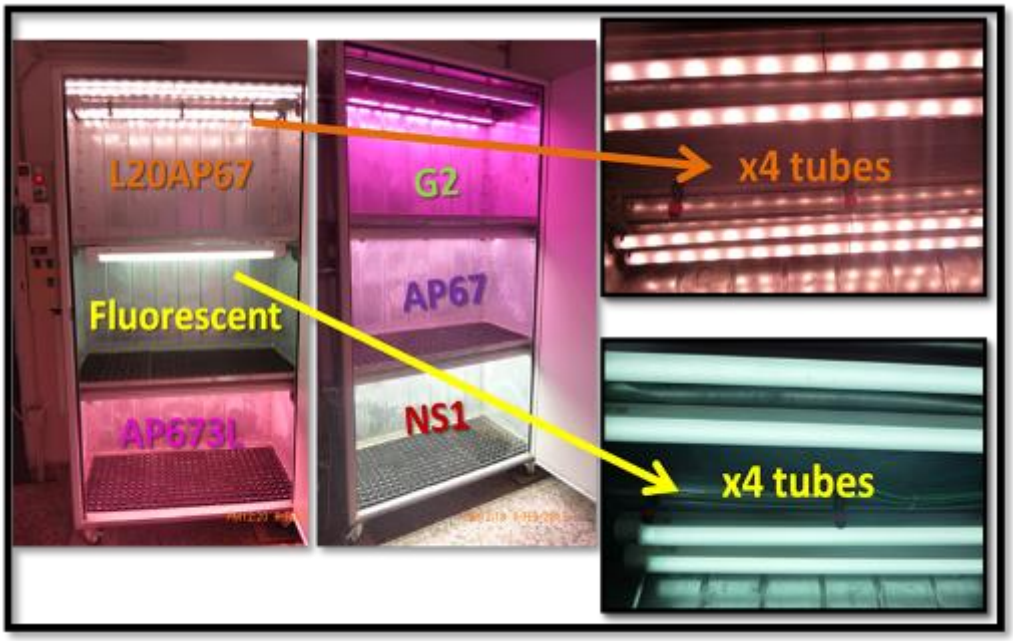


Photo 1. Growth chambers and tested lights FL (x4 tubes), L20AP67 (x4 tubes), AP673L, G2, AP67 & NS1

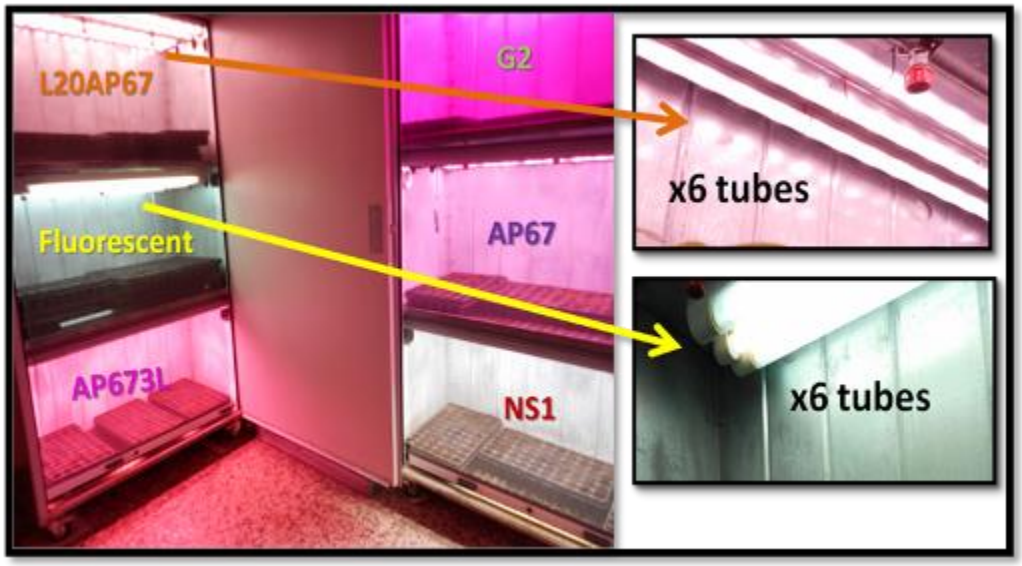


*Photo 2***.** *Growth chambers and tested lights FL (x6 tubes), L20AP67 (x6 tubes), AP673L, G2, AP67 & NS1*

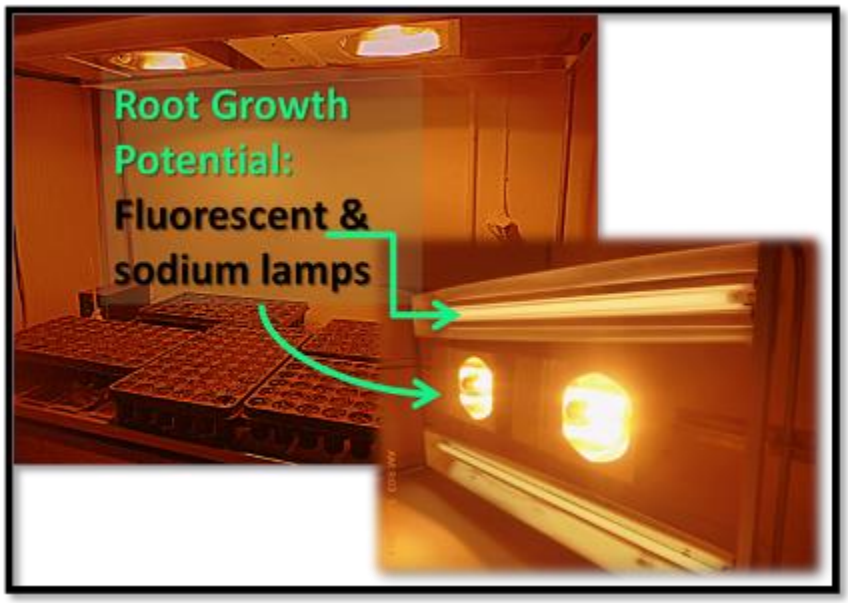


Photo 3. Root Growth Potential light environment (Fluorescent & sodium lamps) that also used as another reference light source for two tested species those of the Castanea sativa and Myrtus communis.

3.2.7. Growth kinetics

Seedlings were kept into the growth chambers for a varied time period depend on the species that lasts from two until five weeks the most. During the cultivation phase,

growth rate was measured based on the seedling height that was computed through a formula entered in the statistical program based on maximum of ten randomly selected seedlings per species and light treatment as follows:

COMPUTE gr_1 = Height.1 / 5. -COMPUTE gr_2 = (Height.2 - Height.1) / 5- …….

…… COMPUTE gr_n= (Height.n – Height.n)/n….. etc.

We measured needle/leaf number and visual evaluation of the needle/leaf colour every week; visual evaluation of leaf/needle colour characterized as (1=pale, 2=light green, 3=dark green, 4=reddish). For the estimation of leaf/needle colour the following R:G:B ratios were used. The **yellow- pale**: i) R:255, G:255, B:204, ii) R:238, G:221, B:130, iii) R:238, G:232, B:170 and iv) R:214, G:219, B:112. The **light green** combinations: i) R:153, G:204, B:0, ii) R:102, G:204, B:0 and iii) R:102, G:153, B:0. The **dark green** combinations, i) R:0, G:100, B:0, ii) R:85, G:107, B:47 and iii) R:110, G:139, B:61. Finally the **reddish** combinations: i) R:221, G:59, B:47. Furthermore, by taking photographs from above and from the side of the trays for all tested species.

The impression approach was used to determine leaf stomatal density, which was expressed as the number of stomata per unit leaf area (Radoglou and Jarvis, 1990). The abaxial epidermis of the leaf was cleaned first using a degreased cotton ball, and then carefully smeared with nail varnish in the mid-area between the central vein and the leaf edge, for approximately 20 min. The thin film (approximately 5 mm x 315 mm) was peeled off from the leaf surface, mounted on a glass slide, immediately covered with a cover slip, and then lightly pressured with finepoint tweezers. All the impressions were taken from at least 3 leaves for each light treatment and examined under a light microscope with camera attachment at x40 magnification using the Axio Vision program (47.1). Four fields per slide were randomly selected and photographed. Stomata and epidermal cells of the species *Q. ithaburensis* and *Castanea sativa* were counted on the photographs and the stomatal density, stomatal index and cell density were calculated. Stomatal index was estimated using the formula [s/(e + s)]x100 where s number of stomata and e is number of epidermal cells (Salisbury, 1927). The guard cells were not included in the number of epidermal cells. Cell density was calculated as the total number of cells (e + s) per unit area of leaf.

Further leaf area of all tested species except from the coniferous and common myrtle was measured by the device LI-3000C Portable Area Meter (LI-COR Biosciences). Chlorophyll content index (CCI) of *Q. ithaburensis* and *Castanea sativa* was measured by the portable CCM-200 (Opti-Sciences, Inc.**,** NH 03051 USA) and the chlorophyll fluorescence by the Photosynthesis Yield Analyzer MINI-PAM (WALZ, Mess und Regeltechnik, Germany).

For the extraction of the total Chlorophyll a (chla), Chlorophyll b (chlb) and Carotenoids content of common myrtle and pomegranate, the seedlings were submersed into liquid nitrogen for 5 min and then placed in 3 ml N,N'-dimethylformamide at 4 °C for 24 h. Absorbance of each related parameter was measured by a UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) at 663, 647 and 480 nm, respectively. Following the obtained concentrations of chla, chlb and carotenoids, were calculated using the equations described by Porra *et al*., 1989 shown below:

Chl a: $C_a = 12 * A_{663.2} – 3.11 * A_{646.8}$

Chl b: $C_b = 20.78 * A_{646.8} – 4.88 * A_{663.2}$

Carotenoids: $C_{x+c} = (1000 * A_{480} – 1.12 * C_a – 34.07 * C_b) / 245$

The Folin-Ciocalteau colorimetric assay (Singleton and Rossi, 1965) was used for the determination of the *Ocimum basilicum* TPC, with gallic acid as calibration standard (R^2 = 0.998). Seedlings were extracted into 10 mL of 80% aqueous methanol followed by centrifugation at 15,000 rpm for 15 min. 2.5 mL of Folin-Ciocalteau's reagent was added in each sample and after 1 min, 2 mL of 7.5% sodium carbonate solution was added. The absorbance of the colored reaction product was measured at 760 nm versus a blank. The results were expressed as mg of Gallic Acid Equivalent per g (mg GAE/g) of fresh basil.

TPC of the common myrtle and pomegranate extracts was measured by a UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) using the Folin-Ciocalteau colorimetric assay (Singleton & Rossi, 1965) with tannic acid as calibration standard. For the extraction, seedlings were submersed into liquid nitrogen for 5 min to perforate the waxy cuticle and rupture cell membranes and then placed in plastic containers with 3 ml 6M HCl : H2O : MeOH (7 : 23 : 70). The containers remained in the dark at 4 °C for 24 h. This was followed by addition of 2.5 ml of Folin- Ciocalteau's reagent and vortex of the mixture. 2 ml of 7.5% sodium carbonate solution was added after 1 min, the mixture was vortexed again and

samples were incubated for 5 min at 50 °C. The absorbance of the colored reaction product was measured at 720 nm versus a blank consisting of 500 µl of methanol, 2.5 ml of Folin- Ciocalteau's reagent and 2 ml of 7.5% aqueous sodium carbonate. The TPC in the extracts was calculated from a standard calibration curve obtained with different concentrations of tannic acid (correlation coefficient: R^2= 0.998) and the results were expressed as mg of Tannic Acid Equivalent per g (mg TAE/g) of fresh weight of myrtle and pomegranate.

Anthocyanin content of the common myrtle and pomegranate extracts was determined spectrophotometrically as A_{530}-0.24A_{563} (Murray & Hackett, 1991) versus a blank containing 6M HCl : H2O : MeOH (7 : 23 : 70). The anthocyanin content in the extracts was calculated from a standard calibration curve obtained with different concentrations of cyanidin glycoside (correlation coefficient: R^2= 0.992) and the results were expressed as µg of cyaniding glycoside per g of fresh myrtle and pomegranate.

The total flavonoid content of pomegranate seedlings was determined according to the Arvouet-Grand *et al*., 1994 method using a 96-well microplate reader. 100 µl of each seedling extract was mixed with a methanolic (2%) solution of aluminium trichloride ($AlCl_3$) (100 µl). The absorbance of each mixture was measured at 510 nm versus a blank reagent of plant extract (100 µl) without $AlCl_3$, and methanol (100 µl). For calibrations, different concentrations of quercetin solution were used and results were expressed as mg of quercetin equivalents (QE) per g.

Also, seedlings of all tested species were measured for the morphological parameters of the Shoot height (SH) (cm) and the Root length (RL) (cm) using a ruler tape.

Scanned photos of the root system of the *Q. ithaburensis* and *Castanea sativa* seedlings were loaded in GiA Roots (www.giaroots.org) which is a software tool to automate and facilitate the large-scale analysis of root networks. GiA Roots was used to quantify the structure of plant root system architecture. Root architecture of seedlings was defined by the number of FOLRs (First Order Lateral Roots) greater than 1 mm diameter (primary FOLR) originating along the length of the taproot and at the base of the taproot (i.e. at the point of undercutting in bare-root stock or air pruning in container stock). A root fibrosity index was devised to provide a relative measure of structural and fine root branching (Tabl. 2 & 3). Individual seedling root systems were assigned a fibrosity class on a 1–5 scale, with five being the most

fibrous. The scale was developed from a fibrosity index devised by Hatchell and Muse (1990) (Wilson *et al*., 2007).

The seedlings of all tested species were also evaluated based on dry weight (g), of the leaves (DWL), shoots (DWS) and roots (DWR) that were assessed after oven-drying at 70 °C for 48 h. The root-to-shoot ratio (R/S) was calculated on a dry weight basis.

The root growth potential (RGP) test was carried out to determine the potential capacity of *P. sylvestris* provenances, *Myrtus communis*, basil varieties, *C. sanguinea*, *P. avium* and *P. granatum* seedlings to initiate new roots. This test was implemented immediately after the end of the cultivation period in the growth chambers using the plant material from the same populations. At random, 10 seedlings per species per light treatment were selected and transplanted into mini-plug containers of same size, following the standardized RGP technique for containerized seedlings described by Mattsson (1986). The containers were placed on top of stainless steel boxes (35 × 26 × 8 cm) filled with equal volumes of peat (Klassmann Base Substrate 250I, Klassmann-Delmann GmbH, Geeste, Germany) and sand. The boxes were immersed in a stainless water bath. The seedlings remained in the RGP bath for one month period, although for the species of *P. sylvestris, O. basilicum and P. granatum* two trials were held, the first harvest was done at the 15th day and the second at the 31st, for the rest of the mentioned species only one harvest was done at the end of 31st day of the test, at an air temperature of 21±2 °C, RH of 60±10%, and 16-h photoperiod, with a PPFD at plant level of 300 μmol m^{-2} s^{-1}. Finally seedlings per species per light treatment were carefully removed from the RGP boxes. All new roots protruding from the root plug (new roots that were formed during the RGP test) were cut and cleared from the peat remains. The root growth potential (RGP) of each seedling was assessed by measuring the new root length (NRL) and new root dry weight (NRDW) of these roots.

Nursery performance attributes that were measured only for *Q. ithaburensis* seedlings: Seedling survival (%), Plant height (cm), Shoot height (cm), Shoot diameter (cm) (5 mm above the root collar), Root collar diameter (cm), Leaf colour & number, Chlorophyll content index (CCI) and finally, three randomly selected seedlings per light treatment were collected for destructive sampling and they were transferred to the Laboratory for biomass measurements. Dry weight of leaves,

shoots and roots, R/S ratio, Dickson's Quality Index (DQI)) (calculated as follows (Bayala *et al*. 2009):

$$\text{Quality index} = \frac{\text{Seedling dry weight (g)}}{\frac{\text{Height (cm)}}{\text{Root collar diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}.$$

3.3. Results

3.3.1. Growth rate

***Pinus sylvestris* L. (provenance Greece-Sweden)**

Among the provenances of *P. sylvestris* no significant differences found for the height growth rate irrespective the light spectrum over time. However significant differences found for each of the provenances alone. Specifically *P. sylvestris* prov. Greece seedlings grown under the FL light showed significantly higher growth rate of 5 mm compared to those grown under the NS1 ($p<.002$) and AP673L ($p< .006$) with height increment of 3.69 mm and 3.78 mm at the first week (Fig.1). The situation was altered at the fourth week where seedlings grown under the AP673L LED light showed significantly higher growth rate of 1 mm, compared to the FL ($p<. 001$) with an average height increment of 0.18 mm (Fig.1). According to Duncan homegeneous subsets each light treatment obtained height growth rate in a descending order 1.31 mm, 1.29 mm, 1.23 mm, 1.22 mm, 1.21 mm and 1.13 mm under the L20AP67, G2, FL, AP673L, AP67 and NS1, respectively. As for the *P. sylvestris* prov. Sweden, significant differences found only at the first week where FL and L20AP67 lights obtained higher growth rate of 5.18 mm and 5.07 mm compared to the NS1 that obtained height increment of 3.85 mm (Fig.2). According to Duncan homegeneous subsets higher height increment was obtained in descending order under the L20AP67, FL, G2, AP67, AP673L and NS1 with average values of 1.32 mm, 1.29 mm, 1.28 mm, 1.24 mm, 1.20 mm and 1.14 (Fig.2).

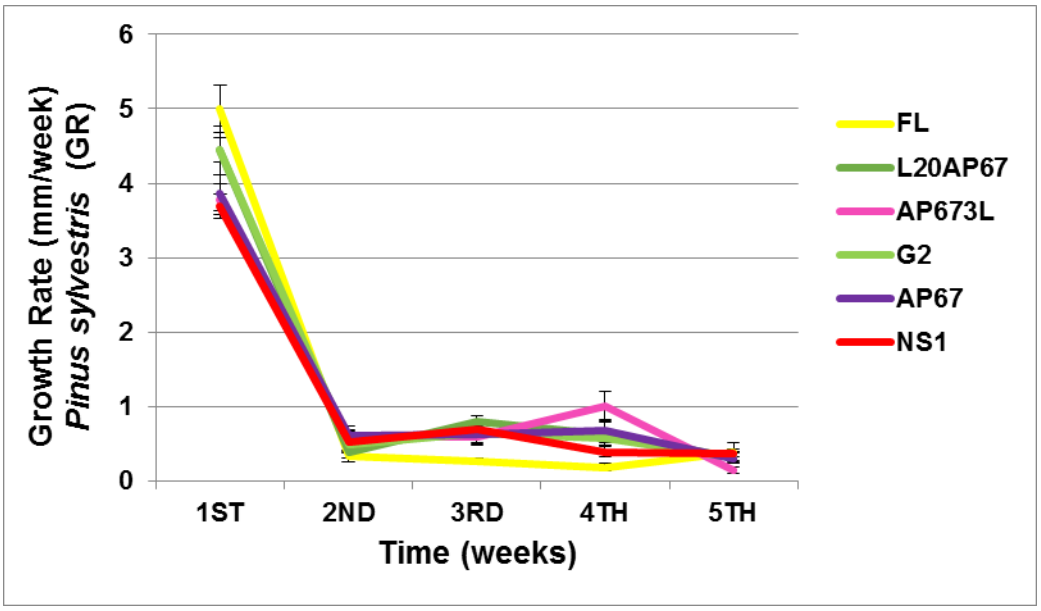


Figure 1. The growth rate of Pinus sylvestris provenance Greece seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size during the 5 week experimental period.

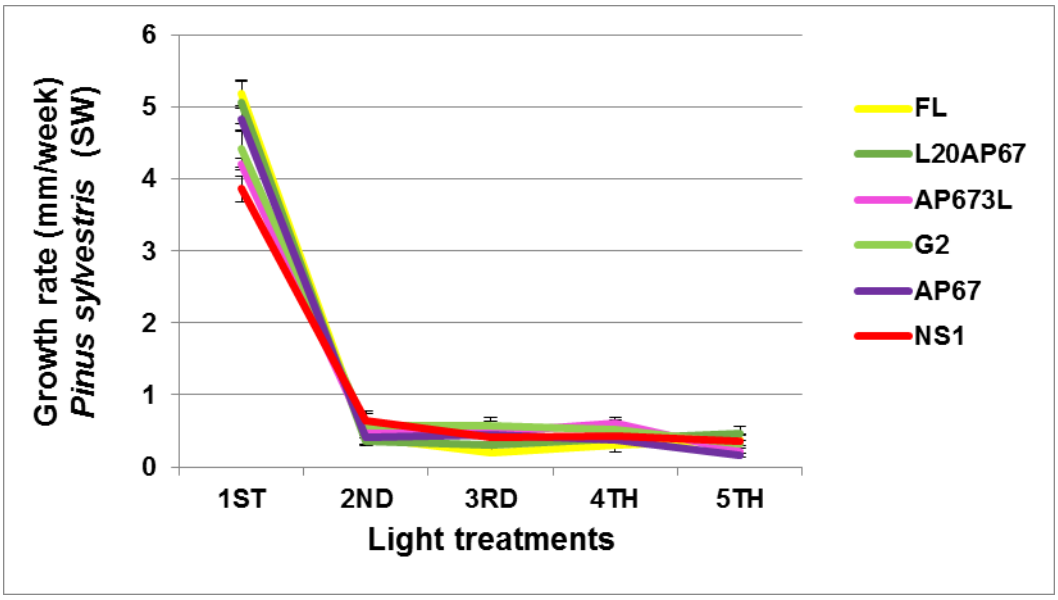


Figure 2. The growth rate of Pinus sylvestris provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size during the 5 week experimental period.

***Picea abies* Karst. (provenance Sweden)**

No significant differences found between the light treatments for the height growth rate of *Picea abies* seedlings over time. However regarding the average values obtained under the lights the highest was for the L20AP67 with 1.28 mm following by

the AP673L, G2 with 1.18 mm, FL with 1.15 mm AP67 with 1.10 mm and NS1 with 1.05 mm, respectively (Fig.3).

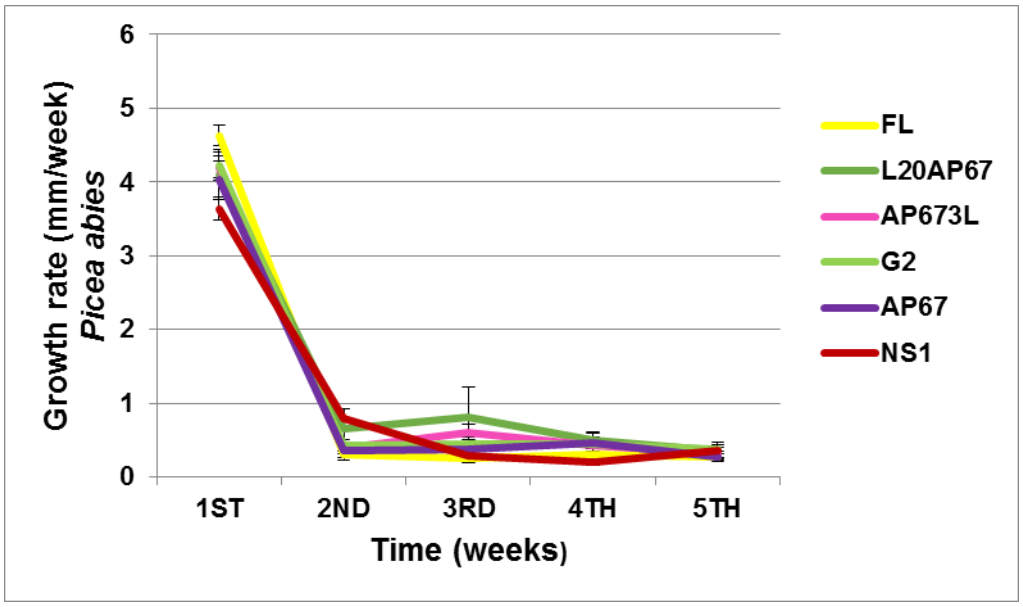


Figure 3. The growth rate of Picea abies provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size during the 5 week experimental period.

***Pinus nigra* Arn.**

Pinus nigra seedlings showed significant differences for the height growth rate only at the first week of the indoor experiment. Specifically FL and L20AP67 lights showed higher height increment of 6.50 mm and 5.84 mm compared to LED lights such as the NS1, AP67 and AP673L with height increment of 3.88 mm, 4.17 mm and 4.29 mm, while G2 obtained 5.08 mm (Fig.4). According to Duncan homogeneous subsets the height increment in descending order obtained under light treatments of FL, L20AP67, G2, AP673L, AP67 and NS1 of 1.51 mm, 1.37 mm, 1.17 mm, 1.04 mm, 1.01 mm and 0.91 mm, respectively.

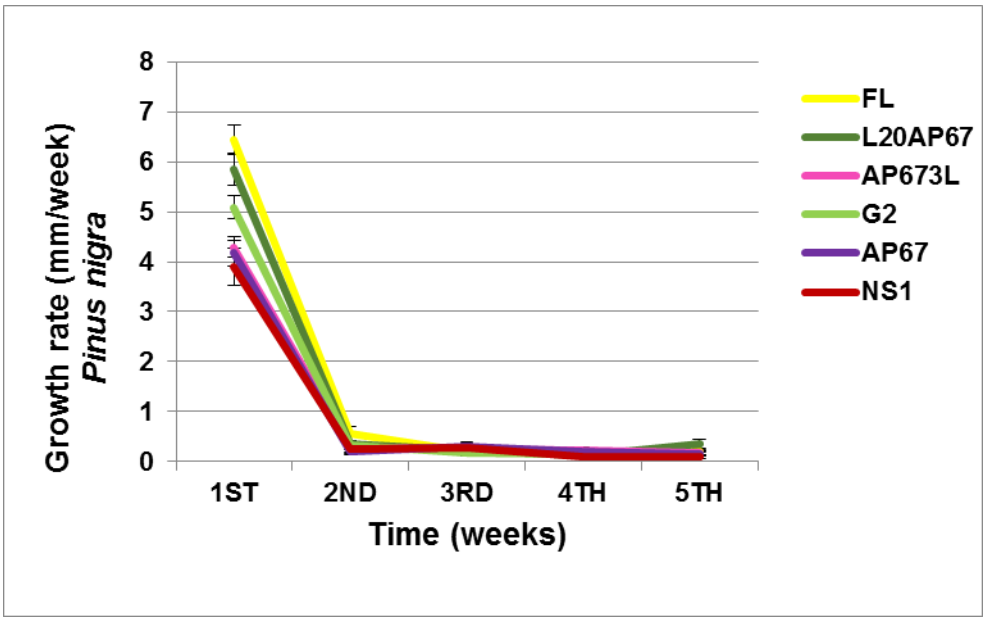


Figure 4. The growth rate of Pinus nigra seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size during the 5 week experimental period.

Quercus ithaburensis ssp. *macrolepis*

Q. ithaburensis seedlings showed no significant differences for the height growth rate over time. Hence all the light treatments presented a similar height increment for each of the four weeks of the indoor experiment. Considering the average values of the height growth rate, the highest was for the L20AP67 with 0.71 cm, following by the FL, G2, AP673L, AP67 and NS1 with 0.63 cm, 0.52 cm, 0.50 cm, 0.44 cm and 0.35, respectively. Time effect is significant (p<. 001) as it shown in Figure 5 where a general reduction of the height increment is observed for all the light treatments until the third week into the growth chambers.

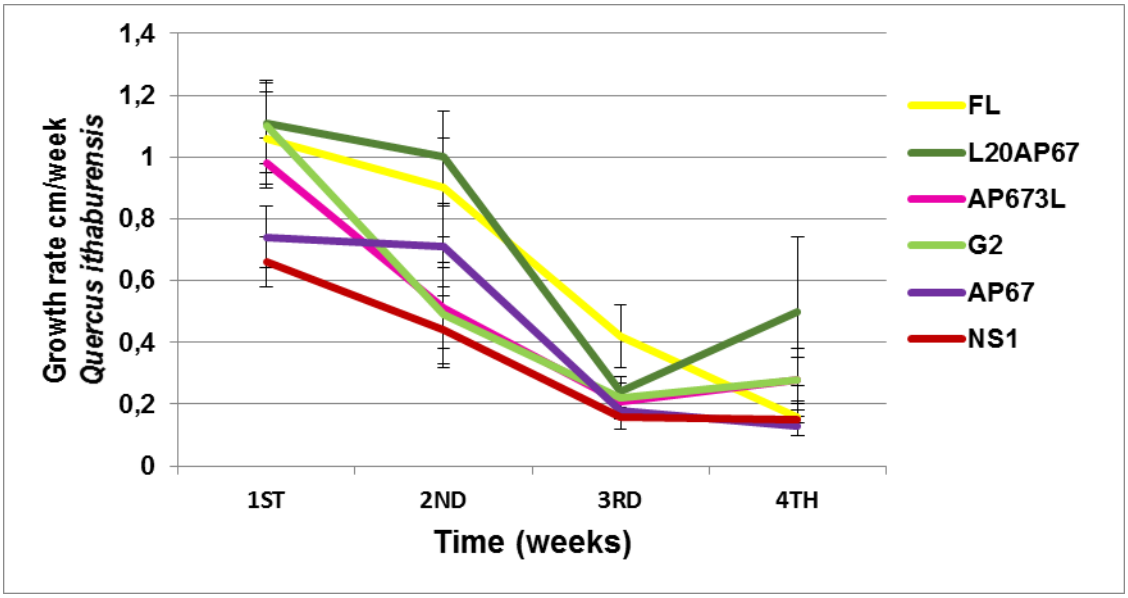


Figure 5. The growth rate of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size during the 4 week experimental period.

***Castanea sativa* Mill.**

Castanea seedlings showed significant differences in height growth rate only at the first week of the experimental period (p<. 001) (Fig.6). Accelerated height increment of 2.81 cm was induced by L20AP67 LED light compared to NS1, FL (p<. 001) and AP673L (p<. 007) with 1.39 cm, 1.58 cm and 1.74 cm, respectively. As for the rest of the light treatments, such as the RGP (FL & sodium lamps), G2 and AP67, their growth rates at the first week were 2.13 cm, 1.84 cm and 1.78 cm. Reduction in the height growth rate was following during the second and the third week with an average increment for all lights of 0.27 cm and 0.36 cm, respectively. Considering only the average values of height increment induced by each of the lights without the time effect were in descending order, 1.11 cm, 0.79 cm, 0.77 cm, 0.75 cm, 0.74 cm, 0.68 cm and 0.66 cm for the G2, RGP, AP673L, AP67, FL and NS1, respectively.

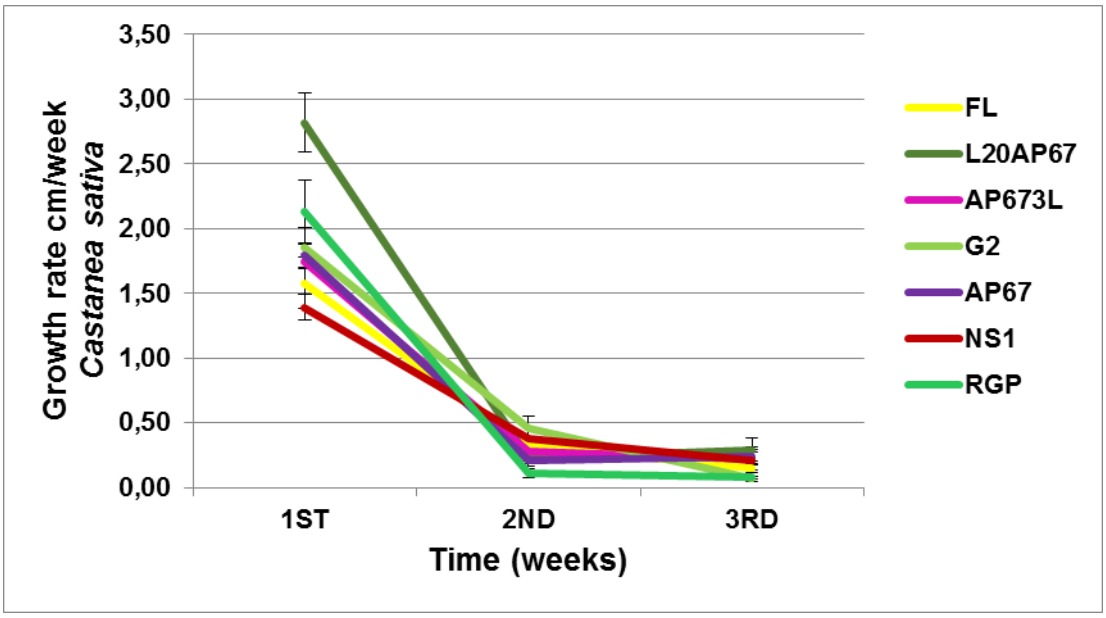


Figure 6. The growth rate of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size during the 3 week experimental period.

***Myrtus communis* L.**

Significant differences found between the different light qualities for the height growth rate of both time intervals for *Myrtus* seedlings (p<. 005). More specifically at the 14th day of the indoor experiment FL light induced significantly higher height increment of 20.27 mm compared to the NS1 LED (p<. 002) that showed height increment of 15.74 mm; the growth rate obtained under the rest of lights in descending order was 19.04 mm, 18.09 mm, 17.66 mm, 17.30 mm and 17.28 under RGP, L20AP67, AP673L, AP67 and G2, respectively (Fig.7). Also at the 28th day, significant increase in height increment was found under the FL and AP673L illuminations with 30.39 mm and 29.65 mm, respectively that further differed significantly with AP67 (p<. 001) and G2 (p<. 007) that obtained height increment of 21.83 mm and 22.83 mm. The rest of lights such as L20AP67, NS1 and RGP showed height increment of 27.55 mm, 27.72 mm and 24.43 mm, respectively (Fig.7).

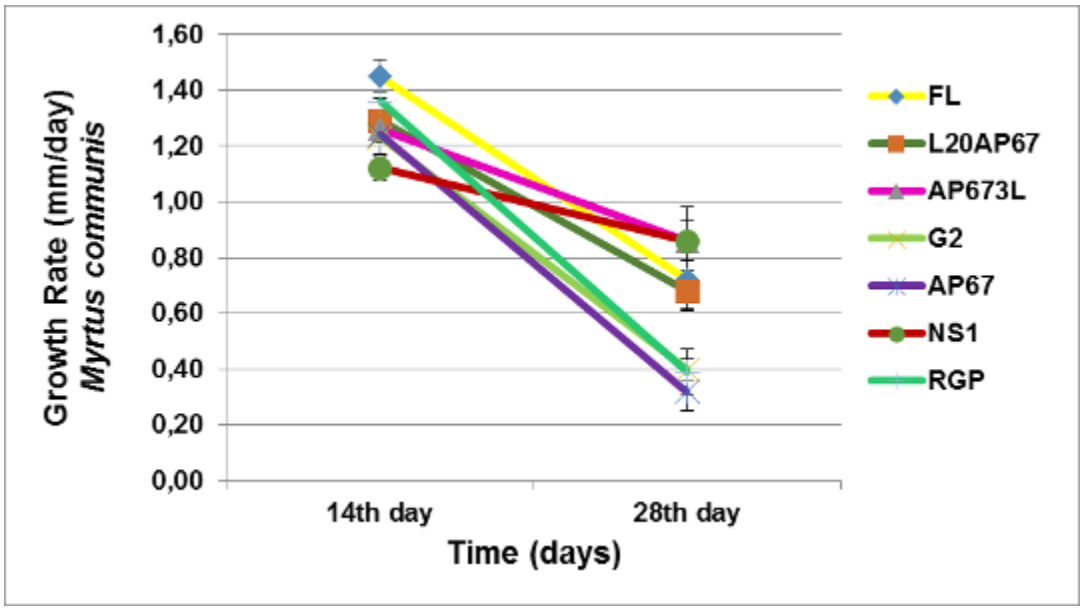


Figure 7. The growth rate of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size during the 3 week experimental period.

***Ocimum basilicum* L.**

At the first week, *Ocimum basilicum* LL seedlings grown under the AP673L and AP67 lights had significantly lower growth rate of 0.77 mm and 1.07 mm compared to the L20AP67, G2 and FL lights with average values of 1.55 mm, 1.51 mm and 1.48 mm, respectively ($p < .001$). Furthermore significant differences also found for the seedlings grown under the NS1 light that had average height increment of 0.86 mm compared to the L20AP67 and FL lights ($p < .001$) (Fig.8). During the second week of the experimental period NS1 and AP673L lights had significantly lower height increment of 0.58 mm than G2 ($p<. 003$), L20AP67 ($p<. 004$) and AP67 ($p<. 009$) shown similar average values about 1.07 mm. At the third week of the experimental period the L20AP67 light showed significantly higher value of 0.87 mm compared to the AP673L ($p < .001$) and NS1 lights ($p<. 003$) with average values of 0.30 mm and 0.45 mm, respectively; while AP673L LED light found also induced significantly lower growth rate compared to FL ($p<. 009$) that showed average value of 0.68 mm. No significant differences were found at the fourth week for the height increment between the light treatments, however higher was found under the G2 with average value of 0.72 mm, following by the AP67, L20AP67, FL, AP673L and NS1 showed average values of 0.64 mm, 0.60 mm, 0.50 mm and 0.46 mm, respectively (Fig.8).

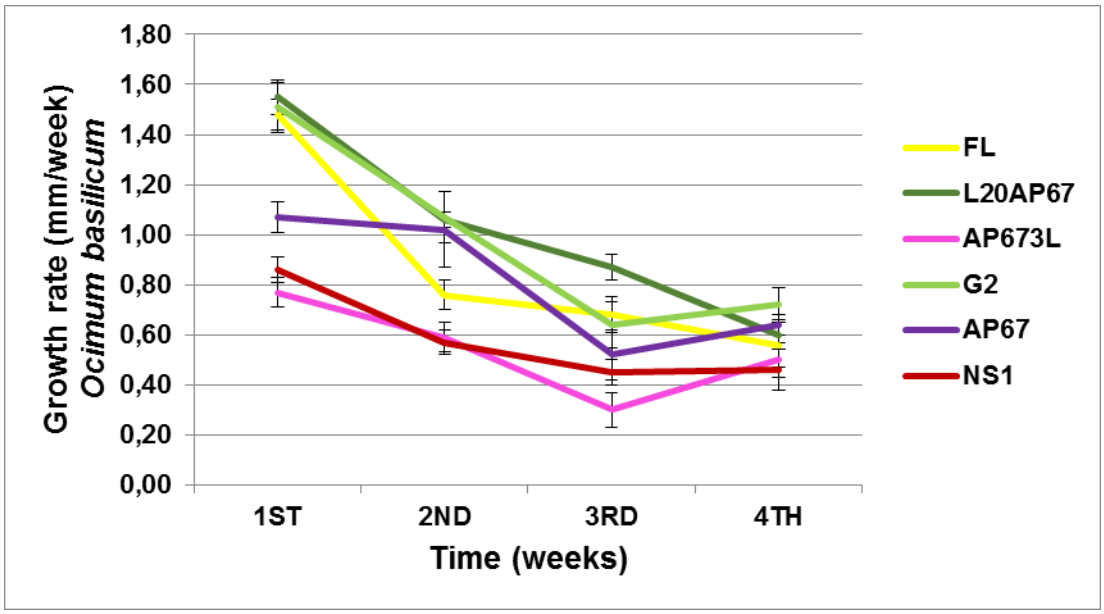


Figure 8 . The growth rate of Ocimum basilicum LL seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size during the 4 week experimental period.

Ocimum basilicum RR hybrid

For the first week of the experimental period, RR hybrid basil seedlings grown under the AP673L light had significantly lower growth rate of 0.88 mm compared to the treatments such as the FL, L20AP67 ($p < .001$) and AP67 ($p < .003$) that shown 1.77 mm, 1.54 mm and 1.50 mm, respectively, while G2 and NS1 lights had average values of 1.37 mm and 1 mm (Fig.9). Furthermore, during the first week the NS1 light also showed significantly lower growth rate compared to the FL ($p < .001$). During the second week of the experimental period the G2 light induced the highest height increment of 1.28 mm and differed significantly with all lights such as the FL ($p < .005$), NS1 ($p < .008$), L20AP67 and AP673L ($p < .009$) that had average values of 0.78 mm, 0.80 mm and 0.81 mm, respectively. No significant differences were observed during the third week of the experimental period with the control light showing the lowest average values of 0.76 mm among LED treatments such as the G2, AP67, NS1, AP673L and L20AP67 that shown average values of 1.22 mm, 1.19 mm, 0.98 mm, 0.86 mm and 0.84 mm. Finally at the end all light treatments obtained

similar growth by means of height and induced in descending order 1.31 mm, 1.12 mm, 0.97 mm and 0.81 by the AP67, AP673L, G2, NS1.

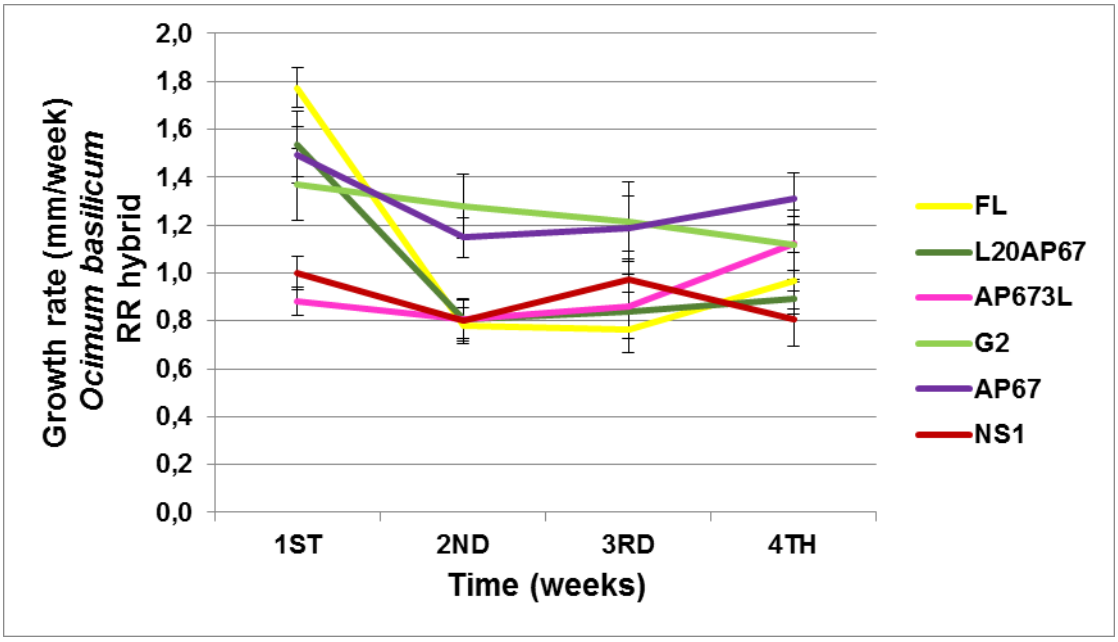


Figure 9. The growth rate of Ocimum basilicum RR hybrid seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size during the 4 week experimental period.

***Cornus sanguinea* L.**

Statistical analysis showed a significant interaction time*light ($p < .001$) during the second, third and the fourth week of the experimental period (Fig.10). At the second week G2 LED light) showed higher growth rate of 1.27 mm among the tested lights however no significant differences found at all. Similar height increment induced for the rest of lights such as the AP673L, AP67, FL and NS1 with average values of 1.10 mm, 0.97 mm, 0.96 mm and 0.83 mm. The lower was for the L20AP67 with an average height increment of 0.60 mm. During the third week NS1 LED light induced significantly lower height increment of 0.68 mm compared to the highest induced by the L20AP67 ($p<. 001$) with average value of 1.33 mm. Finally, at the fourth week of the experimental period L20AP67 ($p<. 001$) LED and FL($p<. 002$) conventional light induced significantly higher height growth rate of 1.05 mm and 0.99 mm than AP673L with the lowest height increment of 0.43 mm. The rest of lights such as the AP67, NS1 and G2 showed similar growth rates about 0.65 mm.

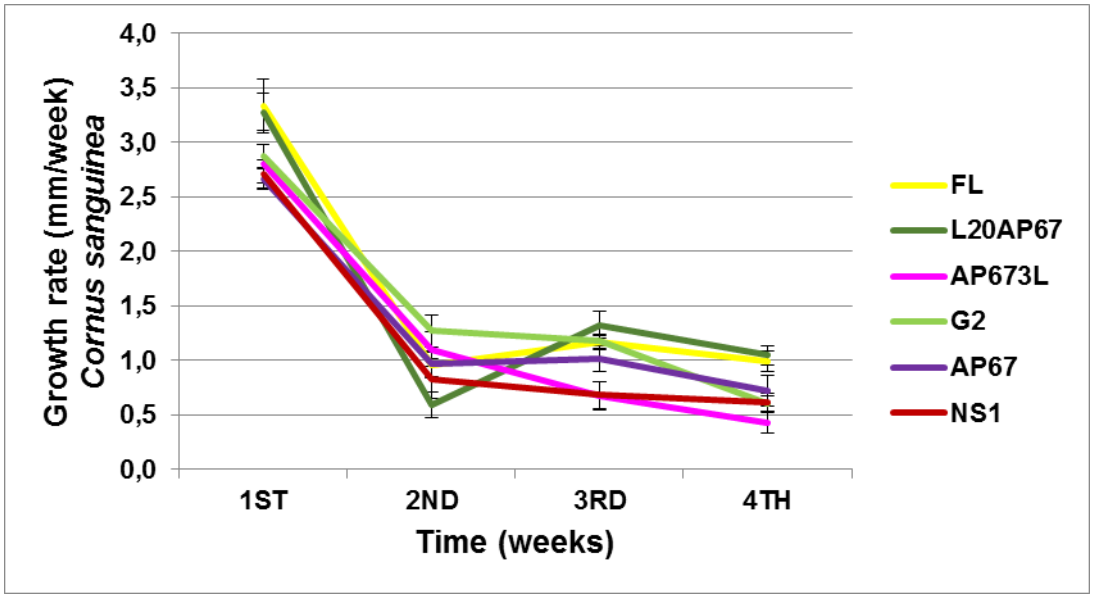


Figure 10. The growth rate of Ocimum basilicum RR hybrid seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size during the 4 week experimental period.

***Prunus avium* L.**

Wild cherry seedlings did not show significant differences for the growth rate in the first 2 weeks stay in the growth chamber. G2 LED light showed the highest average values with 2.58 mm followed by AP67 with 2.47 mm, L20AP67 with 2.44 mm, NS1 with 2.42 mm, FL conventional light with 2.35 mm and AP673L with 2.10 mm (Fig.11).

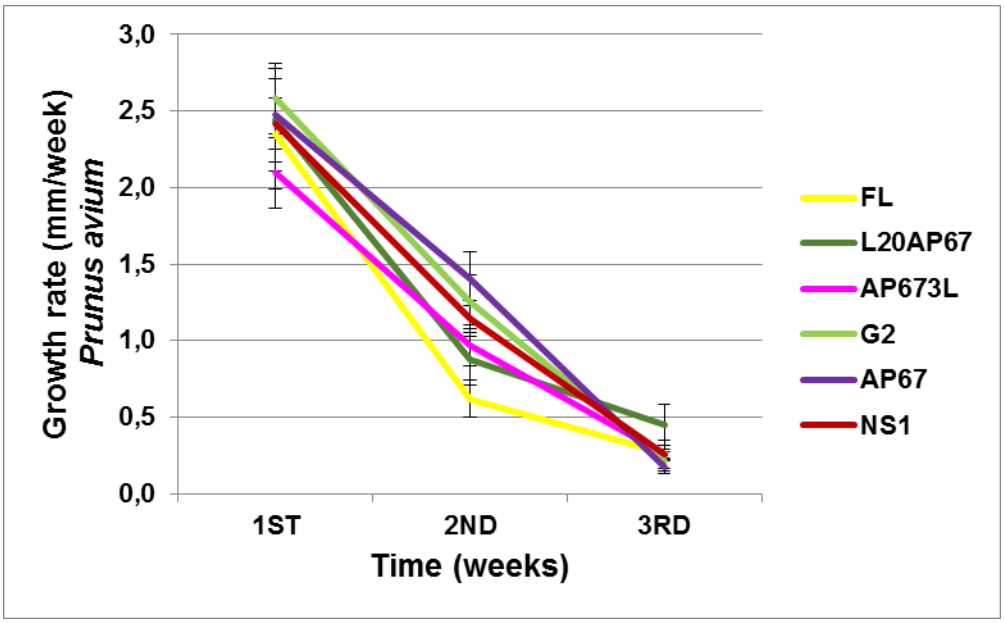


Figure 11. The growth rate of Prunus avium seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size during the 6 week experimental period.

***Punica granatum* L.**

In the first two weeks in the growth chambers L20AP67 LED promoted significantly greater growth rate with an average value of 2.32 mm compared to the rest of the treatments such as AP673L, NS1, G2, AP67 ($p<.001$) and FL ($p<.018$) that obtained 1.20 mm, 1.38 mm, 1.40 mm and 1.7 mm, respectively. The same light treatment also induced significantly higher height growth rate of 1.75 mm during the 3rd and 4th week into the growth chambers. L20AP67 treatment was followed by AP673L 0.84 mm, G2 with 0.66 mm, NS1with 0.60 mm, FL control light obtained 0.52 mm and the lowest for the AP67 with an average of 0.26 mm ($p < .001$). In addition, for the same period, seedlings grown under the AP673L showed significantly higher height increment than those under the AP67 ($p<.003$) light treatment. During the 5th and 6th week G2 LED showed the lowest growth rate with an average value of 0.44 mm and significant differences found with the AP673L ($p<.001$), FL ($p<.003$) and L20AP67 ($p<.00$) that showed similar higher height increment of 1 mm. NS1 and AP67 LEDs obtained an average height increment of 0.67 mm for the aforementioned period (Fig.12).

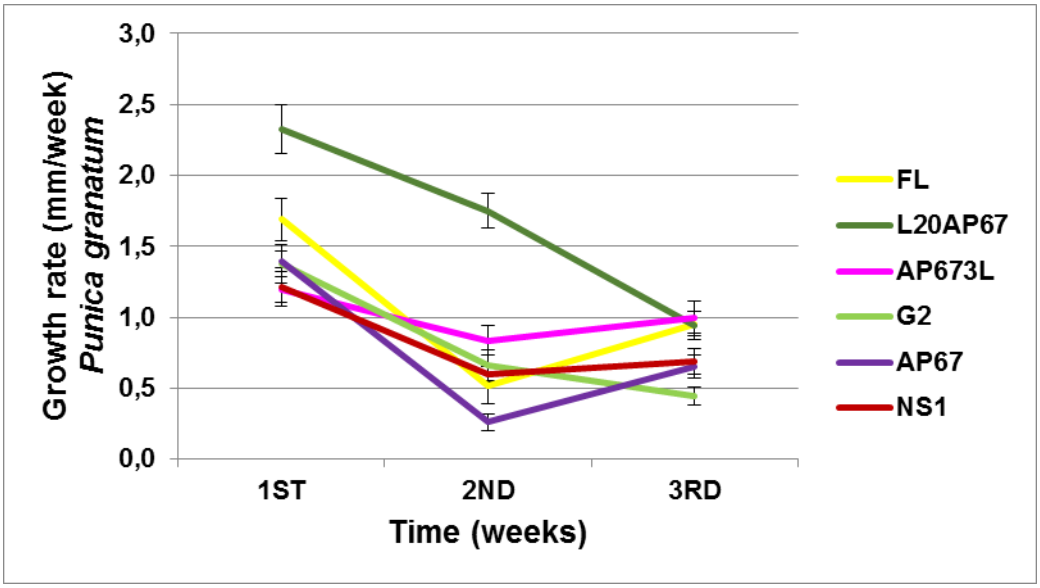


Figure 12. The growth rate of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size during the 6 week experimental period.

3.3.2. Leaf colour rating & Number

***Pinus sylvestris* L. (provenance Greece-Sweden)**

Different light treatments did not induce significant changes in needle colour at any of the coniferous tested species

Needle formation pattern of two provenances of *P. sylvestris* was similar. However we found significant differences under the light treatments for each of the provenance over time. Specifically for the prov. Greece, significantly more needles were formed under the AP67 with 9 needles compared to FL, L20AP67 ($p<.001$) with 6 needles NS1, AP673L ($p<.002$) with 7 needles (Fig.13). Further at the second week AP67 and G2 LEDs induced significantly more needles 20 and 18 compared to FL, L20AP67 ($p<.001$) and AP673L ($p<.003$) that formed in average 11, 12 and 14 needles, respectively. Following at the third week both FL and L20AP67 lights induced significantly less needles formed, 14 and 16, compared to the rest of LED treatments ($p<.001$) such as G2, AP67, NS1 and AP673L that formed 25, 24, 23 and 21 needles. At the fourth week FL light obtained the least number of needles, 20 compared to all LED lights and significant differences found to all ($p<.001$) except from the L20AP67 that obtained 23 needles. The highest number of needles was formed under the G2 of 33 needles, following by the AP67, AP673L and NS1 with 31, 29 and 28 needles. At the end of the indoor experiment significantly more needles were formed under the G2 and AP67 illuminations with 37 and 36 needles that differed from those formed under the FL and L20AP67 ($p<.001$) with 27 and 28 needles. As for the rest of LEDs, such as the NS1 and the AP673L 28 needles were formed.

P. sylvestris prov. Sweden seedlings grown under the AP67 LED formed threefold number of needles and significant differences found with those grown under the FL, L20AP67 and AP673L that formed 6 needles during the first week (Fig.14). At the second week LED lights of AP67, NS1 and G2 induced significantly more needles 19, 18 and 17 compared to the FL and L20AP67 ($p<.001$) with 12 needles. At the third week LEDs of AP67, G2, NS1 and AP673L induced significantly more needles compared to FL and L20AP67 ($p<.001$). At the last two weeks of the indoor experiment LED lights such as AP67 and G2 accelerate the needle formation compared to the FL light that showed 10 needles less been formed (Fig.14).

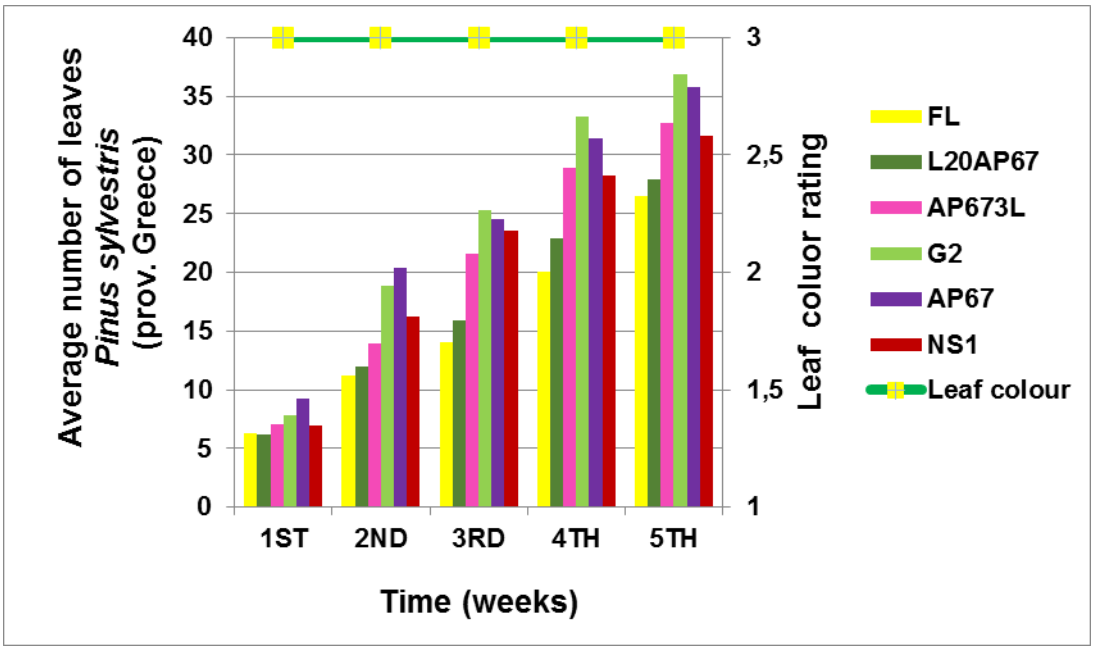


Figure 13. The leaf colour rating (1=pale, 2=light green, 3=dark green, 4=reddish) and the number of Pinus sylvestris provenance Greece seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size during the 5 week experimental period.

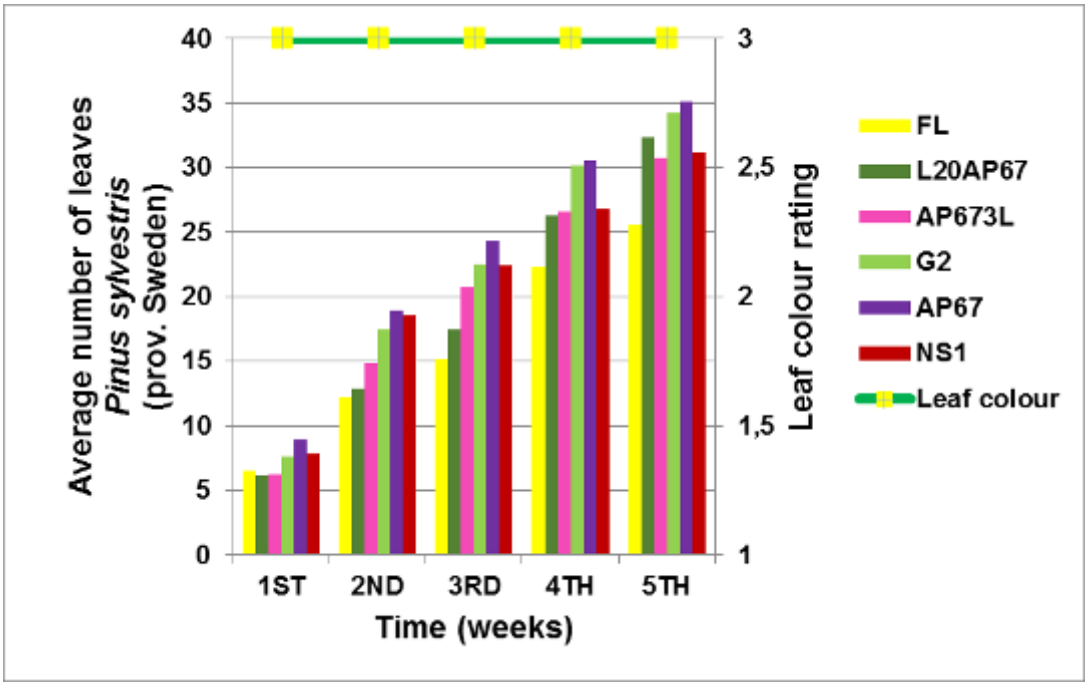


Figure 14. The leaf colour rating (1=pale, 2=light green, 3=dark green, 4=reddish) and the number of Pinus sylvestris provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size during the 5 week experimental period.

***Picea abies* Karst. (provenance Sweden)**

Significant differences in needle formation of *Picea* seedlings were shown at the second week where AP67 and AP673L LEDs formed 17 needles compared to the FL and L20AP67 that induced 12 needles (p<. 005). Following at the third week AP67 and G2 LEDs formed significantly more needles specifically 28 and 26 compared to FL, L20AP67 and NS1 (p<. 001) that formed 15, 18 and 20 needles. At the fourth week FL light quality induced the least number of needles formed 24, compared to all lights (p<. 002) except from L20AP67 with 27; while the rest of the lights formed more than 30 needles. Finally at the fifth week all LEDs induced significantly more needles compared to the FL light (p<. 001). Also L20AP67 LED had less needles formed than AP673L, AP67 (p<. 001) and G2 (p<. 007) that showed in average 44, 42 and 40 needles (Fig.15).

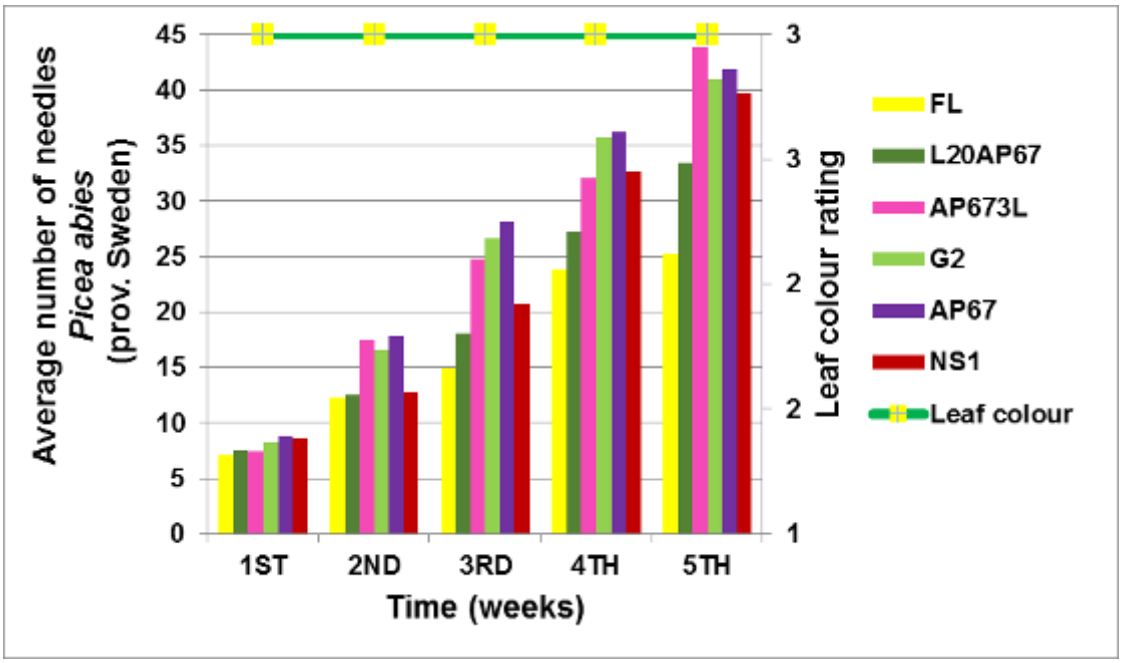


Figure 15. The leaf colour rating (1=pale, 2=light green, 3=dark green, 4=reddish) and the number of Picea abies provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size during the 5 week experimental period.

***Pinus nigra* Arn.**

No significant differences found between the different light treatments for the needle formation of *P. nigra* seedlings over time; however more needles were formed under the illumination of G2 with an average of 21 needles, following by the AP673L,

AP67, NS1, FL and L20AP67 with 21, 20, 19, 18 and 17 needles, respectively (Fig.16).

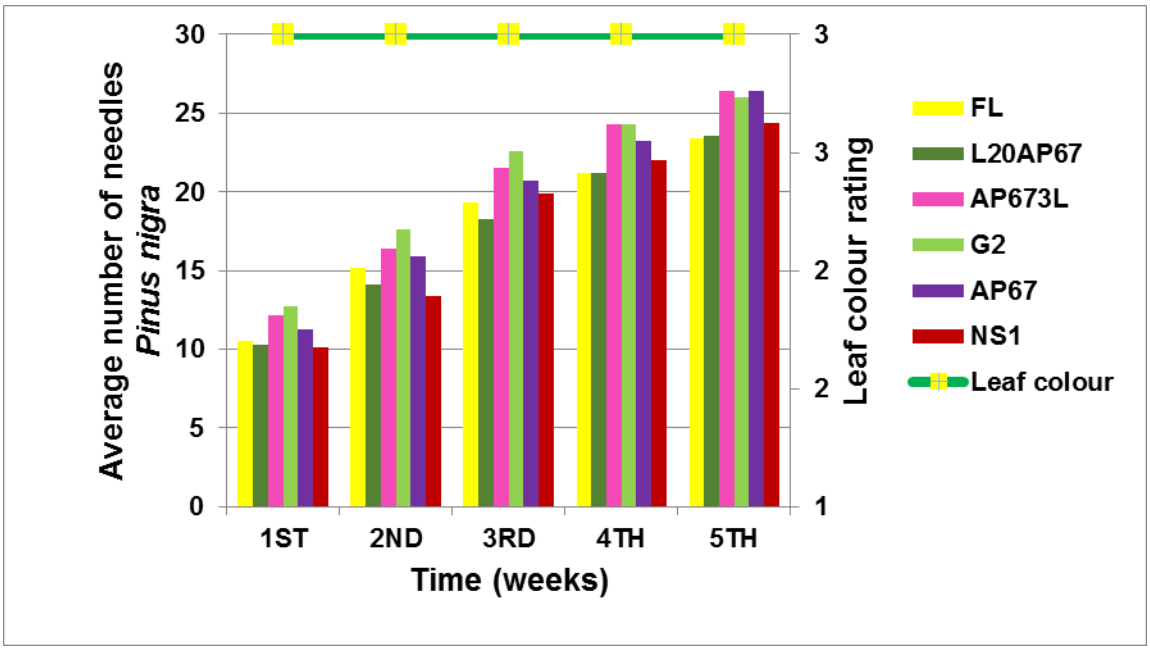


Figure 16. The leaf colour rating (1=pale, 2=light green, 3=dark green, 4=reddish) and the number of Pinus nigra seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size during the 5 week experimental period.

Quercus ithaburensis ssp. *macrolepis*

Different light qualities induced the same effect on seedlings leaf colour rating during the indoor experiment; eventually all seedlings characterized with dark-green colour irrespective the light spectrum (Fig. 17). In contrast significant differences were found between the light treatments for the leaf formation over time (p<. 005). Specifically, at the second week AP67 LED light induced significantly more leaves (16) compared to the G2 (10) (p<. 014) and FL (11) (p<. 034). Also at the third week significantly more leaves were found under the AP67 LED (17) compared to the G2 (11) (p<. 007). As it is presented in Figure 2, the slowest leaf formation is observed both for the FL and the G2 light qualities, while AP67 and AP673L LEDs stimulated leaf development throughout the experiment.

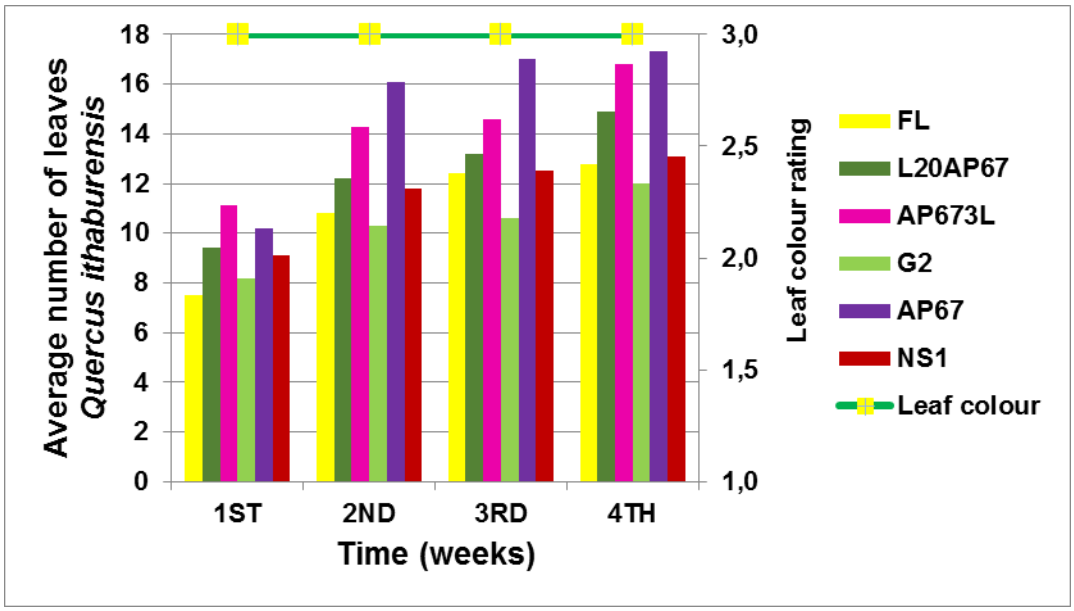


Figure 17. The leaf colour rating (1=pale, 2=light green, 3=dark green) and the number of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size during the 4 week experimental period.

***Castanea sativa* Mill.**

Leaf colour rating of *Castanea* seedlings showed no significant difference between the light treatments but there was a gradual enhancement during the three weeks of the experimental period, thus all seedlings characterized with dark green colour (Fig.18). Also light qualities did not induce significant effect on the leaf formation over time. However was observed faster leaf formation under the combined effects of both L20AP67 and G2 that eventually formed 11 leaves. The slowest was observed under FL and RGP (FL & sodium lamps) with an average of 8 leaves. The rest of LEDs, such as AP673L, AP67 and NS1 showed 10, 9 leaves, respectively (Fig.18).

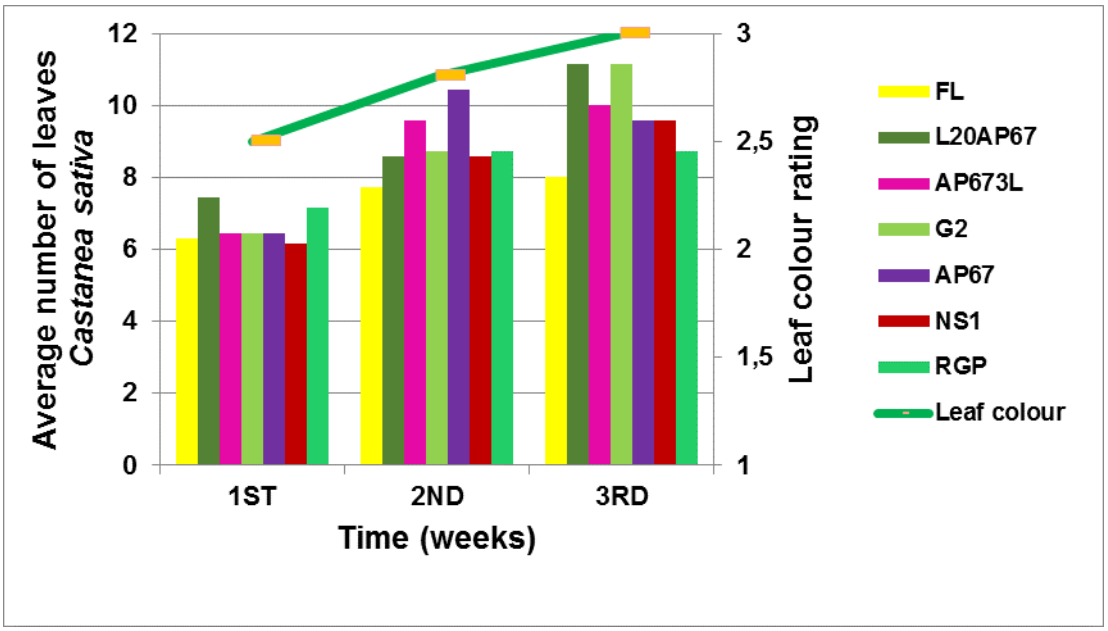


Figure 18. The leaf colour rating (1=pale, 2=light green, 3=dark green, 4=reddish) and the number of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size during the 3 week experimental period.

***Myrtus communis* L.**

Leaf colour of *Myrtus* seedlings showed no significant differences between the tested lights; however less beneficial light source for that characteristic found to be the RGP than LED lights. Besides, significant differences found for the leaf formation under the different lights for both time intervals. At the first time AP67 and RGP lights formed significantly faster leaves in average 7, compared to FL and L20AP67 that formed 5. At the end of the indoor experiment, significantly more leaves were formed under the effect of NS1 LED treatment with 12 leaves compared to the L20AP67 ($p<.001$), RGP ($p<.001$) and FL ($p<.004$) with 8 leaves. The rest of LED lights, such as the AP67, AP673L, and G2 formed 11 and 10 leaves (Fig.19).

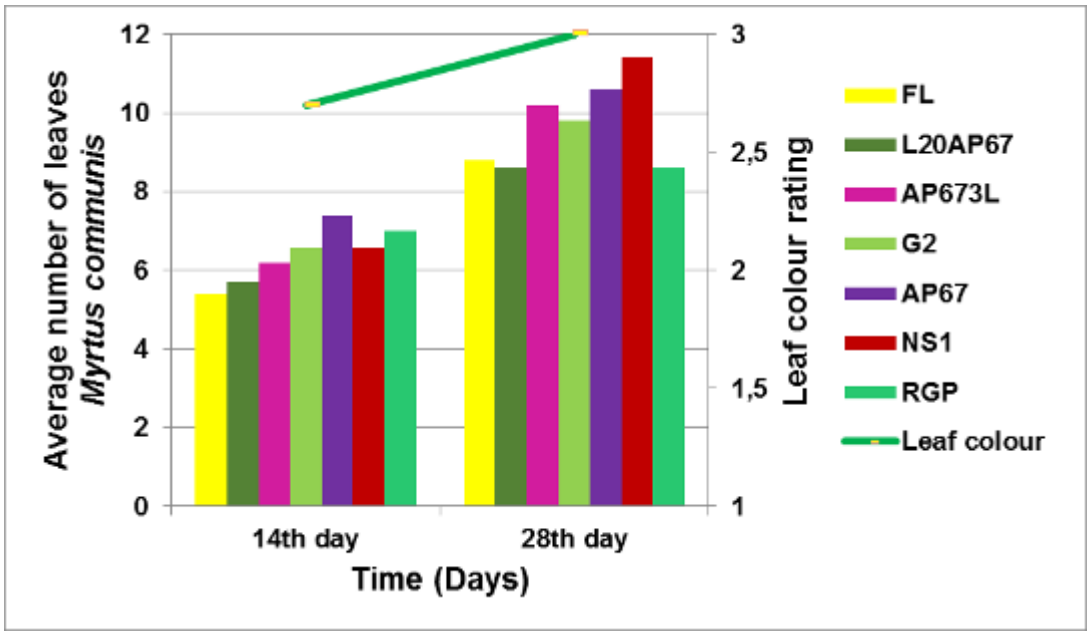


Figure 19. The leaf colour rating (1=pale, 2=light green, 3=dark green, 4=reddish) and the number of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size during the 3 week experimental period.

***Ocimum basilicum* L.**

Results for the leaf colour showed no significant differences between the light treatments as basil seedlings maintained a light green colour throughout the 4 week experimental period (Fig.20). Basil seedlings, showed significant differences though for the leaf formation that observed only at the first week, where the FL and L20AP67 lights (p<. 001) induced the formation of two leaves, while LEDs of AP673L, G2, AP67 and NS1 LED lights formed almost 4 leaves on average.

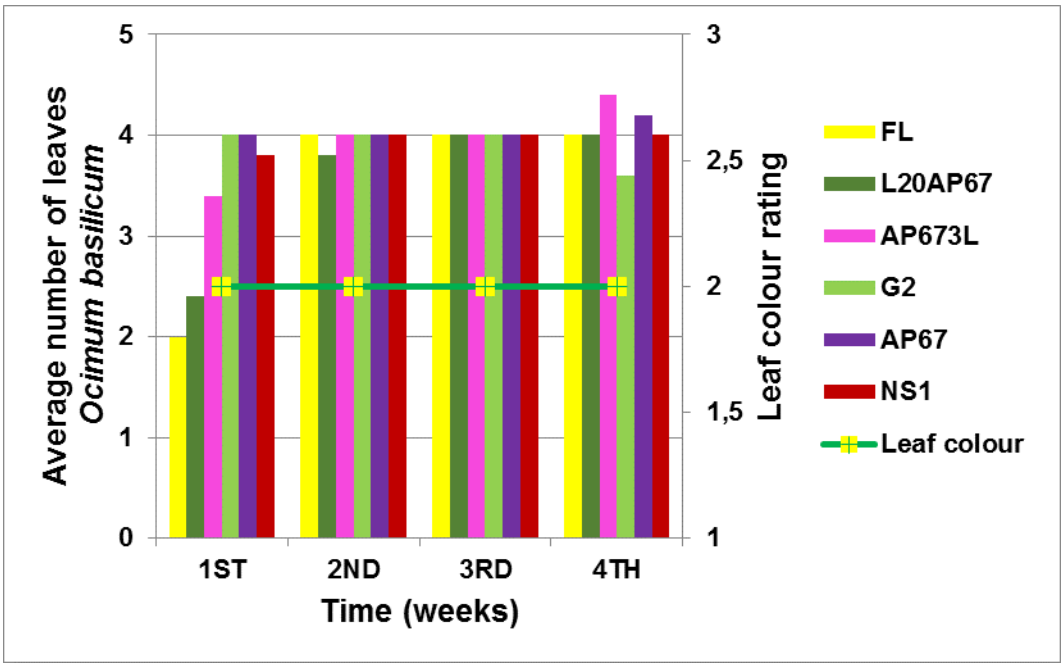


Figure 20. The leaf colour rating (1=pale, 2=light green,3=dark green) and number of Ocimum basilicum LL seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 VW mini-plug size during the 4 week experimental period.

Ocimum basilicum RR hybrid

The leaf colour showed no significant differences between the light treatments as the basil's hybrid seedlings maintained purple colour throughout the 4 week experimental period (Fig.21). As for the leaf number of RR basil seedlings, the results showed significant differences only at the first week where both NS1 and AP67 LED lights formed 4 leaves, compared to the AP673L, FL ($p < .001$) and L20AP67 lights ($p < .004$) that formed 2 to 3 leaves on average. Furthermore, the seedlings grown at the G2 light formed significantly more leaves (almost 4 leaves) than the FL ($p < .004$) and L20AP67 lights ($p < .001$) (Fig.21).

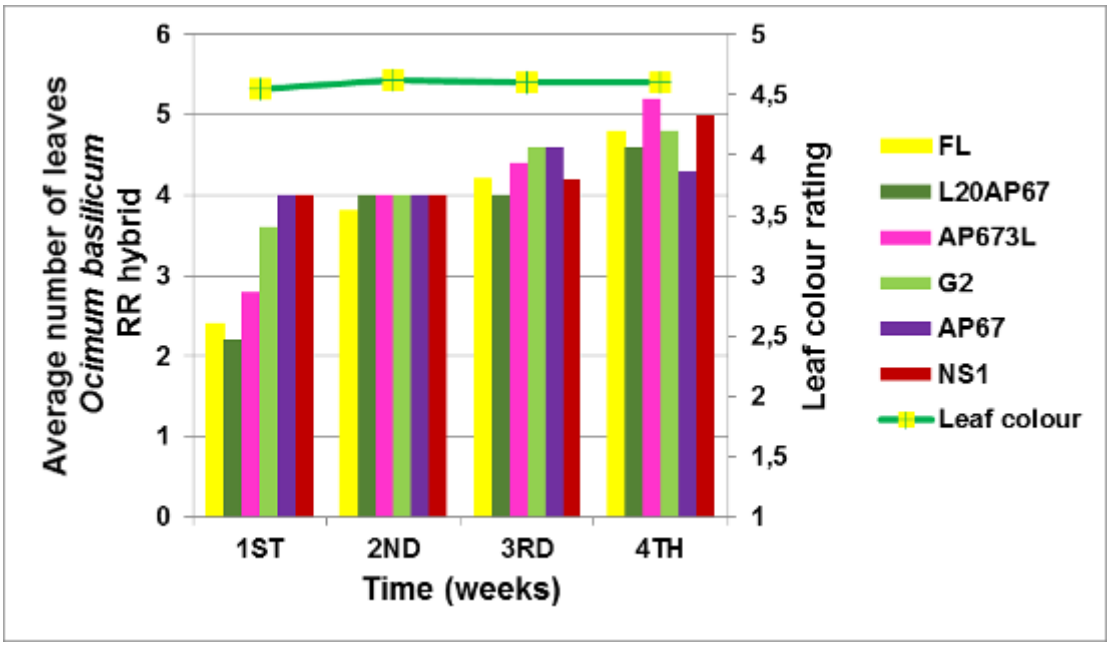


Figure 21. The leaf colour rating (1=pale, 2=light green,3=dark green, 4= purple-green, 5= purple) and number of Ocimum basilicum RR hybrid seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 VW mini-plug size during the 4 week experimental period.

***Cornus sanguinea* L.**

The leaf colour of the seedlings was characterized as dark green under all the light treatments during the first and the second week. Although at the third week seedlings grown under the AP673L, G2, AP67 and NS1 LED lights induced the appearance of reddish colour on the leaves compared to the totally dark green obtained under the FL and L20AP67. Finally, at the fourth week seedlings showed significantly different colour on leaves, specifically under the AP673L, G2, AP67 and NS1 lights was reddish compared to the dark green obtained under both the FL and L20AP67 lights ($p < .001$) (Fig.22). Meanwhile for the leaf formation no significant differences were found during the whole period of the indoor cultivation under different light qualities, however both G2 and AP67 LEDs promoted more the leaf development than the FL and L20AP67 lights (Fig.22)

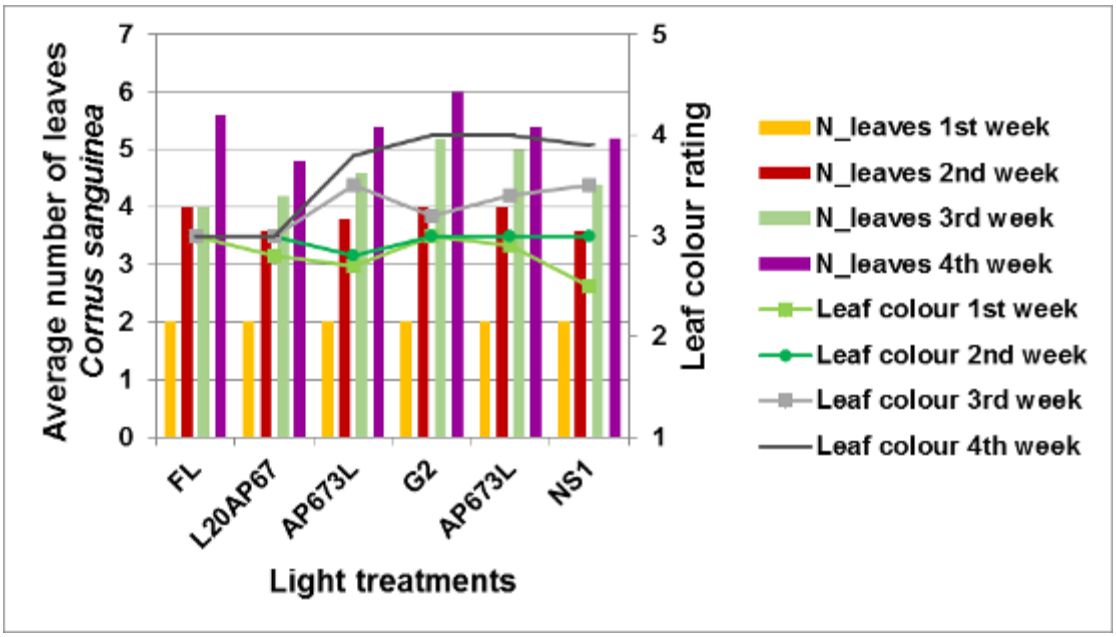


Figure 22. The leaf colour rating (1=pale, 2=light green,3=dark green, 4=reddish) and number of Cornus sanguinea seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 VW mini-plug size during the 4 week of experimental period.

***Prunus avium* L.**

As far as the leaf colour is concerned, there were no significant differences between the light treatments for the first 4 weeks into the growth chambers. In that period all seedlings maintained dark green colour. However, in the final 2 weeks FL and L20AP67 lights preserved their dark green colour but the rest of the LED ($p<.001$) treatments showed light green colour (Fig.23). The results showed that there was no significant effect on the leaf formation of the seedlings grown under the different light treatments throughout the experimental period. Specifically, wild cherry seedlings formed 4-5 leaves in the first 2 weeks under all the light treatments. Following during the 3rd and 4th week 6-7 leaves were formed, while in the 5th and 6th week the leaves were around 5-7 under all the light treatments (Fig.23).

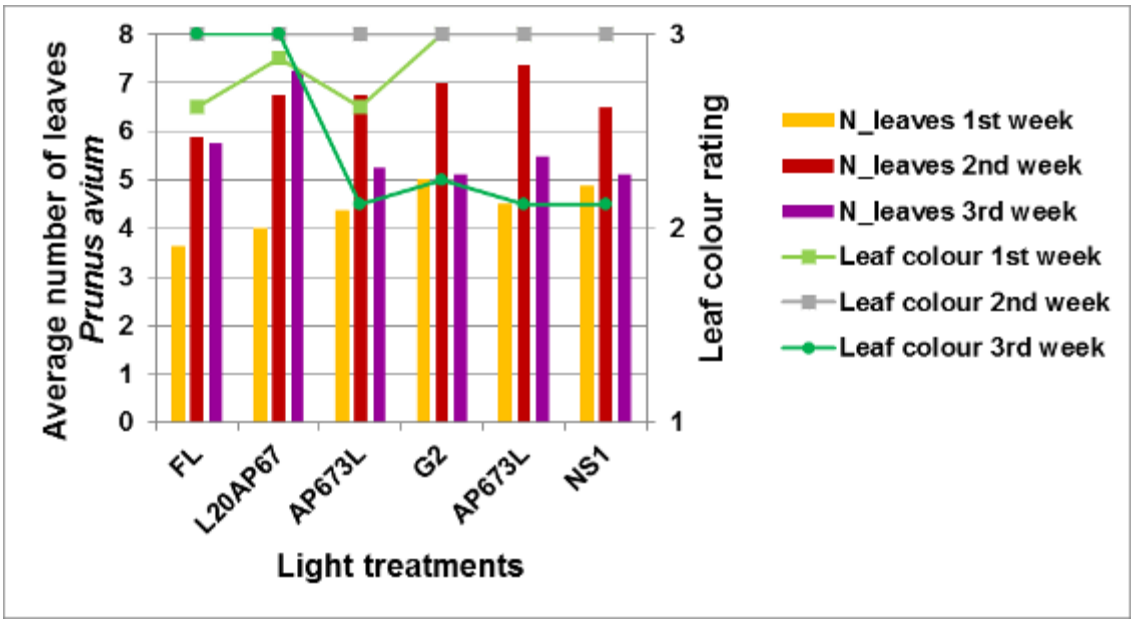


Figure 23. The leaf colour rating (1=pale, 2=light green,3=dark green) and number of Prunus avium seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 VW mini-plug size during the 6 week experimental period.

***Punica granatum* L.**

Leaf colour was light green for all light treatments during the 1st and 2nd week into the growth chambers. The results showed significant differences only at the 3rd and the 4th week between NS1 LED that induced red leaves compared to both the FL and L20AP67 ($p < .005$) that had light-green leaves. No significant differences were observed in the last two weeks with FL, L20AP67, AP673L, G2 and AP67 inducing a light green leaf colour, while NS1 mostly promoting the formation of red leaves. In the 1st and 2nd week in the growth chambers G2 and L20AP67 LED lights induced the formation of more than 6 leaves on average compared to the FL ($p < .001$) and the AP673L ($p < .005$) that induced 4 leaves, while NS1 induced 5 leaves. During the3rd and 4th week L20AP67 light promoted the formation of 12 leaves than FL ($p < .001$) with 6-7 leaves, AP67 and AP673L ($p < .006$) with 8 leaves, while G2 and NS1 LEDs induced the formation of 10 and 9 leaves on average. Finally, in the last 2 weeks no significant differences were observed between the light treatments however NS1 induced higher leaf formation of 15 leaves while FL showed the lowest of 11 leaves (Fig.24).

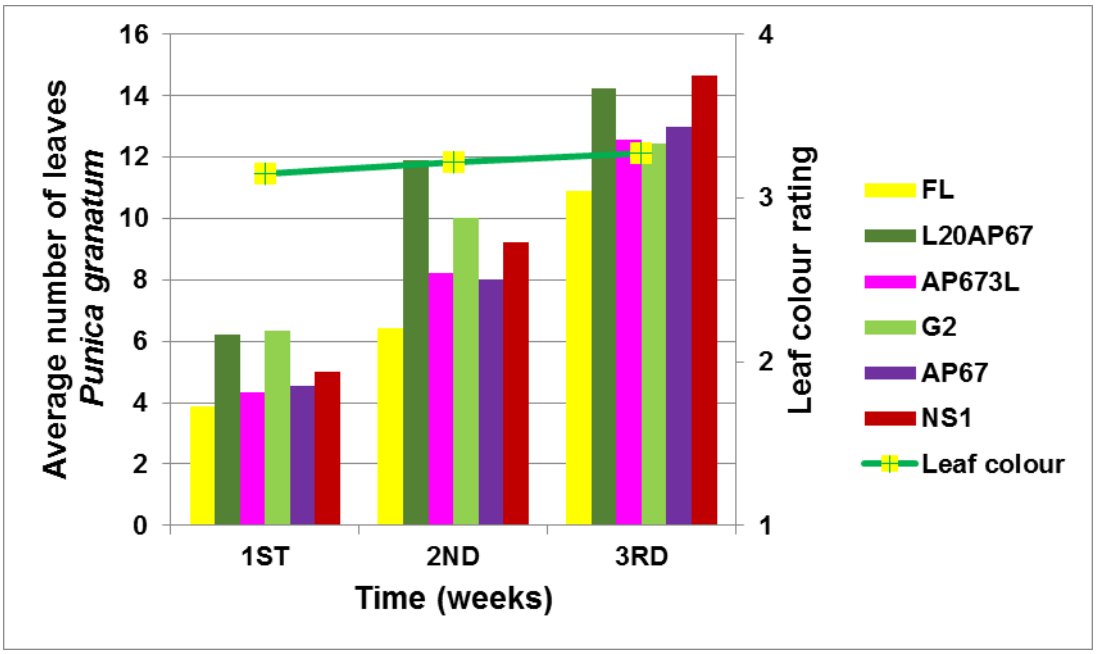


Figure 24. The leaf colour rating (1=pale, 2=light green,3=dark green, 4=reddish) and number of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in QPD 104 VW mini-plug size during the 6 week experimental period.

3.3.3. Number of leaf stomata & epidermal cells

Quercus ithaburensis* ssp. *macrolepis

Different light qualities resulted in significantly different number of stomata (p<. 001) in the abaxial surface of leaves but that was not the case for the epidermal cells. G2 LED light (33 stomata) combined effects induced significantly higher stomatal development compared to the FL (15 stomata) (p<. 002) and L20AP67 (17 stomata) (p<. 004) lights (Fig.25). Epidermal cells did not show any significant differences between the light treatments however more cells were formed under LED lights, especially for the G2 & NS1 qualities with an average of 132 cells, while FL and L20AP67 lights showed an average of 105 cells (Fig.25).

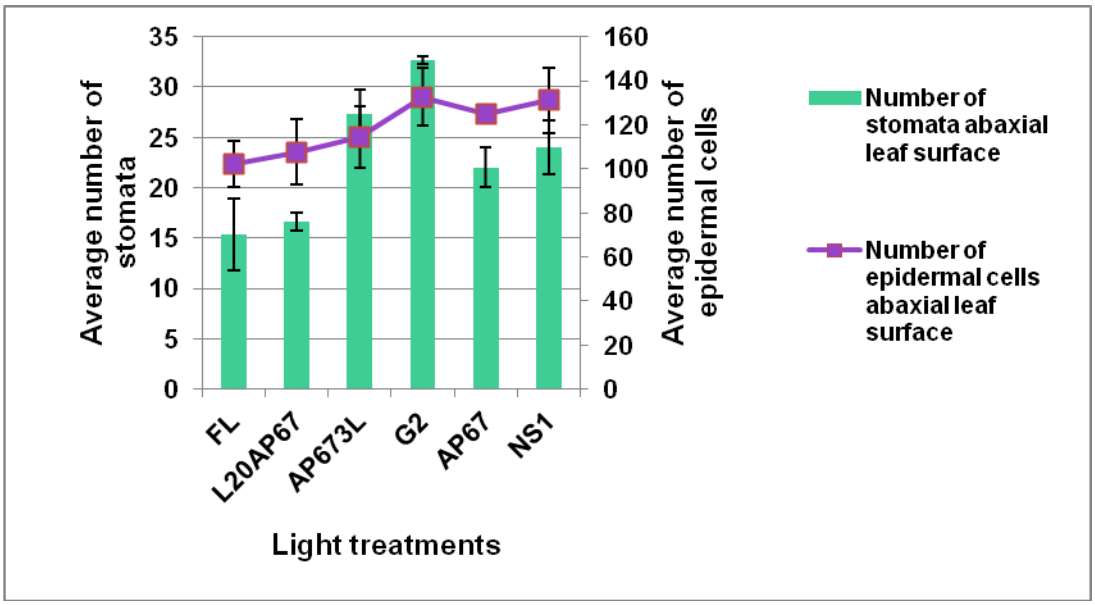


Figure 25. Average number of stomata & epidermal cells on the abaxial leaf surface of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

***Castanea sativa* Mill.**

Significant differences found both for the number of stomata and epidermal cells (p<. 003). Among the different light environments G2 LED showed significantly more stomata 14 in average, compared to the FL and L20AP67 (p<. 008) that induced 8 stomata (Fig.26). As for the rest of the lights such as AP67, AP673L and RGP showed 13, 12 and 11 stomata, respectively. Furthermore NS1 and AP67 LEDs had significantly created more epidermal cells 88 and 86, respectively compared to the FL conventional light that had 59.25. More epidermal cells also were formed under the rest of light treatments those of G2, AP673L, RGP and L20AP67 with average values of 78, 73, 68 and 67, respectively (Fig.26).

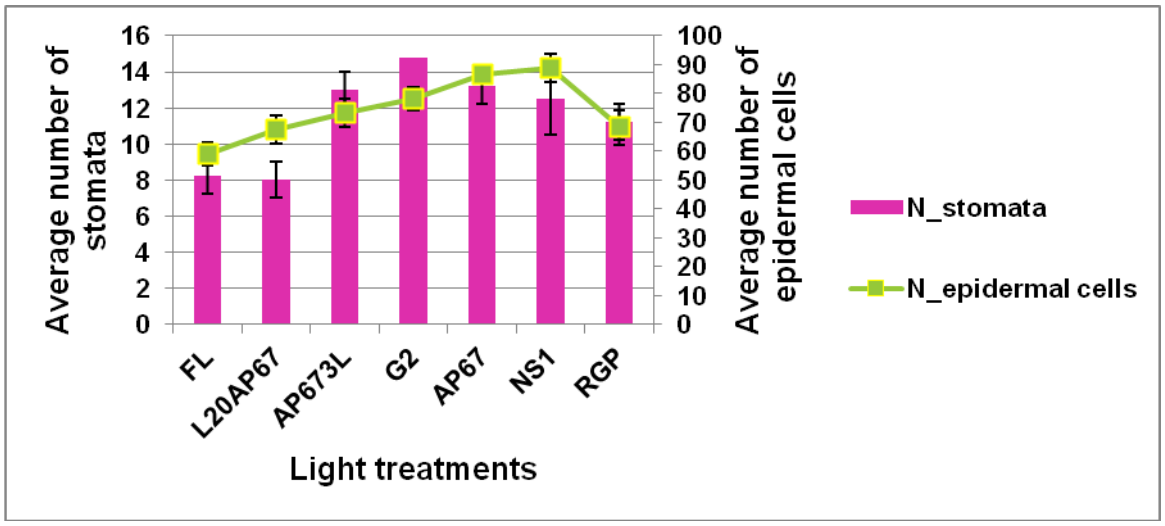


Figure 26. Average number of stomata & epidermal cells on the abaxial leaf surface of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

3.3.4. Stomatal density (SD) - Stomatal index (%) (SI) - Cell density (CD)

Quercus ithaburensis* ssp. *macrolepis

Contribution of the abaxial surface of leaves was similar for all light qualities investigated, demonstrating that the hypostomatous character of the quercus leaves was unaffected by light quality. SD was significantly higher on leaves grown in the presence of G2 LED light (488 stomata /mm^2). This higher SD was not due to an increased production of stomata, as SI (17.8%) was not substantially affected by the G2 light (Fig.27). Instead, this higher SD was due to the highest CD (2723) (i.e. a lower epidermal cell size) on leaves that developed, compared to the rest of lights (Fig.28). Lowest average values for SD, SI and CD were found for the FL light, 254 stomata/ mm^2, 12.42% and 1986, respectively. As for the rest of light treatments, such as AP673L,NS1, AP67 and last L20AP67, the average values for SD, SI and CD were 413 stomata/ mm^2, 18.9 % and 2347, 401 stomata/ mm^2, 15.30% and 2618, 370 stomata/ mm^2, 14.82% and 2486 274 stomata/ mm^2, 13.30% and 2096 (Fig.27) (Fig.28).

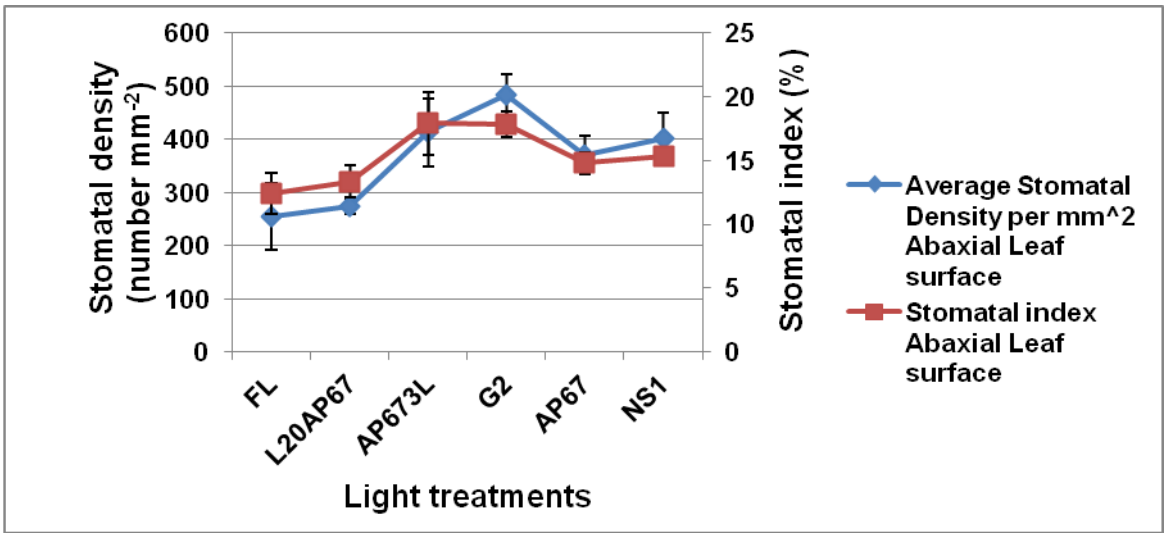


Figure 27. Stomatal density (SD) and Stomatal index (SI) (%) on the abaxial leaf surface of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

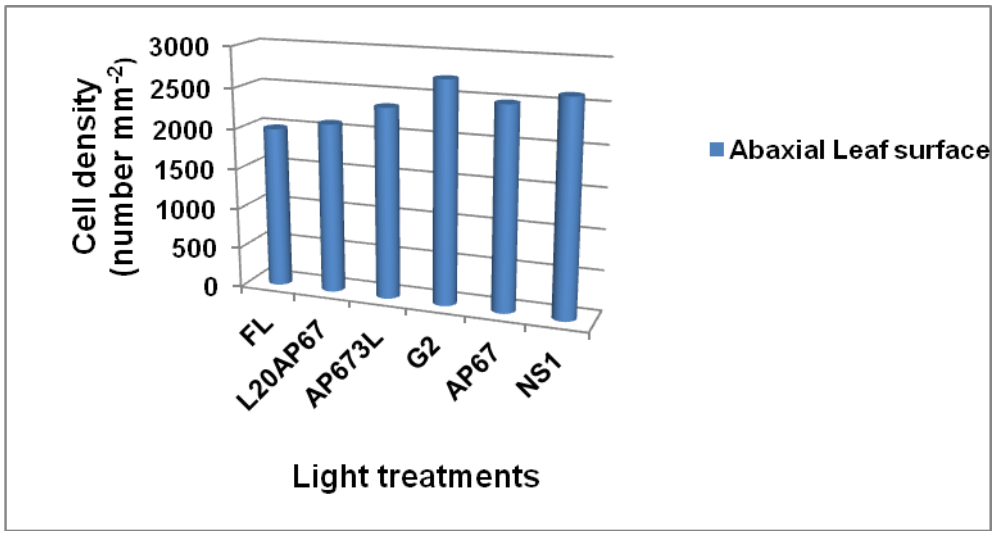


Figure 28. Cell density (CD) on the abaxial leaf surface of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

***Castanea sativa* Mill.**

Hypostomatous character of *Castanea l*eaves showed significant differences for the SD and SI between the different light qualities (p<. 003). G2 light with 251 stomata/mm^2, showed significantly higher density compared to the L20AP67 and FL light that induced 137 stomata/mm^2 and 139 stomata/mm^2 (p<. 009), respectively

(Fig.29). The rest of the lights showed also higher SD than the later lights such as 186.5 stomata/mm^2, 211.7 stomata/mm^2, 217.7 stomata/mm^2 and 225 stomata/mm^2 for the RGP, NS1, AP673L and AP67. Furthermore G2 LED also induced significantly higher SI with 16.03% compared to the L20AP67 with 10.6% (p<. 003). No significant differences found for the rest of lights; however still showed higher values compared to the L20AP67, such as 15%, 13.9%, 13.2% and 12.15% for the AP673L, RGP, AP67 and FL lights, respectively (Fig.29). FL light quality induced significantly lower CD of 1140 compared to NS1 (p<. 004) and AP67 (p<. 006) LED lights that had 1714 and 1688.7, respectively (Fig.30). Also the rest of the light qualities showed higher values of CD than the FL conventional light, such as the G2, AP673L, RGP and L20AP67 with 1573.5, 1452, 1339 and 1276 (Fig.30).

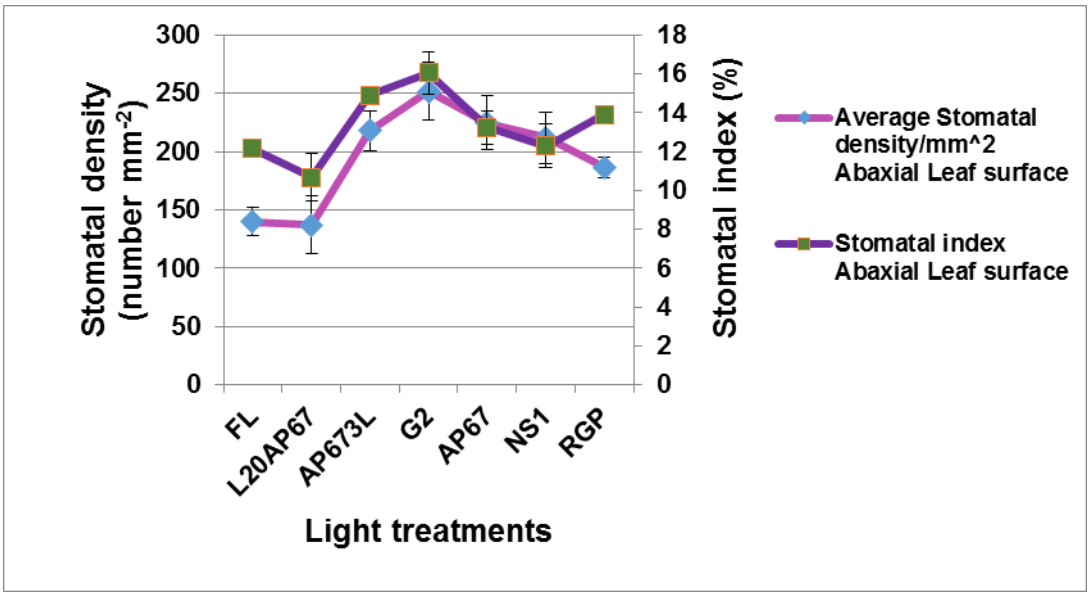


Figure 29. Stomatal density (SD) and Stomatal index (SI) (%) on the abaxial leaf surface of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1, RGP (Fl & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

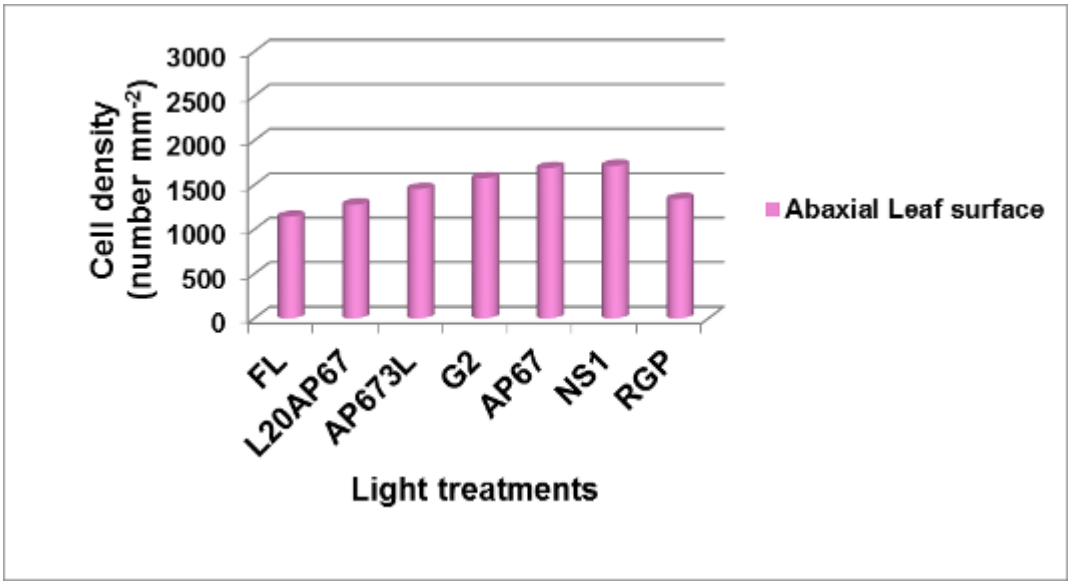


Figure 30. Cell density (CD) on the abaxial leaf surface of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

3.3.5. Leaf area

Quercus ithaburensis* ssp. *macrolepis

Leaf expansion under different light treatments showed no significant differences, however the combined effects of L20AP67 LED promoted leaf formation thus leaf area has the greatest aerial occupation with an average of 121.98 cm^2 (Fig.31); following by the lights AP67 with 87.21 cm^2, FL with 78.93 cm^2, AP673L with 77.33 cm^2, G2 with 68.31 cm^2 and the lowest for the NS1 with 65.63 cm^2.

Findings in *Arabidopsis* seedlings shown that blue light suppresses hypocotyl elongation and induces cotyledon expansion, and red light induces hypocotyl elongation and cotyledon expansion (Whitelam and Halliday, 2007). In our case L20AP67 that induced higher leaf number and area had 10.5% in blue range and 48.9% in red region, contrast to the NS1 that shown the lowest and had 20.2% and 35.7%, respectively.

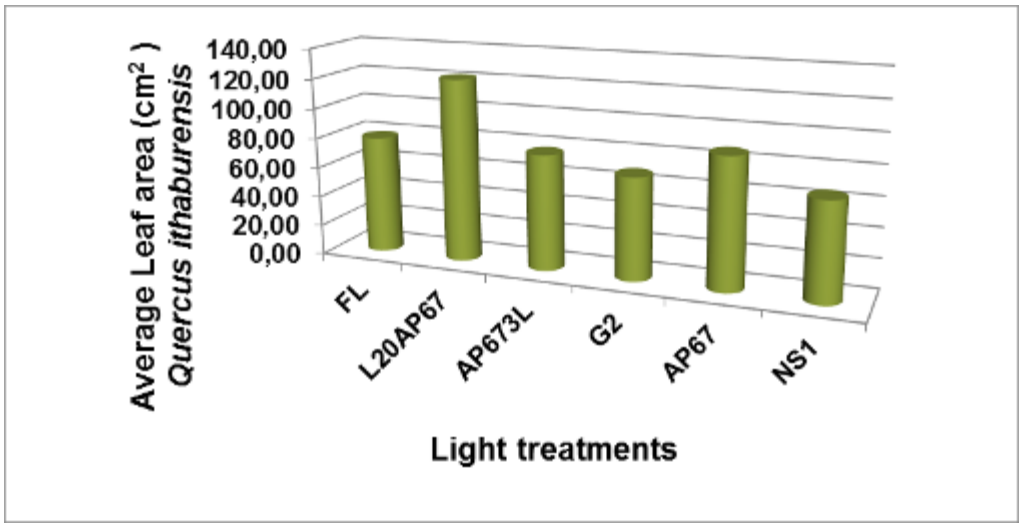


Figure 31. Average Leaf area (cm^2) of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

***Castanea sativa* Mill.**

Leaf area of different light treatments was similar; however the highest was formed under the AP673L with 269.42 cm^2, following by the NS1, L20AP67, AP67, G2, FL and RGP with 216.75 cm^2, 215.8 cm^2, 203.37 cm^2, 188.55 cm^2, 182.65 cm^2 and 178,42 cm^2, respectively (Fig.32).

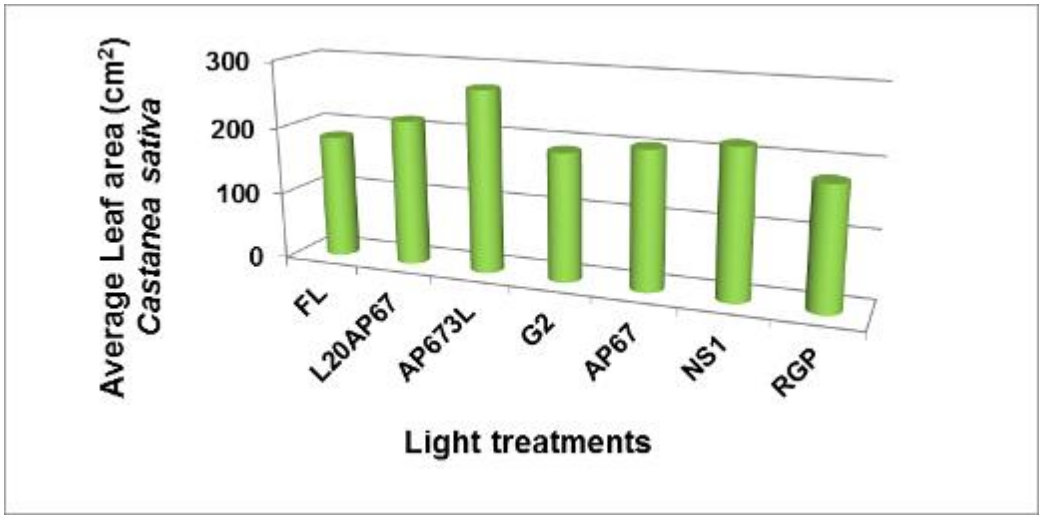


Figure 22. Average Leaf area (cm^2) of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

***Ocimum basilicum* L.**

According to the results significantly greater leaf area was found under the FL light showed an average value of 10.02 cm^2 compared to all LED treatments such as the NS1, G2, AP673L, AP67 ($p<.001$) and barely to the L20AP67 ($p<.025$) that showed average values of 5.70 cm^2, 6.56 cm^2, 6.91 cm^2 and 7.85 cm^2, respectively (Fig.33).

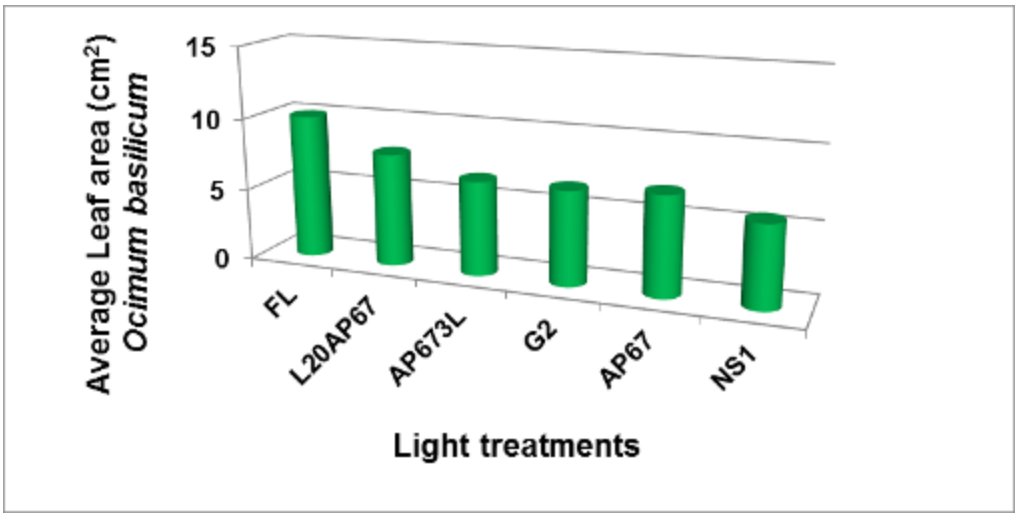


Figure 33. Average leaf area (cm^2) of Ocimum basilicum LL seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

Ocimum basilicum RR hybrid

L20AP67 and FL significantly promoted the leaf expansion of RR hybrid seedlings showed average values of 8.28 cm^2 and 8.25 cm^2 compared to the AP673L ($p<.003$) and AP67 ($p<.025$) LEDs that induced lower of 5.59 cm^2 and 6.05 cm^2; As for the NS1 and G2 LEDs had average values of 7.05 cm^2 and 6.70 cm^2 (Fig.34).

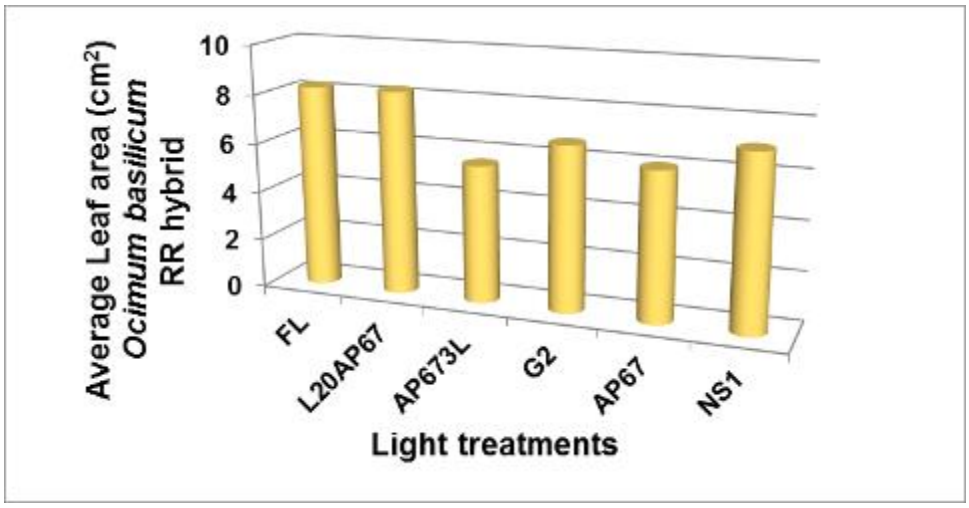


Figure 34. Average leaf area (cm^2) of Ocimum basilicum RR hybrid seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

***Cornus sanguinea* L.**

No significant differences found for the leaf area of *C. sanguinea* seedlings; however larger was found under the AP67 with an average value of 9.21 cm^2 followed by FL conventional light with 8.84 cm^2, L20AP67 with 8.06 cm^2, G2 with 7.63 cm^2, AP673L with 7.00 cm^2 and the lowest value was obtained from NS1 LED light with 6.77 cm^2 (Fig.35).

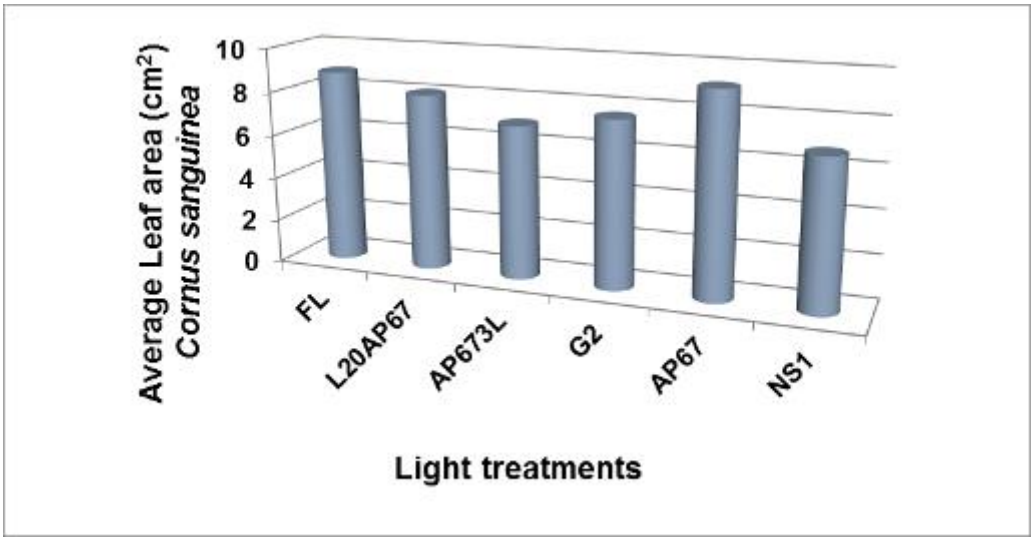


Figure 35. Average leaf area (cm^2) of Cornus sanguinea seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

***Prunus avium* L.**

Regarding the leaf area of *Prunus* seedlings, no significant differences were found. However, larger leaves were formed under L20AP67 light with 69.62 cm^2, followed by AP67 with 57.93 cm^2, G2 with 55.89 cm^2, AP673L with 51.47 cm^2, NS1 with 49.38 cm^2 and FL control light with 40.31 cm^2 (Fig.36).

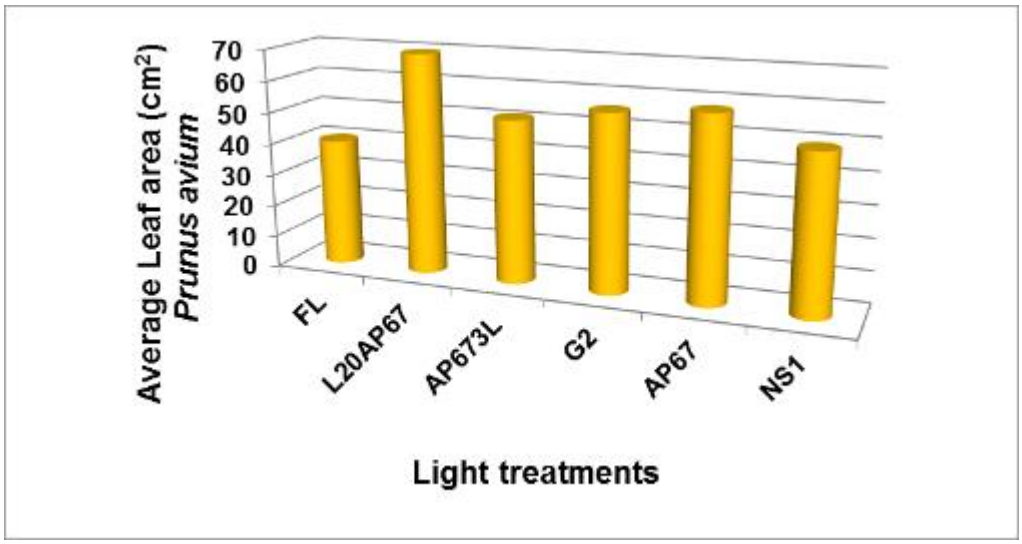


Figure 36. Average leaf area (cm^2) of Prunus avium seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6week experimental period.

***Punica granatum* L.**

Leaf area of pomegranate seedlings was favored under L20AP67 treatment that had an average value of 13.55 cm^2. This value was significantly higher compared to the rest of the lights such as the G2 that showed 5.54, FL 5.71 cm^2, AP67 5.77 cm^2, AP673L 6.80 cm^2 ($p < .001$) and NS1 7.99 cm^2 ($p = .001$) (Fig.37).

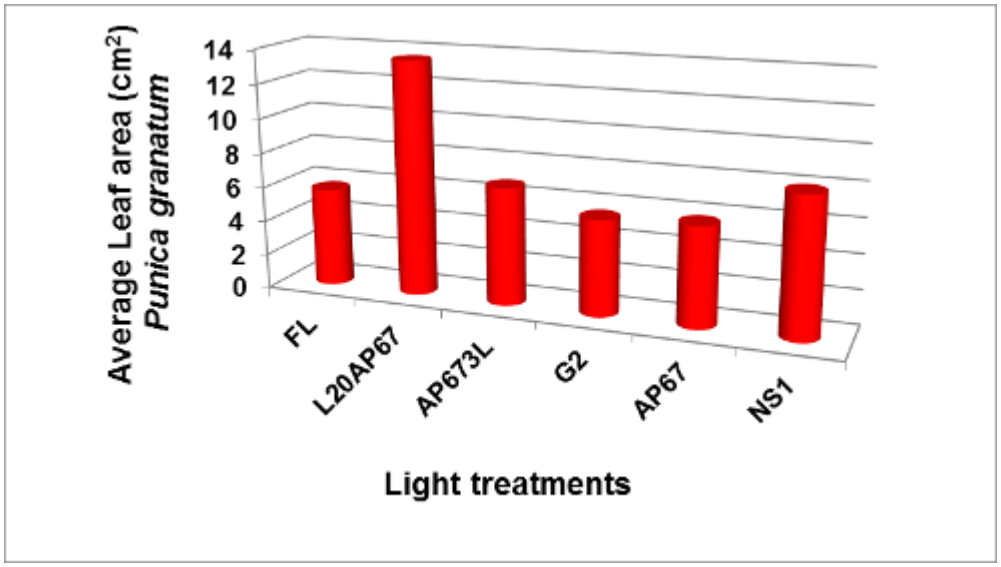


Figure 37. Average leaf area (cm^2) of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6week experimental period.

3.3.6. Chlorophyll content index (CCI) & Chlorophyll fluorescence

Quercus ithaburensis* ssp. *macrolepis

Different light irradiations did not induce significant changes either in the chlorophyll content or the chlorophyll fluorescence values. Positive effects of blue light have been found, activating cryptochromes system and matching chlorophyll and carotenoids absorption spectra on green vegetable morphology, growth and photosynthesis (Yanagi *et al*., 1996). In our case FL conventional light has 34.8 % in blue range, which is the highest compared to the rest of the light treatments, thus found to have the highest CCI=18.46; Following by the G2, L20AP67, NS1, AP67 and AP673L with CCI average values of 17.86, 17.02, 16.14, 15.87 and 13.62, respectively (Fig.38). However G2 light that has the lowest percentage in blue range (7.7%) compared to the rest of lights, showed also high CCI value. This might cause of the highest percentage in red region of 64.4%.

In plants of Triticum aestivum, formation of green pigments in plants was inhibited by red light, but this was reversed when plants were exposed to blue light or a mixture of blue and red light. Thus exposure to blue and red light caused plants to accumulate normal levels of carotenoids and chlorophyll pigments, as compared with plants exposed only to red or far-red light (Gupta & Tripathy, 2010).

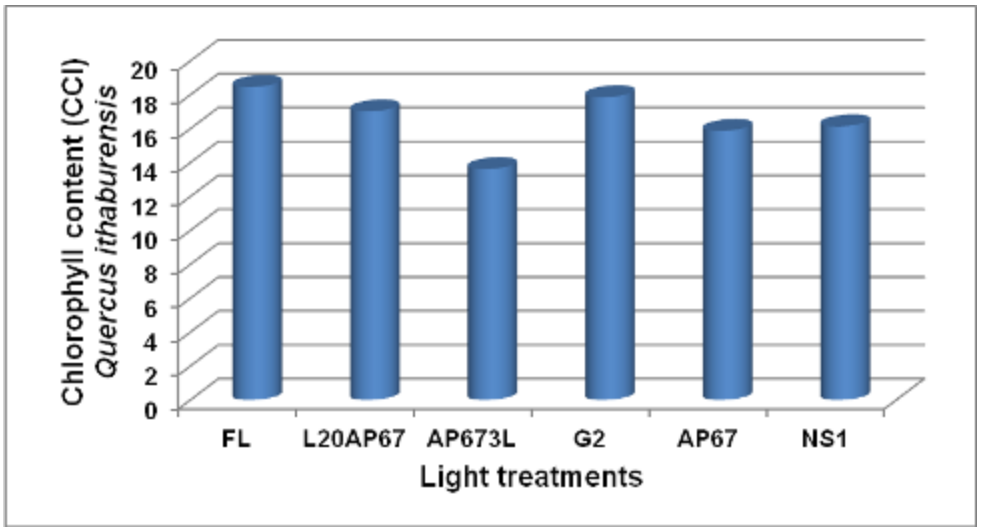


Figure 38. Chlorophyll concentration per unit leaf area measured as CCI of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

F_v/F_m is a quantitative measure of photochemical efficiency (Kitajima & Butler 1975) or optimal quantum yield (Schreiber & Bilger 1993) of Photosystem II (PSII). In particular, it is a good indicator of photoinhibition (a lowering of photosynthetic activity by excess light) (Bolhår-Nordenkampf & Lechner 1988a, Öquist & Wass 1988). Photo-inhibition is interpreted to be indicative of photodamage and/or protection in plant tissues (Schreiber and Bilger 1993). Björkman and Demmig (1987) found that for a wide variety of C3 species, including tree species, an average value of 0.832 for F_v/F_m was typical of well-functioning photosynthetic apparatus. In our case the optimal quantum yield was 0.82, 0.80, 0.79, 0.78 and 0.76 for the G2, FL, L20AP67, AP673L, AP67 and NS1 lights, respectively (Fig.39). Thus it could be assumed that there was not photochemical damage of PSII system.

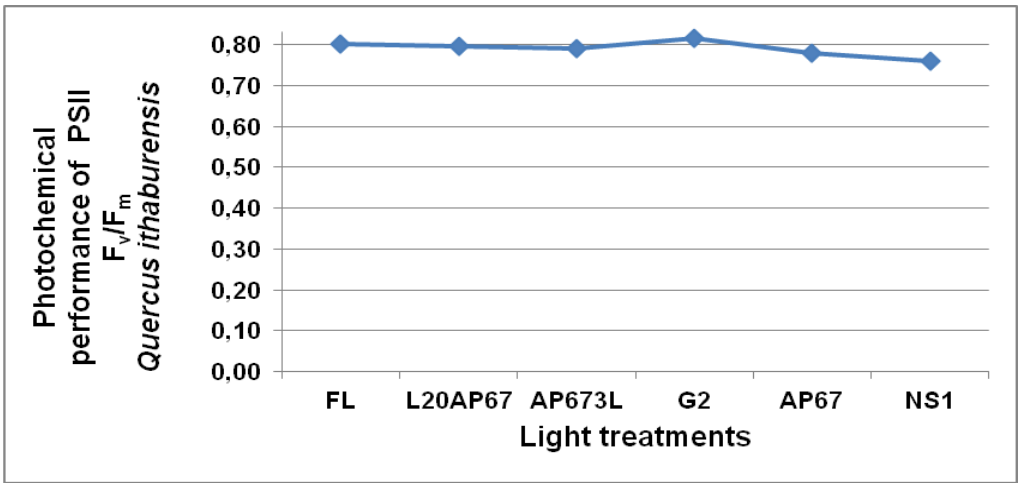


Figure 39. Photochemical quantum yield Fv/Fmax of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

***Castanea sativa* Mill.**

Significant difference found for the CCI between two LED lights, the L20AP67 that showed the highest among lights with 13.72 and NS1 that had the lowest with 6 ($p<.010$) (Fig.40) the rest of the lights had the following CCI values of 10.7, 9.42, 8.47,8.2 and 7.35 for the FL, G2, AP673L, AP67 and RGP, respectively. *Castanea* seedlings showed no significant differences for the effective quantum yield between light treatments; however the values were among 0.66-.0.80 such as the AP67 with F_v/F_m 0.66, G2, AP673L with 0.67 and RGP with 0.69. The highest values of F_v/F_m were found for the L20AP67 and the FL lights with 0.80 and 0.74, respectively (Fig.41).

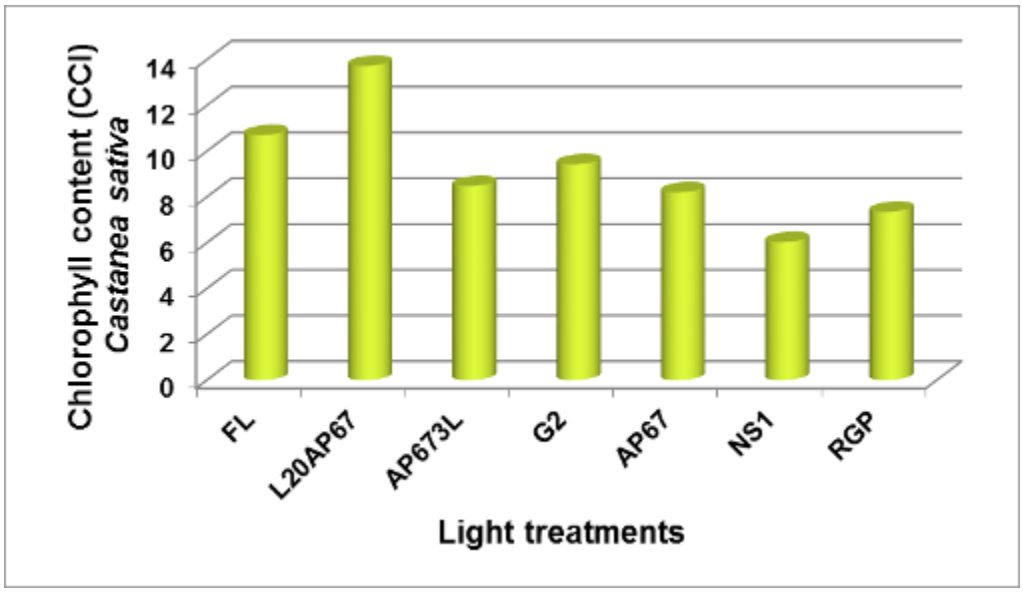


Figure 40. Chlorophyll concentration per unit leaf area measured as CCI of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

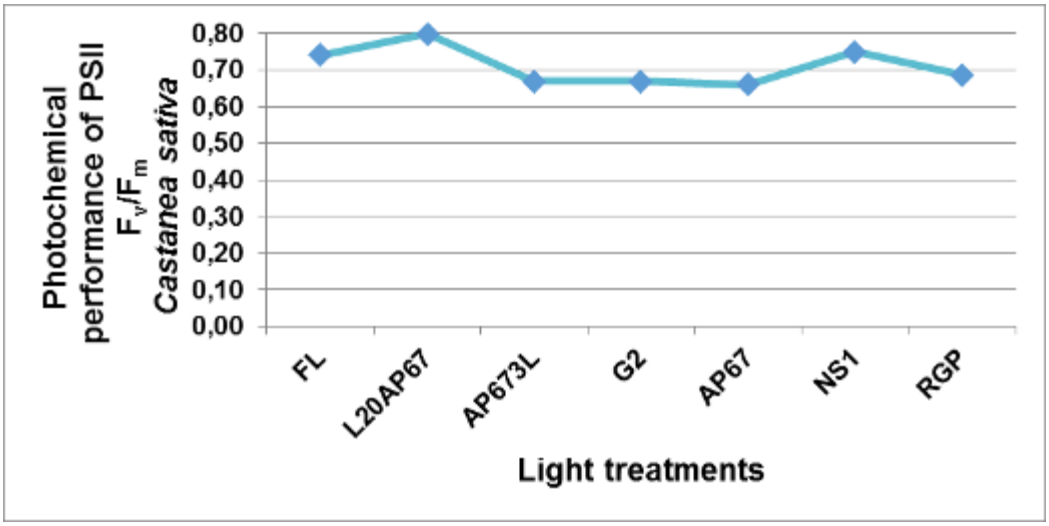


Figure 41. Photochemical quantum yield Fv/Fmax of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

3.3.7. Phytochemicals

3.3.7.1. Chlorophyll & Carotenoid content

***Myrtus communis* L.**

Lighting system is a very important element for chlorophyll synthesis. Light sources with different wavelengths affect different photoreceptors of plants to control pigment synthesis (Stuefer and Huber, 1998). According to our analysis no significant differences found for the chla content under different light illuminations of *Myrtus* seedlings (Fig.42). However higher chla content of 1125 μg/g and 968 μg/g was found under the RGP and FL lights, respectively. Among LEDs the higher chla content, was induced by the AP673L that showed average value of 691 μg/g following by the NS1, G2, L20AP67 and AP67 with average values of 649 μg/g, 626 μg/g, 518 μg/g and 470 μg/g, respectively (Fig.42).That was not the case for chlb content synthesis of *Myrtus,* significant increase was induced under the FL with 699 μg/g compared to AP67 (p<. 002) and NS1 (p<. 003) LEDs with values of 293 μg/g and 299 μg/g. Among the rest of lights, higher chlb synthesis of 468 μg/g was obtained by the AP673L, following by RGP, G2 and L20AP67 that showed average values of 432 μg/g, 274 μg/g and 244 μg/g, respectively (Fig.42).

Carotenoid is the auxiliary pigment of antenna Chls in chloroplasts and can help Chl to receive light energy (Zheng *et al.,* 2008). In our case different light qualities used showed no significant differences for the carotenoid content. However higher synthesis was induced under the RGP and FL lights with average values of 404 μg/g and 361 μg/g, respectively (Fig.43). Among LED treatments, AP673L showed the higher content of 293 μg/g following by the G2, L20AP67, NS1 and AP67 that had average values of with 274 μg/g, 244 μg/g, 241 μg/g and 207 μg/g (Fig.43).

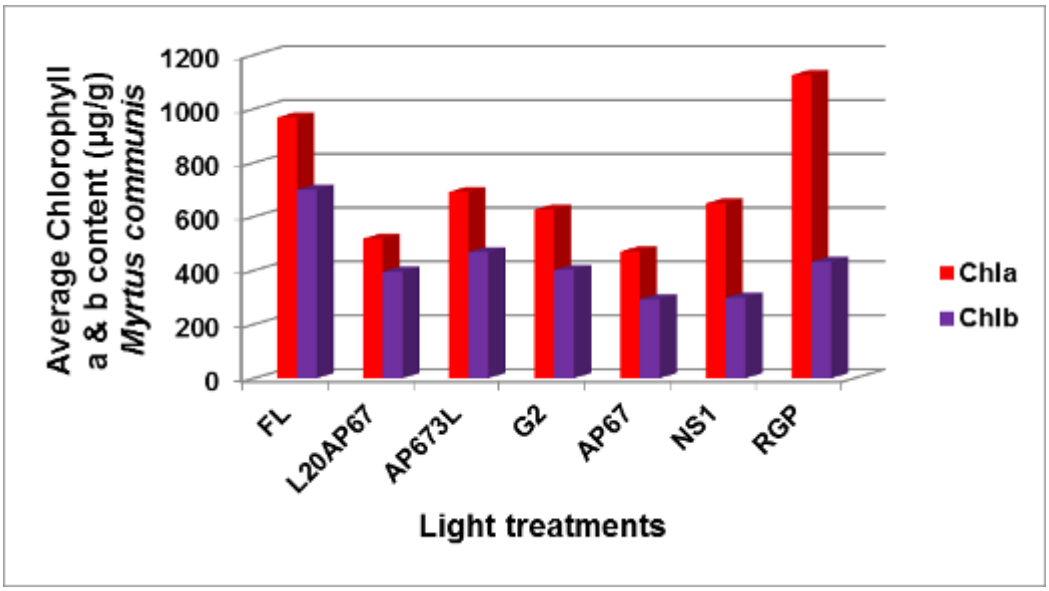


Figure 42. Average Chlorophyll a & b content (µg/g) of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size at the end of the 3week experimental period.

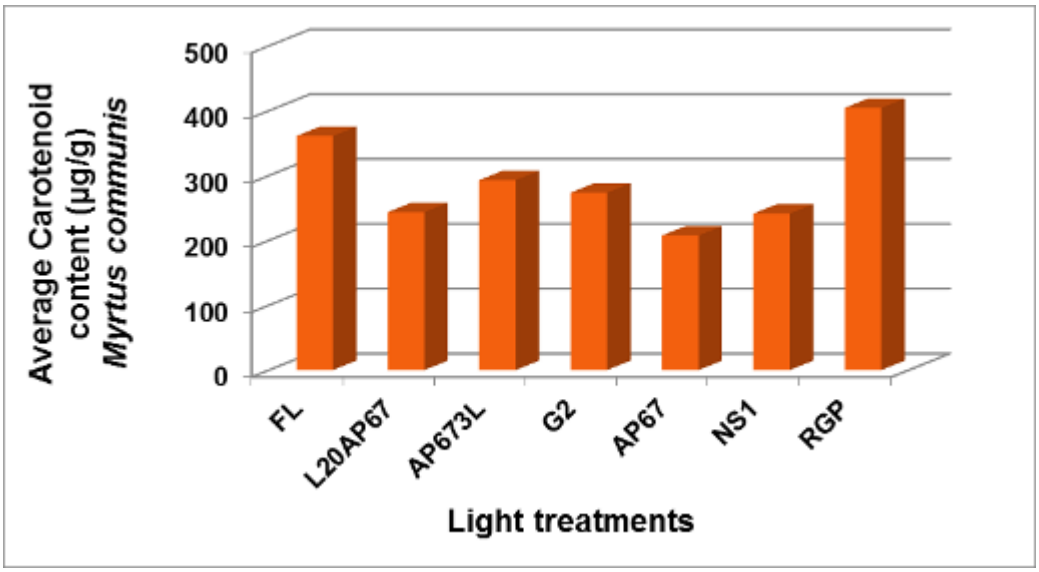


Figure 43. Average Carotenoid content (µg/g) of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size at the end of the 3week experimental period.

***Punica granatum* L.**

FL conventional light showed significantly greater chla & chlb content of 1165 µg/g and 429 µg/g, compared to the rest of the lights such as NS1 and AP673L ($p < .001$) that showed the lowest contents of 367 µg/g &194 µg/g and 433 µg/g &181 µg/g, following by the AP67, G2 and L20AP67 ($p < .001$) that showed 501 µg/g & 263 µg/g, 510 µg/g & 233 µg/g and 654 µg/g & 277 µg/g, respectively (Fig.44). Carotenoid content was similar to the chla and chlb content for pomegranate seedlings. Specifically, FL control light promoted more the induction of carotenoids than the rest of the treatments showed significantly greater average value of 263 µg/g, than NS1, AP673L, G2 ($p < .001$) and AP67 ($p < .004$) that showed average values of 142 µg/g, 144 µg/g and 170 µg/g, respectively. As for the L20AP67 showed an average carotenoid content of 193 µg/g (Fig.45).

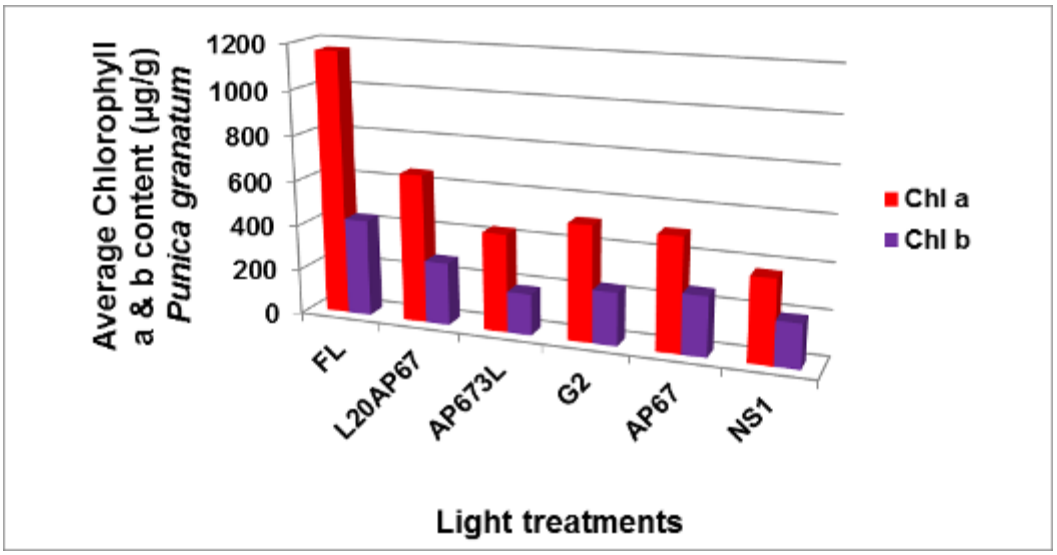


Figure 44. Average Chlorophyll a & b content (µg/g) of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6week experimental period.

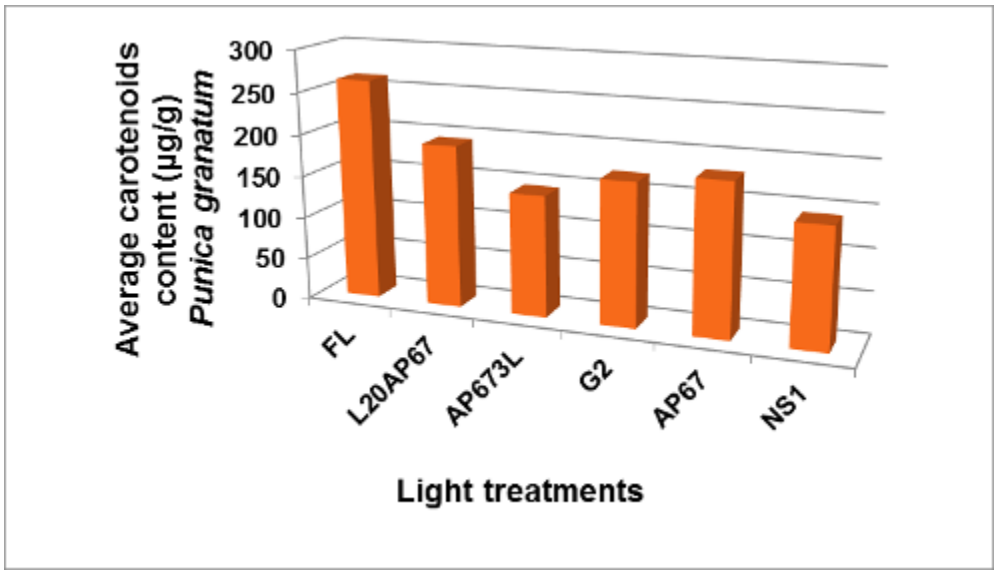


Figure 45. Average Carotenoid content (µg/g) of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size at the end of the week experimental period.

3.3.8. Total phenol content

***Myrtus communis* L.**

In plants antioxidant defense systems include various antioxidants such as phenolic compounds, anthocyanin and flavonoids production that play important role in protection from photooxidative damage (Samuoliene *et al*., 2009; Wang *et al.,* 2010).

LED treatments significantly increased the total phenol content of *M. communis* seedlings. Specifically NS1 LED that is high in blue-green spectrum compared to the rest of lights (20.2% in blue and 38.9% blue-green) induced significantly increase of 25.57 mg/g in total phenol content compared to FL, G2, RGP, AP67 and AP673L ($p < .001$) that showed average values of 13.40 mg/g, 13.38 mg/g, 13.01 mg/g, 11.58 mg/g and 8.17 mg/g, respectively (Fig.46).

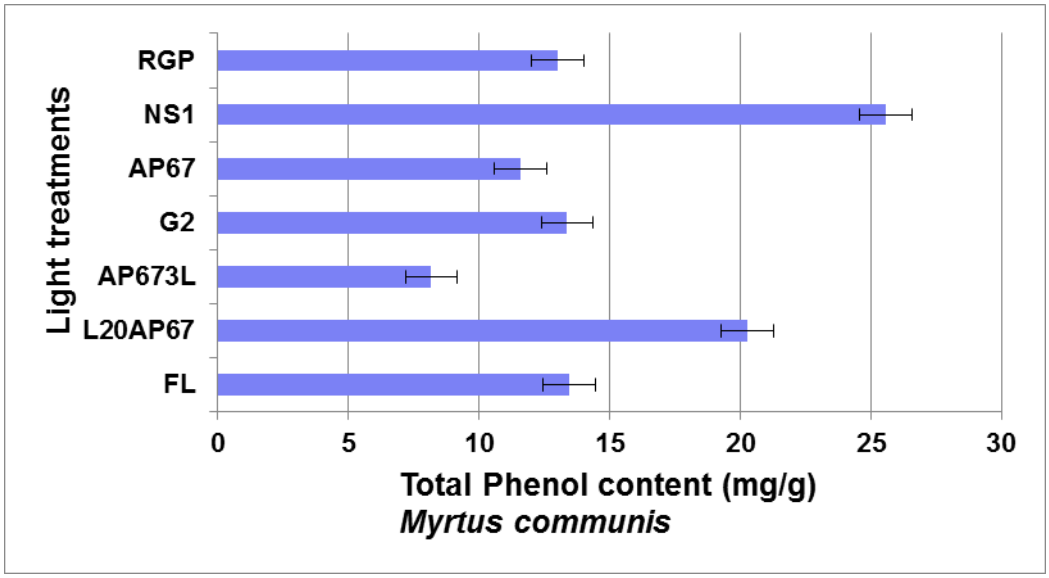


Figure 46. Average total phenol content (mg/g) of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size at the end of the 3week experimental period.

***Ocimum basilicum* L.**

Seedlings grown under the NS1 (p< .001) LED light resulted in significantly higher total phenol content that showed average value of 18.74 mg/g compared to all the other treatments. Specifically FL light induced the lowest content of 4.77 mg/g, following by the L20AP67, G2, AP67 and AP673L that showed average values of 6.15 mg/g, 9.12 mg/g,12.47 mg/g and 13.76 mg/g, respectively (Fig.47). Furthermore, LEDs such as the AP673L and AP67 showed significantly higher total phenol content than the FL and L20AP67 (p< .001) lights. G2 LED light showed significantly higher phenol content than the FL (p< .003) but significantly lower than the AP673L (p< .001) (Fig.47).

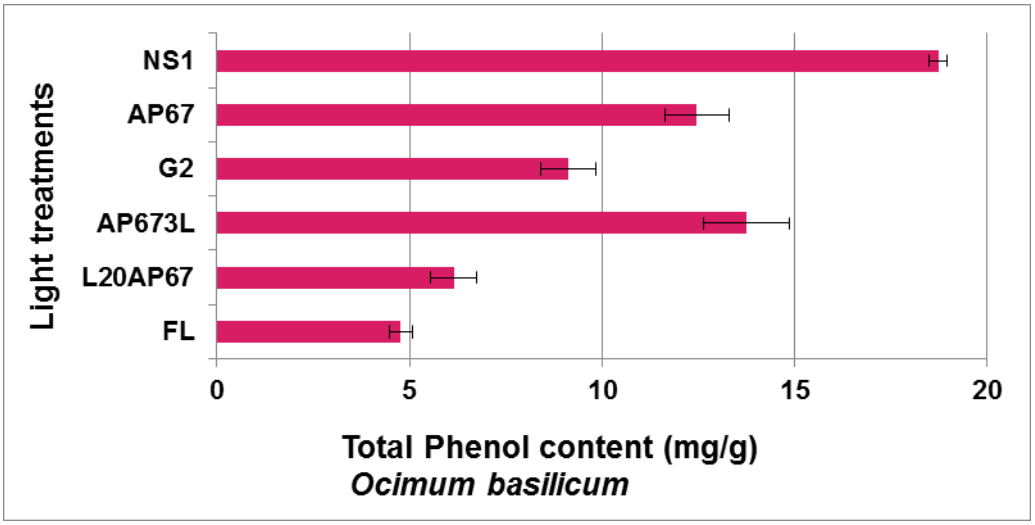


Figure 47. Average total phenol content (mg/g) of Ocimum basilicum LL seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

Ocimum basilicum **RR hybrid**

FL light (p< .001) quality induced the lowest total phenol content of 4.62 mg/g for *basilicum* RR hybrid seedlings compared to all LED treatments such as the NS1, AP67, AP673L, G2 and L20AP67 that showed average values of 17.02 mg/g, 14.61 mg/g, 14.39 mg/g, 13.19 mg/g and 9.48 mg/g, respectively (Fig.48). However among LEDs, L20AP67 obtained the lowest total phenol content than AP673L, AP67 and NS1 (p < .001) and G2 (p< .006), while NS1 showed higher total phenol content than the G2 light (p< .005) (Fig.48).

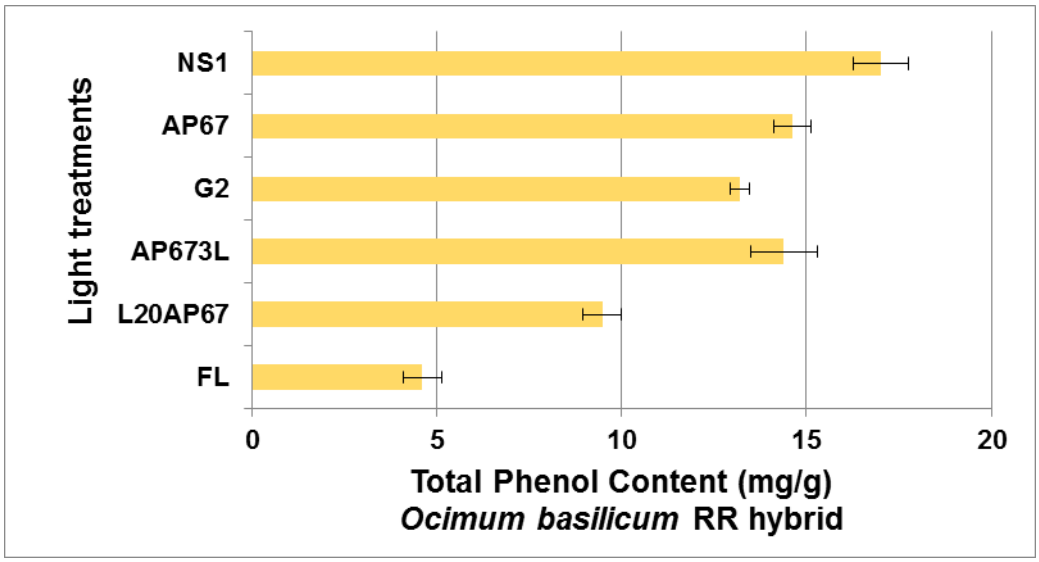


Figure 48. Average total phenol content (mg/g) of Ocimum basilicum RR hybrid seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the of the 4week experimental period.

***Punica granatum* L.**

Significantly more phenols were induced for the pomegranate seedlings grown under the NS1 LED that showed an average value of 43.45 mg/g compared to the FL ($p < .001$) control light and the L20AP67 ($p < .007$) LED that had 28.62 mg/g and 30.72 mg/g, respectively. Further G2 LED showed significantly higher total phenol content of 40.66 mg/g ($p < .013$) than the FL light, while AP67 and AP673L LEDs showed average values of 35.25 mg/g and 31.96 mg/g (Fig.49).

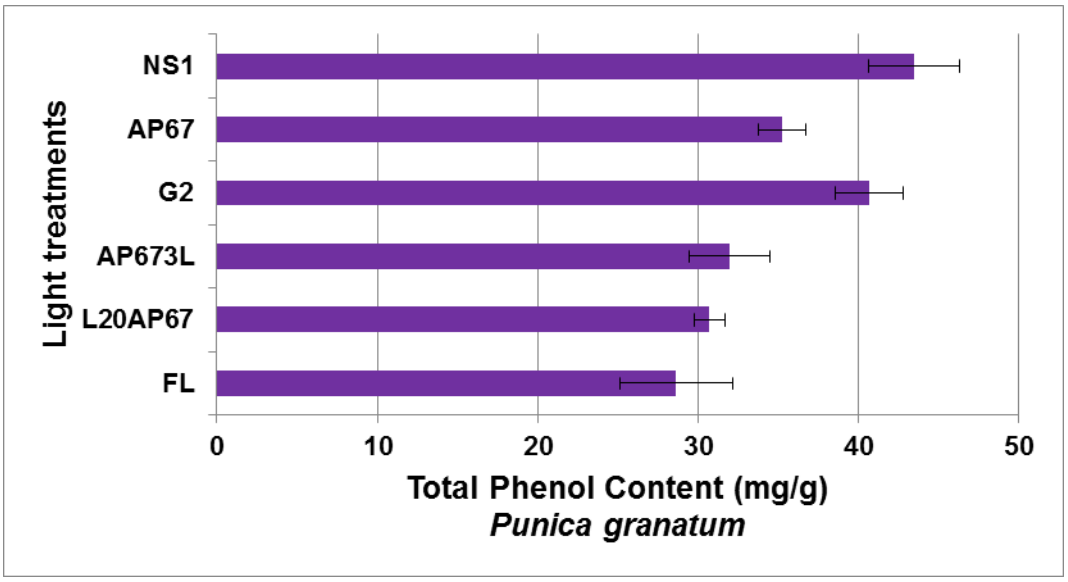


Figure 49. Average total phenol content (mg/g) of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size at the end of the experimental period at the of the 6week experimental period.

3.3.8.1. Anthocyanin content

***Myrtus communis* L.**

As for the anthocyanin content of *M. communis* seedlings, LEDs such as L20AP67, AP67, G2 and NS1 with 27.13 µg/g, 26.64 µg/g, 23.85 µg/g and 21.63 µg/g induced significantly increase compared to RGP ($p<.001$) with an average of only 3.15 µg/g. FL and AP673L showed similar values of 16.03 µg/g and 14.47 µg/g (Fig.50).

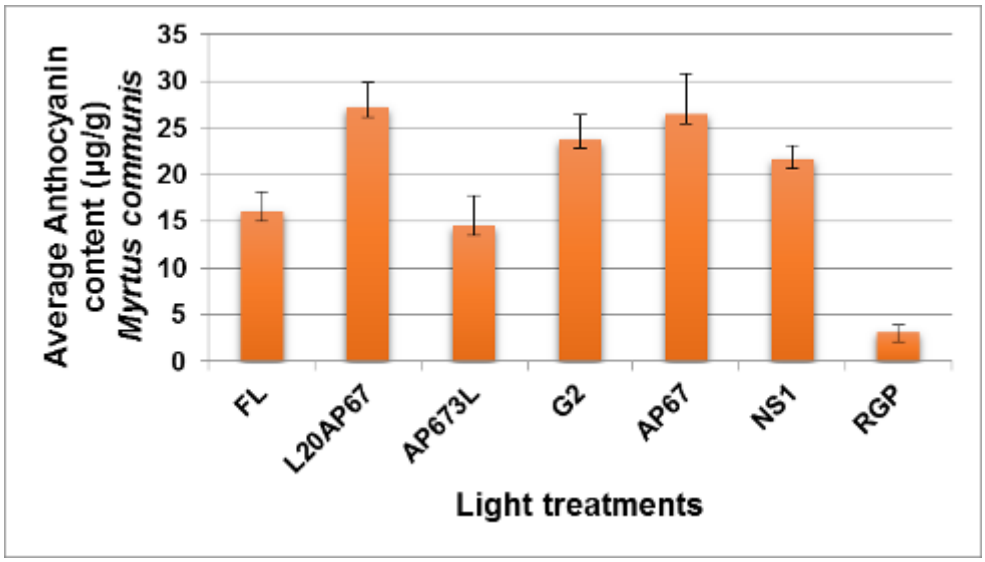


Figure 50. Average Anthocyanin content (µg/g) of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size at the end of the 3week experimental period.

***Punica granatum* L.**

Significantly higher anthocyanin content was found for the G2, AP67 and NS1 LED treatments that showed average values of 81.31 µg/g, 63.74 µg/g and 59.77 µg/g, respectively, compared to the rest of lights such as the FL ($p< .001$) conventional light that had the least beneficial effect showing an average value of only 19.12 µg/g

following by the L20AP67(p< .007) (p< .001) and AP673L (p< .001) (p< .012) that showed average values of 33.02 µg/g and 30.68 µg/g (Fig.51).

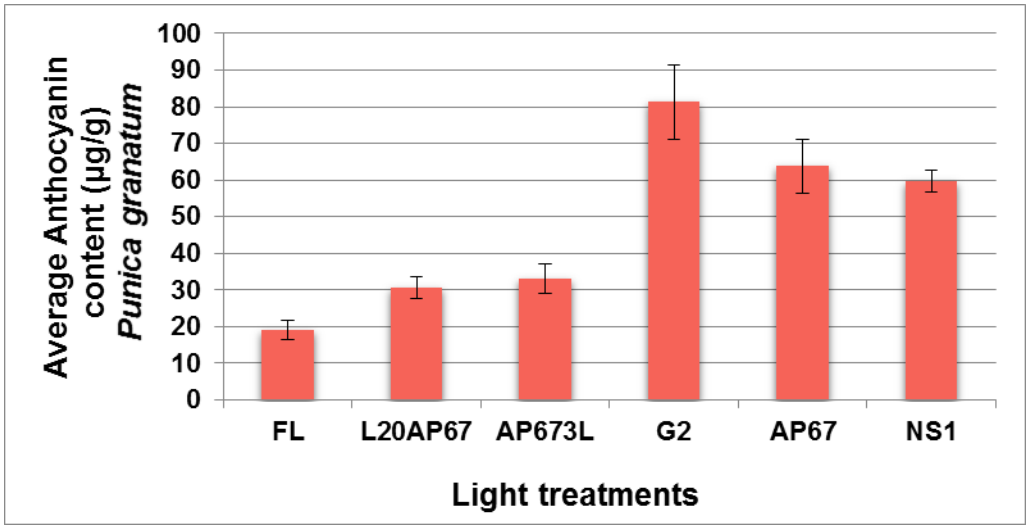


Figure 51. Average Anthocyanin content (µg/g) of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6week experimental period.

3.3.8.2. Flavonoids content

***Punica granatum* L.**

Pomegranate seedlings grown under G2 and AP67 illuminations contained significantly more flavonoids in their tissues of 261.87µg/g and 190.92µg/g respectively than the rest of the treatments. FL (p< .001) control light induced the lowest content of only 20.09 µg/g, following by the L20AP67, NS1 and AP673L (p< .001) with an average content of 51.87 µg/g, 62.32 µg/g and 87.86 µg/g, respectively (Fig.52).

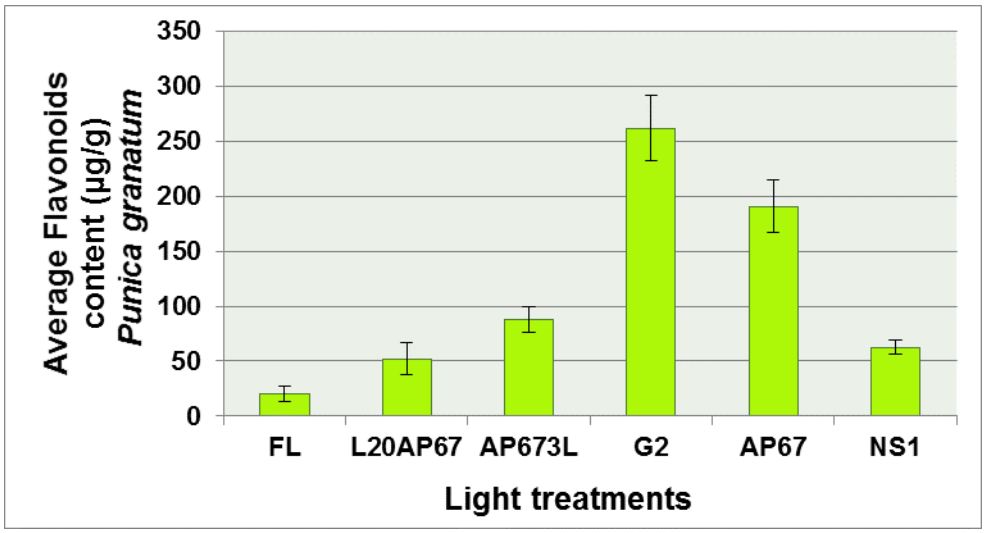


Figure 52. Average Flavonoids content (µg/g) of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6week experimental period.

3.3.9. Morphological parameters

3.3.9.1. Shoot height (SH) & Root length (RL)

***Pinus sylvestris* L. (provenances Greece-Sweden)**

Between the two provenances of *P. sylvestris* no significant differences were found both for the morphological parameters tested.

Examining the response of the Greece provenance, seedlings showed similar shoot development under the different light treatments; considering only the average values the highest was for the AP67 inducing 4.14 cm following by the FL of 4.12 cm, G2 of 4.09 cm, NS1 of 4 cm, L20AP67 of 3.9 cm and AP673L of 3.7 cm, respectively (Fig.53). In contrast significant effect on the RL was found under LEDs such as the G2 that showed the longest roots with 13.56 cm, NS1 with 12.10 cm, AP673L with 12 cm and AP67 with 10 cm compared to the shortest induced by the FL and L20AP67 LED ($p<.001$) with 4.45 cm and 5.5 cm (Fig.53).

Seedlings of Scots pine provenance Sweden grown under the L20AP67 LED induced the highest shoot development of 4.5 cm and significant difference found with those grown under the NS1 LED ($p<.003$) that showed SH of 3.53 cm. As for the rest of the lights similar shoot development was obtained of 4.25 cm, 4.13 cm, 4 cm and 3.9 cm under AP67, FL, G2 and AP673L, respectively (Fig.54). FL and L20AP67 LED induced less beneficial effect on the root development with 4.54 cm and 5.4 cm

compared to AP673L, NS1 (p<. 002) and AP67 (p<. 004) that showed RL of 11.7 cm, 10.77 cm and 10.23 cm, while G2 showed RL of 7 cm (Fig.54).

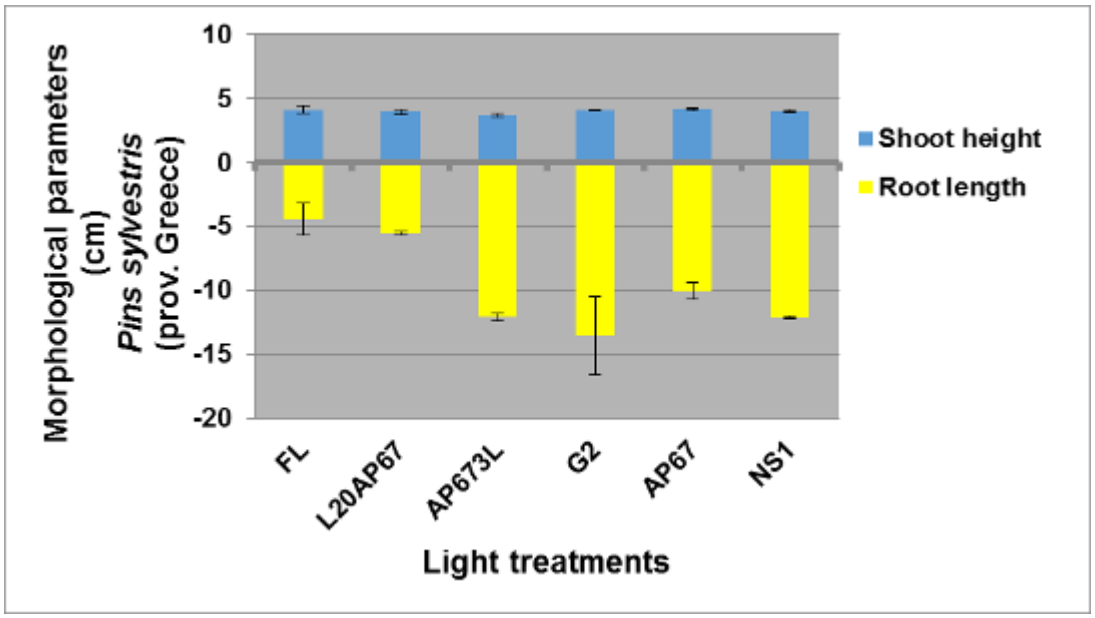


Figure 53. Morphological parameters (cm) of Pinus sylvestris provenance Greece seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

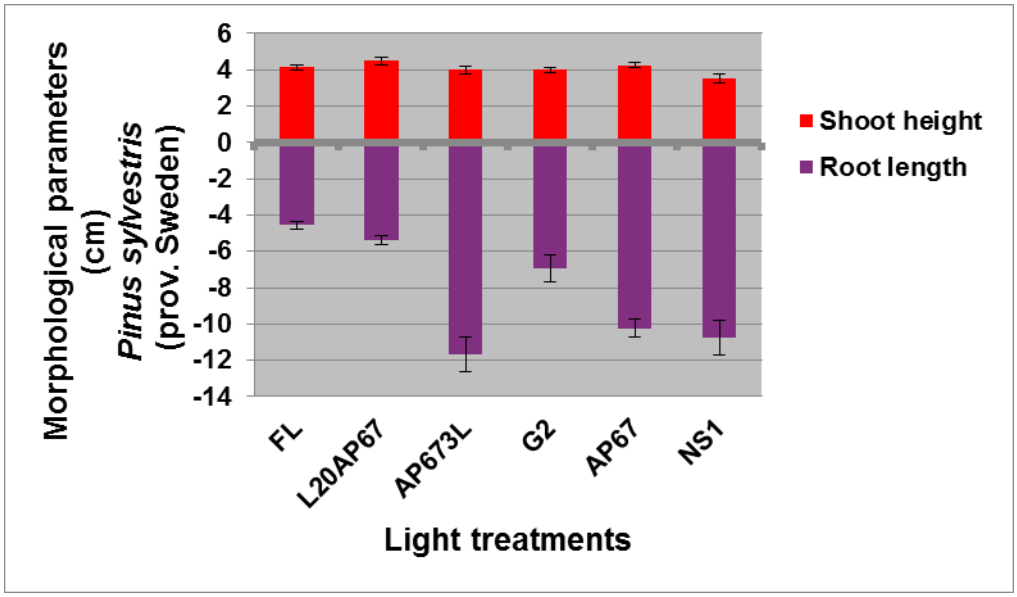


Figure 54. Morphological parameters (cm) of Pinus sylvestris provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

***Picea abies* Karst.**

There were significant differences both for the SH (p<. 004) and the RL (p<. 001) between the different light qualities for the *Picea* seedlings. More specifically L20AP67 LED induced significantly taller seedlings with SH of 4.23 cm compared to the seedlings grown under the NS1 with SH of 3.31 cm. The rest of lights induced similar effect on the SH, thus FL, AP673L, G2 and AP67 obtained average values of 3.82 cm, 3.47 cm, 3.44 cm and 3.43 cm, respectively (Fig.55). Further LEDs promoted the root development especially the NS1 with 6.75 cm and significant differences found with the FL, AP67 and G2 lights (p<. 001) that shown average values of 2.83 cm, 4.20 cm and 4.53 cm. FL light that induced the shortest roots also differed significantly with LEDs such as AP673L (p<. 001) and L20AP67 (p<. 003) that shown average values of 5.7 cm and 4.9 cm (Fig.55).

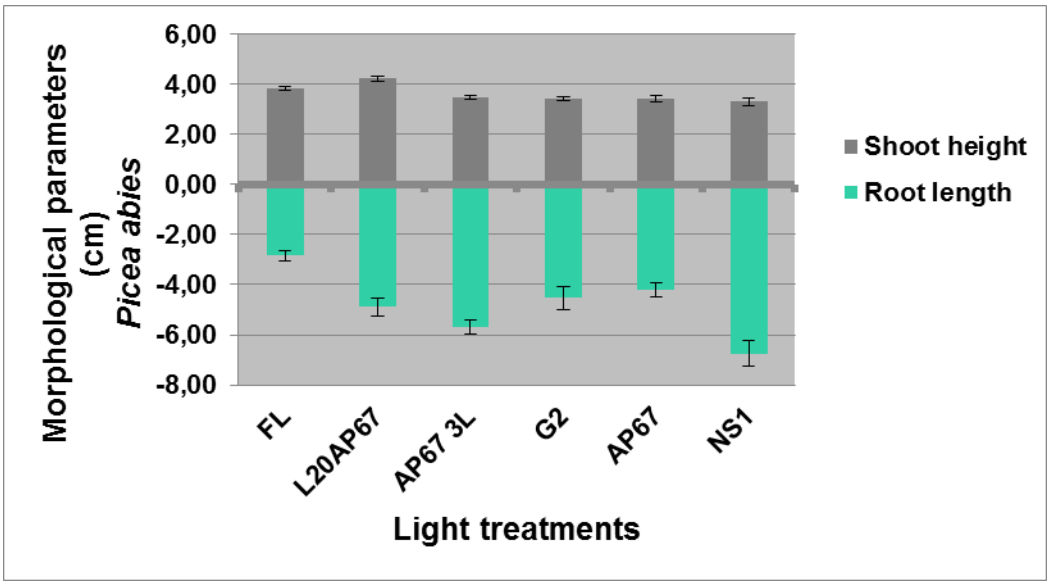


Figure 55. Morphological parameters (cm) of Picea abies provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

***Pinus nigra* Arn.**

Different light treatments induced significantly differences for the SH and RL (p<. 001) of *Pinus nigra* seedlings. FL and L20AP67 lights induced taller seedlings with SH average values of 5.54 cm and 5.40 cm compared to the more compact seedlings grown under LEDs such as the NS1, AP673L and AP67 that had average values of 3.9 cm and 4.06 cm; while G2 LED shown average value of 4.46 cm (Fig.56). FL did not benefit the root development had an average value of 5.54 cm

and significant differences found with LED lights such as the AP673L, AP67 (p<. 001) and NS1 (p<. 003) that had average values of 9.8 cm, 8.9 cm and 8.46 cm. G2 and L20AP67 LEDs also induced longer roots than the FL light with average values of 7.5 cm and 5.83 cm, respectively (Fig.56).

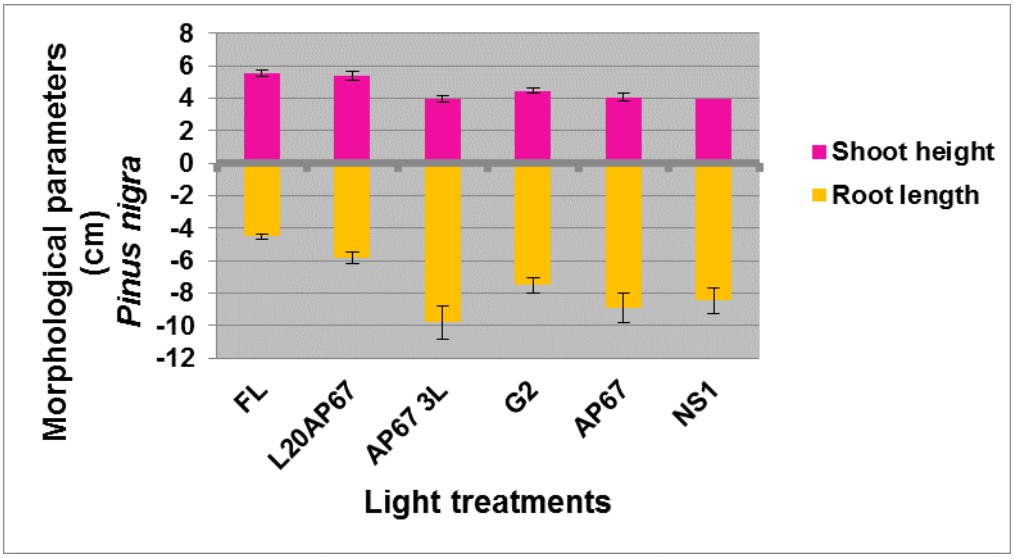


Figure 56. Morphological parameters (cm) of Pinus nigra seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

Quercus ithaburensis ssp. *macrolepis*

No significant differences found both for the SH or RL of *Q. ithaburensis* seedlings, irrespective the light spectrum. However taller seedlings were found under the L20AP67 and FL lights with 25.46 cm and 19.33 cm, respectively; following by the G2, AP67, NS1 and AP673L with 18.13 cm, 16.83 cm, 15.56 cm and 14.43 cm (Fig.57). AP673L illumination showed the shortest seedlings but had beneficial effect on root development with 46.37 cm RL, which is the highest, compared to the rest of the lights. For the rest of the lights such as G2, NS1, FL, AP67 and L20AP67, average values for the RL were 41.40 cm, 39.4 cm, 36.33 cm, 35.86 cm and 30.33, respectively (Fig. 57).

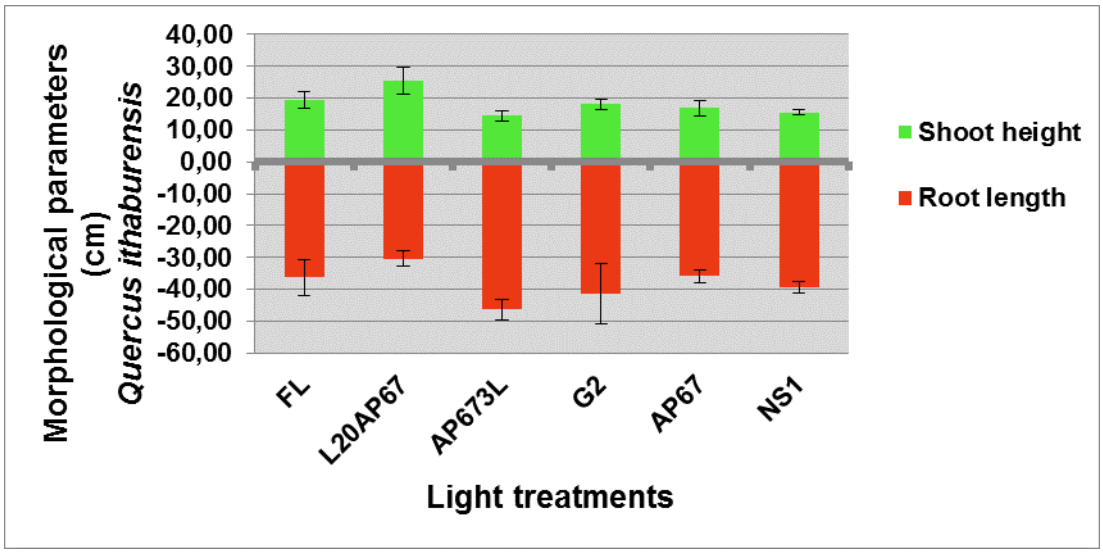


Figure 57. Morphological parameters (cm) of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

***Castanea sativa* Mill.**

SH of *Castanea* seedlings showed no significant differences between the different irradiation environments; however L20AP67 exhibited higher SH of 25 cm. The rest of lights showed similar shoot development, specifically AP673L with an average value of 19.78 cm, NS1 with 19.53 cm, RGP with 19.10 cm, FL with 18.94 cm, AP67 with 18.64 cm and G2 with 18.53 cm (Fig.58). Moreover L20AP67 exhibited significantly longer roots of 27.64 cm than the conventional radiation sources such as the FL and RGP with 14.87 cm and 13.79 cm (p<. 008), respectively. Similar effect on the root development was shown under the rest of LEDs, AP673L with 25.13 cm, NS1 with 24.62 cm, AP67with 24.21 and G2 21.28 cm (Fig.58).

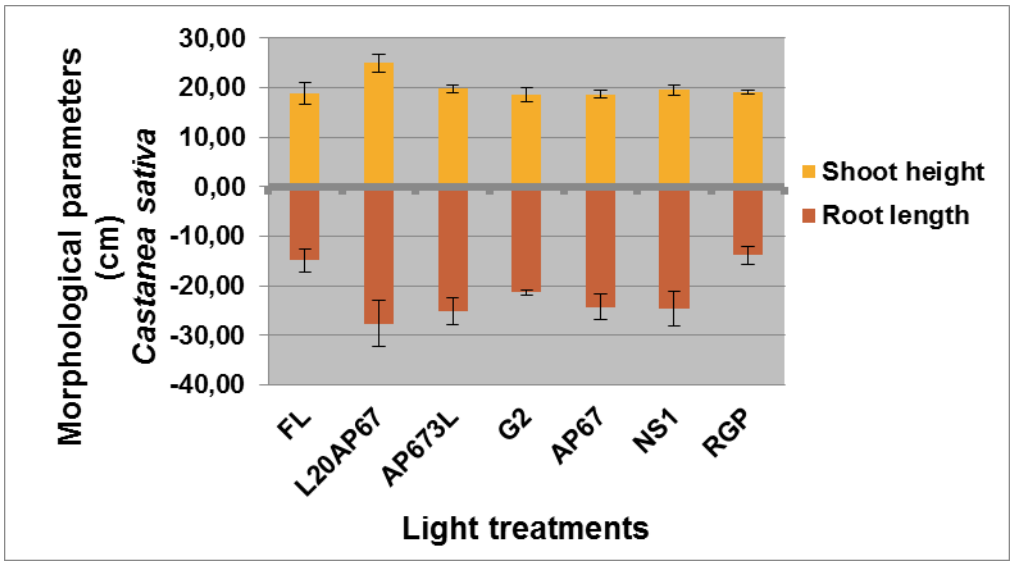


Figure 58. Morphological parameters (cm) of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

***Myrtus communis* L.**

Significantly taller seedlings found for those grown under the AP673L and FL lights that formed shoots with an average height of 37.63 mm and 33.40 mm, respectively, compared to the shorter ones formed under the illuminations of RGP, G2 and AP67 (p<. 001) with average SH of 25.10 mm, 25.41 mm and 27.81 mm. L20AP67 light shown an average SH of 28.76 mm (Fig.59). AP673L and NS1 light qualities significantly increased the RL of *Myrtus* seedlings with average values of 95.40 mm and 82.73 mm, compared to G2 that induced the shortest with 45.29 mm, following by FL with 48.16 mm, L20AP67 with 49.94 mm and RGP (p<. 001) with 55.93 mm, respectively. AP67 LED had average RL of 73.52 mm (Fig.59).

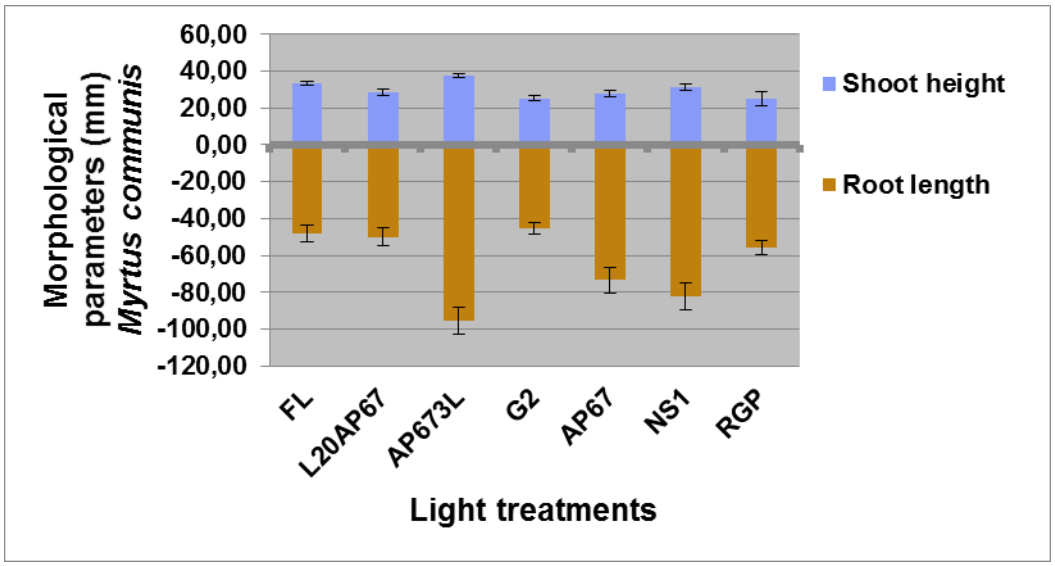


Figure 59. Morphological parameters (mm) of Myrtus communis under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size at the end of the 3week experimental period.

***Ocimum basilicum* L.**

According to the results for the morphological parameters of LL basil's seedlings there were significant differences. Significantly shorter seedlings were obtained under both AP673L and NS1 seedlings with average values for the SH of 2.34 cm and 2.43 cm, compared to the taller obtained under the L20AP67 (p<. 001) and G2 (p<. 004) (p<. 011) showed 3.78 cm and 3.52 cm, respectively (Fig.60). For the rest of lights similar values were found for the SH such as 3.42 cm and 3.22 cm by the AP67 LED and FL control light. Meanwhile AP673L showed more compact seedlings but induced greater root development of 5.7 cm only compared to the NS1 (p<. 014) illumination that showed less beneficial effect with an average value of 3.73 cm (Fig.60). The rest of the light treatments such as the L20AP67, G2, AP67 and FL showed average values for the RL in descending order of 5.08 cm, 4.5 cm and 4.03 cm, respectively.

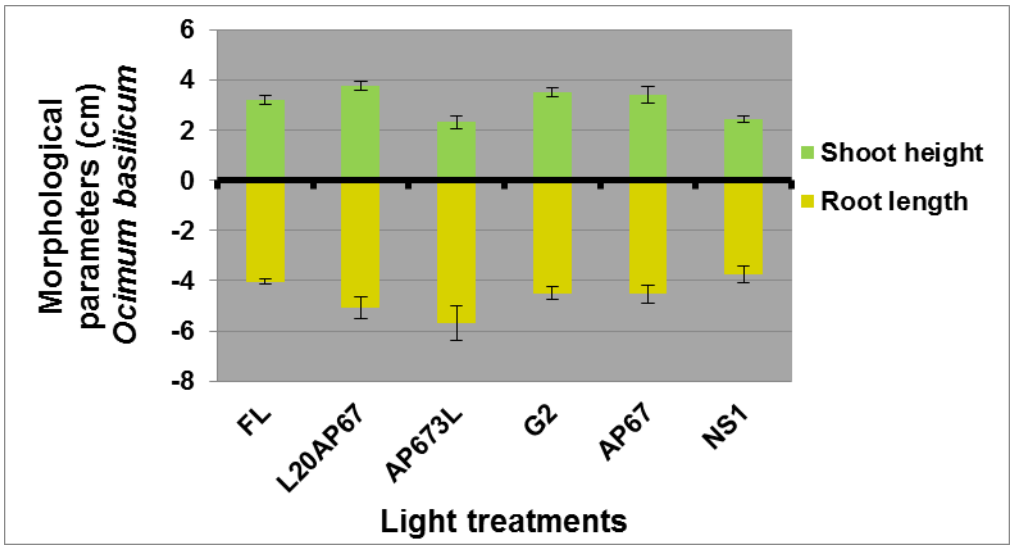


Figure 60. Morphological parameters (cm) of Ocimum basilicum LL seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 VW mini-plug size at the end of the 4week experimental period.

Ocimum basilicum RR hybrid

Taller seedlings were formed under the G2 LED light with an average value of 5.46 cm compared to the AP673L ($p < .001$) that induced 3.50 cm, L20AP67 ($p < .002$) with 3.88 cm, and NS1 ($p < .003$) with 3.93 cm, while the AP67 light also induced higher shoots of 5.08 cm than the AP673L ($p < .002$) (Fig.61). FL light showed an average SH of 4.09 cm. Cultivation of basil RR hybrid seedlings showed no significant effect on the root length at any of the different light treatments. However, NS1 light treatment induced the formation of longer roots with an average of 6.53 cm followed by the AP673L, L20AP67, AP67, FL and G2 with 6.23 cm 5.81 cm, 4.94 cm 4.59 cm and 4.42 cm, respectively (Fig.61).

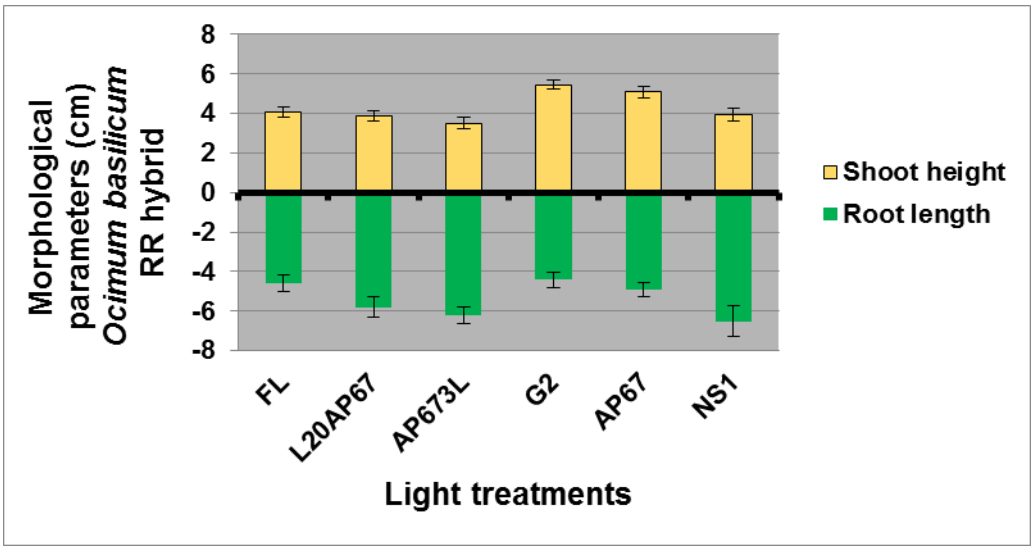


Figure 61. Morphological parameters (cm) of Ocimum basilicum RR hybrid seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 VW mini-plug size at the end of the 4week experimental period.

***Cornus sanguinea* L.**

Significant differences were found among the light treatments for the SH of *C. sanguinea* seedlings (p< .001). FL (p<. 001) conventional light and L20AP67 (p<. 004) (p<. 006) LED induced significantly taller seedlings with an average of 6.60 cm and 6.05 cm compared to NS1 with 4.53 cm and AP67 with 4.57 cm (Fig.62). Finally, AP673L and G2 LEDs had similar average values for the SH of 5.32 cm and 5.22 cm, respectively. *Cornus* seedlings showed no significant differences for the RL. However longer roots were found under the AP67 light with an average of 6.48 cm and the shortest under the L20AP67 with 5.00 cm. The rest of the light treatments such as the NS1, G2, AP673L and FL showed average values for the RL of 6.09 cm, 5.20 cm, 5.17 cm and 5.13 cm (Fig.62).

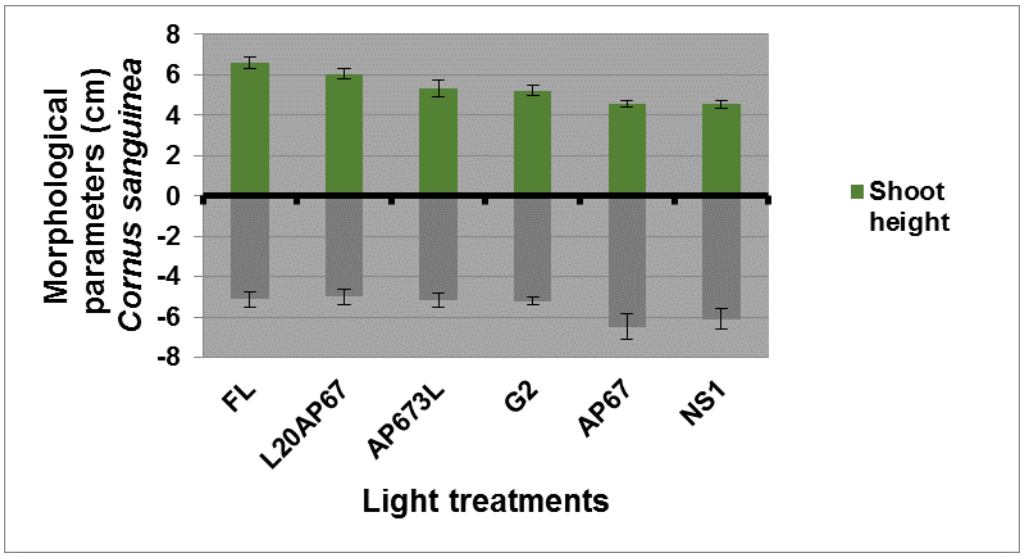


Figure 62. Morphological parameters (cm) of Cornus sanguinea seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 VW mini-plug size at the end of the 4week experimental period.

***Prunus avium* L.**

At the end of the 6 week experimental period no significant differences were observed for the SH of *Prunus* seedlings. Although taller seedlings were observed under the AP67 with average SH of 6.47 cm, following by the G2, L20AP67, NS1, AP673L of 6.39 cm, 6.17 cm, 6.13 cm, 5.71 cm, while FL conventional light showed the shortest of 5.23 cm (Fig.63). In addition, NS1 (p<. 001) LED significantly increased the RL of cherry seedlings showed an average value of 12.57 cm compared to the rest of the light treatments such as the FL that showed the shortest roots of 4.84 cm, following by the L20AP67 with 6.07 cm, G2 with 6.25 cm, AP67 with 6.72 cm and AP673L with 7.20 cm, respectively (Fig.63).

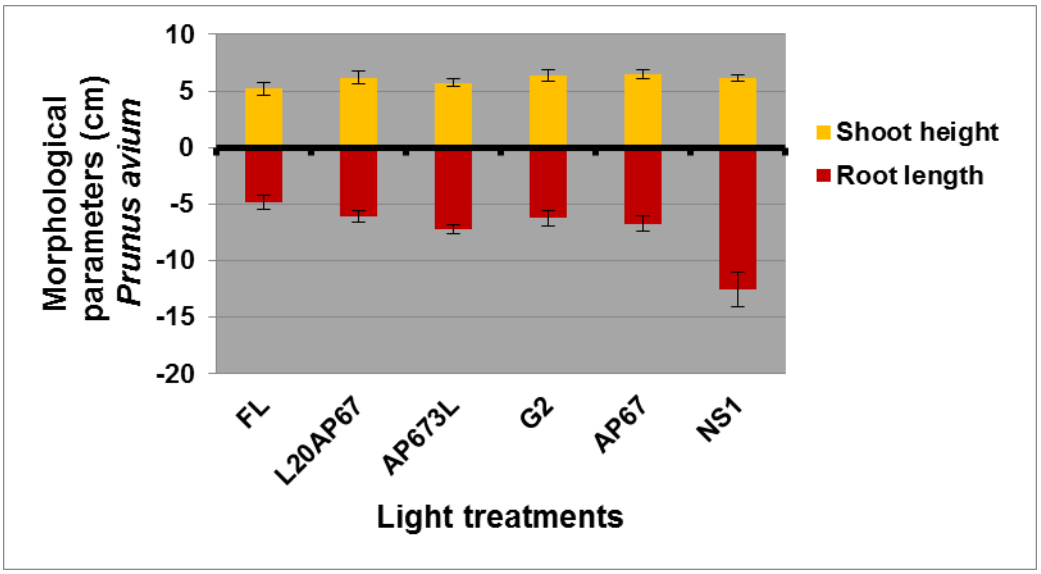


Figure 63. Morphological parameters (cm) of Prunus avium seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD 104 VW mini-plug size at the end of the 6week experimental period.

***Punica granatum* L.**

Eventually L20AP67 (p<. 001) LED induced significantly taller seedlings showed average SH of 7.73 cm compared to the rest of the lights (Fig.64). Shorter seedlings were found under the AP67 with SH of 4.10 m, following by the AP673L with 4.30 cm, G2 with 4.51 cm, NS1 wit 4.53 cm and FL with 4.68 cm. Highly significant differences for the RL were found for the L20AP67 (p=. 001) (p<. 001) that had an average value of 9.33 cm compared to the FL conventional light that had the least beneficial effect forming RL average value of 4.5 cm and to AP67 with 5.19 cm (Fig.64). AP673L, G2 and NS1 LEDs showed the following average values for the RL of 6.5 cm, 6.44 cm and 6.14 cm, respectively.

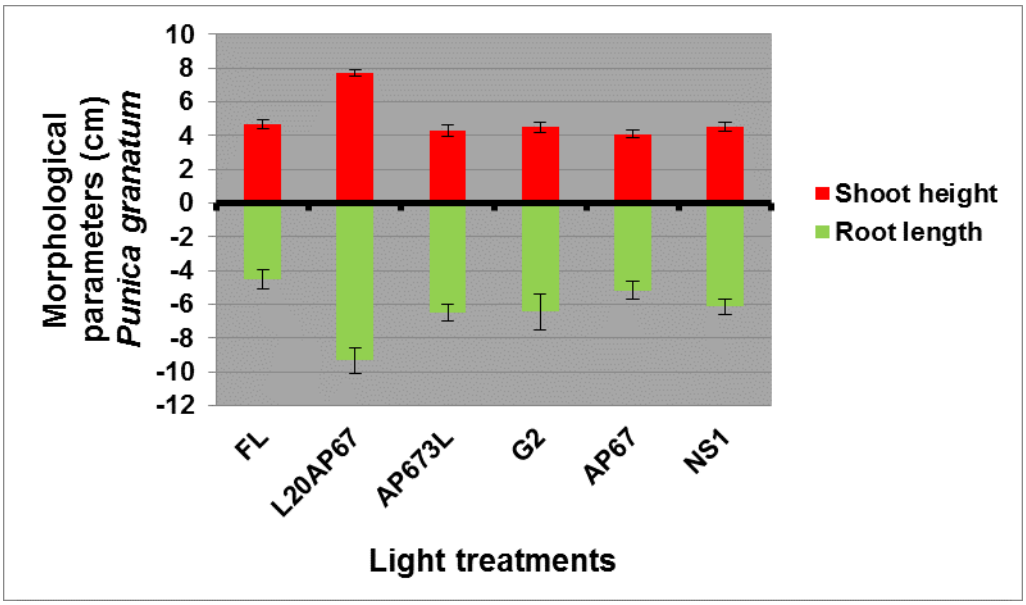


Figure 64. Morphological parameters (cm) of Punica granatum seedlings under FL, L20AP67, AP673L,G2, AP67 & NS1 light treatments in enriched peat soil substrate and in QPD 104 VW mini-plug size at the end of the 6week experimental period.

3.3.10. Root architecture

3.3.10.1. Root density

Quercus ithaburensis* ssp. *macrolepis

Results for the root density of *Q. ithaburensis* seedlings indicated no significant differences between the light treatments. However greater root density was found for the seedlings grown under LEDs than the FL light; specifically under L20AP67 with 1.38, AP67 with 1.32, NS1 with 1.27, G2 with 1.26, AP673L with 1.09 and for the FL 0.75 (Fig.65).

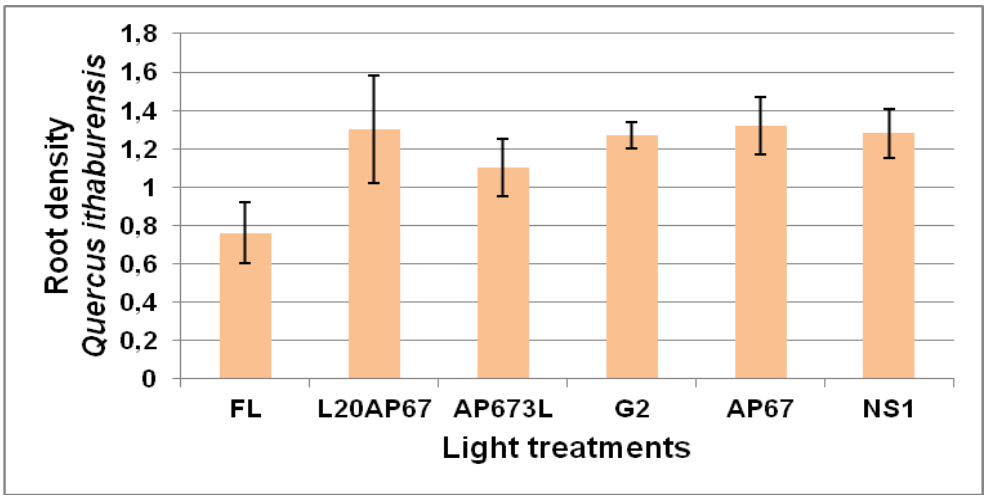


Figure 65. Root Density (RD) calculated by the number of lateral roots to the main root length of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat (SP) soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

Castanea sativa Mill.

Different light qualities did not induce significant effect on the root density; however more roots were formed along the primary length of the seedlings grown under the G2 light with a ratio of 1.52 following by the NS1 with ratio of 1.45, AP67 with 1.41, RGP with 1.38, AP673L with 1.30, L20AP67 with 1.13 and the lowest for the FL light with a ratio of 0.85 (Fig.66).

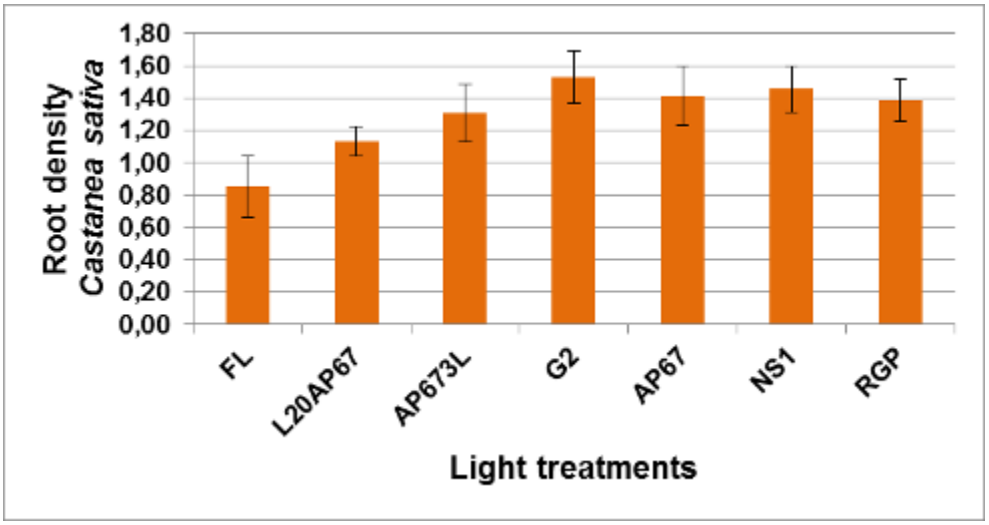


Figure 66. Root Density (RD) calculated by the number of lateral roots to the main root length of Castanea sativa under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

3.3.10.2. Root fibrosity

Quercus ithaburensis* ssp. *macrolepis

Greater root system fibrosity (branchiness) (Tanaka *et al.,* 1976; Schultz and Thompson, 1996) has been related to increased root growth capability (Hallgren and Tauer, 1989) and in certain instances increased survival (Schultz and Thompson, 1996; Li *et al*., 2011). Greater root system fibrosity can lead to greater water movement capability through the root system (Carlson, 1986) thereby reducing seedling water stress (Nambiar, 1984). In our case there were no significant differences in the average number of FOLR but there were in the fibrosity of seedling root systems between the light treatments.

Greater number of FOLR (double) was found under LEDs than the FL light, in descending order under G2, NS1, AP673L, AP67, L20AP67 and FL with 53, 50, 45, 40 and 25, respectively (Fig.67).

Significant differences were found for the number of FOLR with diameter >1 mm between LEDs of NS1 (p<. 001) and AP67 (p<. 002), with the FL light. Also significant differences were found between LED lights, specifically fewer roots oh higher order found for the L20AP67 compared to NS1 and AP67 (Fig.67)

Eventually seedlings grown under LED lights characterized as more fibrous compared to the FL light with NS1 and AP67 shown the greatest effect (Tabl. 2).

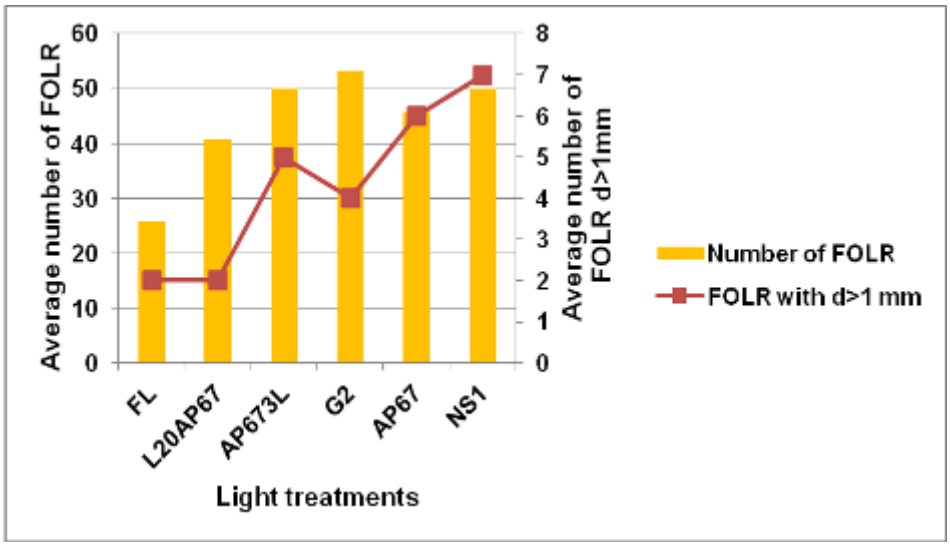


Figure 67. Average number of First Order Lateral Roots (FOLR) & number of FOLR with d> 1mm of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

Light treatment	Rating	Fibrosity class	Description of root system appearance
FL	1.3	Very low	No 2nd order long roots; zero or few short roots present
L20AP67	3	Moderate	3-5 2nd order long roots; moderate density of higher order long and short roots
AP673L	4	High	>5 2nd order long; moderate density of higher order long and short roots
G2	5	Very High	5>5 2nd order long roots; high density of higher order long and short roots
AP67	>5	Very High	5>5 2nd order long roots; high density of higher order long and short roots
NS1	>5	Very High	5>5 2nd order long roots; high density of higher order long and short roots

Table 2. Rating system for root fibrosity of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 at the end of the 4week experimental period. The rating is based on visual assessment of the approximate number and type of high order lateral roots per 10 cm segment of primary first order lateral roots (those with a diameter >1 mm, branching from the taproot). Long roots are >5 mm and are likely to contain branches of the next highest order. Short roots are <5 mm; they do not support roots of higher order (Hatchell and Muse, 1990).

***Castanea sativa* Mill.**

Significantly less FOLR were formed under the effect of FL and RGP illuminations (p<. 001) compared to all LED treatments. FL light had an average of 11 FOLR and RGP showed 18. The highest number of FOLR formed under NS1 with 34 (p<. 001), AP67 with 33 (p<. 001), G2 with 32 (p<. 002), AP673L with 31 (p<. 004) and L20AP67 with 30 (p<. 013) (Fig.68). Also AP673L and NS1 LED lights formed significantly more FOLR with d>1 mm and characterized as more fibrous compared to the FL light that obtained lowest number of all lights (Fig.68). The rest of light treatments, especially LEDs had high or very high fibrosity (Tabl.3)

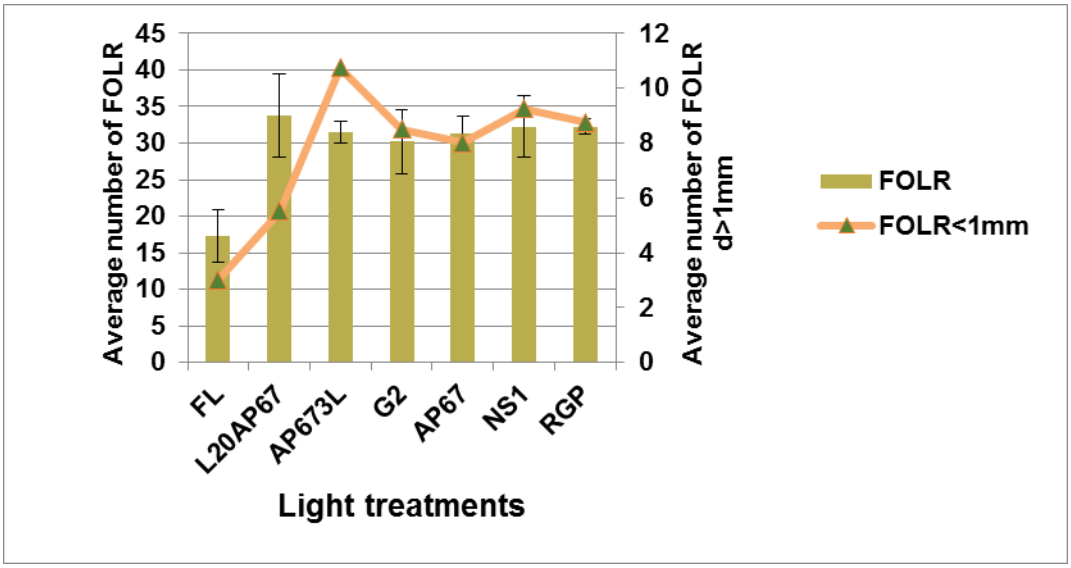


Figure 68. Average number of First Order Lateral Roots (FOLR) & number of FOLR with d> 1mm of Castanea sativa under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments at the end of the 3week experimental period.

Light treatment	Rating	Fibrosity class	Description of root system appearance
FL	3	Moderate	3–5 2nd order long roots; moderate density of higher order long and short roots
L20AP67	4	High	>5 2nd order long roots; moderate density of higher order long and short roots
AP673L	>5	Very High	5 >5 2nd order long roots; high density of higher order long and short roots
G2	5	Very High	5 >5 2nd order long roots; high density of higher order long and short roots
AP67	>5	Very High	5 >5 2nd order long roots; high density of higher order long and short roots
NS1	>5	Very High	5 >5 2nd order long roots; high density of higher order long and short roots
RGP	4	High	>5 2nd order long roots; moderate density of higher order long and short roots

Table 3. Rating system for root fibrosity Castanea sativa under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments at the end of the 3week experimental period. The rating is based on visual assessment of the approximate number and type of high order lateral roots per 10 cm segment of primary first order lateral roots (those with a diameter >1 mm, branching from the taproot). Long roots are >5 mm and are likely to contain branches of the next highest order. Short roots are <5 mm; they do not support roots of higher order (Hatchell and Muse, 1990).

3.3.10.3. Dry weight

***Pinus sylvestris* L. (provenances Greece-Sweden)**

No significant differences found between the two provenances but there were for each of them individually.

FL and L20AP67 lights induced the lowest dry weight mass of leaves, shoots and roots compared the rest of LEDs (p<. 001). Specifically for the DWL FL and L20AP67 light showed average values of 0.010 g and 0.014 g. In contrast the rest of LEDs such as AP67, G2, AP673L and NS1 showed similar values such as 0.040 g, 0.038 g, 0.037 g and 0.035 g (Fig.69). The same was observed for the DWS where AP67 LED showed the highest average value of 0.0085 g, following by the G2 of 0.0084 g, AP673L of 0.0082 g and NS1 of 0.0077 g, while the lowest was found for the FL and L20AP67 with average values of 0.0035 g, 0.0040 g, respectively. AP673L LED showed the highest average value for the DWR of 0.0155 g, following by the NS1 with 0.0133 g, AP67 with 0.0130 and G2 with 0.0120 g, while FL and L20AP67 showed the lowest values of 0.0014 g and 0.0024 g (Fig.69).

Different light qualities had the same effect on the dry weight mass of the Swedish provenance. Specifically FL and L20AP67 (p<. 001) lights obtained the lowest average values for the DWL, DWS and DWR of the seedlings with average values of 0.009 g and 0.016 g, 0.003 g and 0.004 g and 0.0007 g and 0.0028 g, respectively (Fig.70). In contrast AP67 and AP673L LEDs induced the highest average values for the DWL, DWS and DWR of 0.0417 g, 0.0092 g and 0.0158 g and 0.0415 g, 0.009 g and 0.0157 g, respectively. As for the NS1 and G2 LEDs showed also significant differences with the FL and L20AP67 and had average values for the DWL, DWS and DWR of 0.0363 g, 0.007 g and 0.0144 g and 0.0354 g, 0.0078 g and 0.0106 g, respectively (Fig.70).

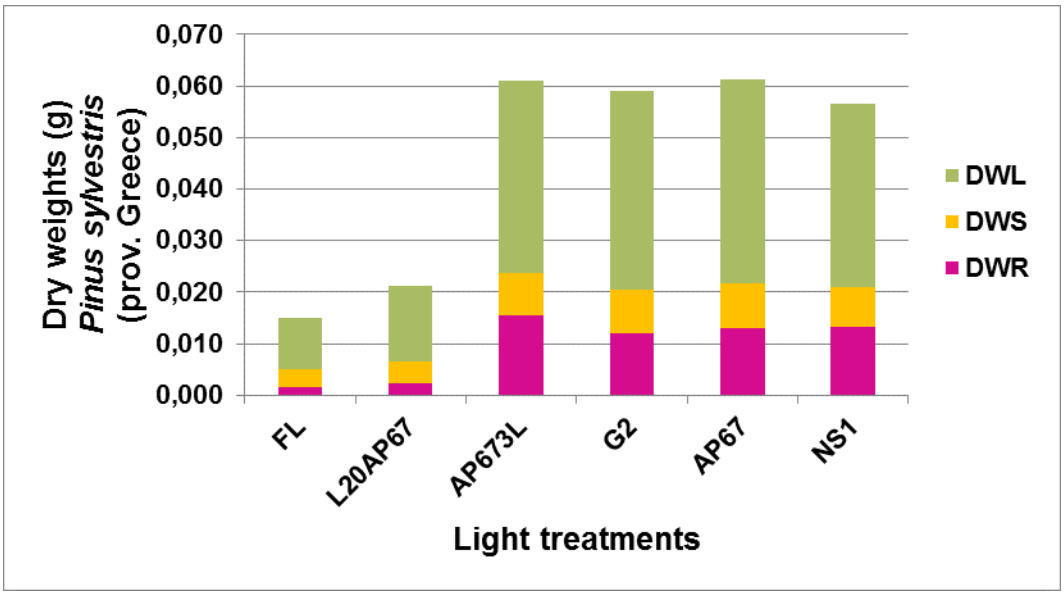


Figure 69. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Pinus sylvestris provenance Greece seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

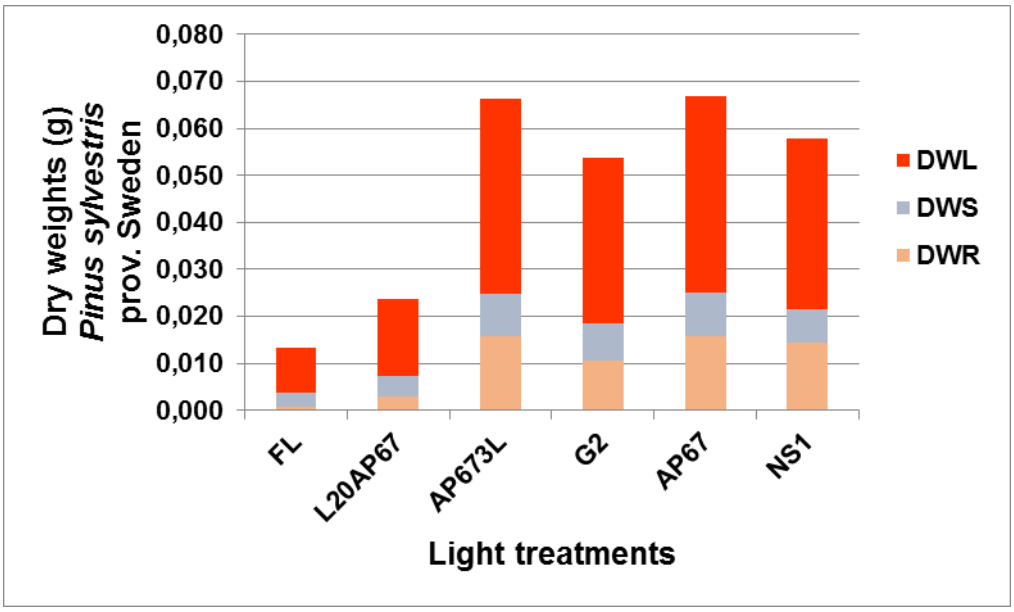


Figure 70. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Pinus sylvestris provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

***Picea abies* Karst.**

LED lights highly promoted the dry weight mass of *Picea* seedlings compared to the FL conventional light. However less beneficial effect on dry weight was induced by the L20AP67 LED light. More specifically for the DWL, FL and L20AP67 (p<. 001) induced significanlty the lowest dry mass with average values of 0.0077 g and 0.01304 g compared to the rest of LEDs. The highest accumulation of dry weight mass on leaves were induced under the AP67 LED with an average value of 0.0253 g, following by the AP673L, G2 and NS1 with average values of 0.0244 g, 0.0240 g and 0.0224 g, respectively (Fig.71). Further for the DWS FL induced the lowest mass with an avergage value of 0.0025 g and significant differences found with the AP673L, NS1 (p<. 001), AP67 (p<. 003) and G2 (p<. 005) that showed avergae values of 0.0049 g, 0.0042 g, 0.00405 g and 0.00401 g, respectively. As for the L20AP67 (p<. 001) showed lower DWS of 0.0033 g than the rest of LEDs but significant differences found only compared to the AP673L (Fig.71). In addition FL light induced the lowest mass allocated to the roots with an averge value of 0.0008 g compared to the AP67, G2, NS1 and AP673L (p<. 001) with average values of 0.0089 g, 0.0071 g, 0.0063 g and 0.0058 g, respectively. Also L20AP67 LED showed less beneficial effect for the DWR with average value of 0.0027 g among the rest of LEDs, thus significant differences found with AP67, G2 (p<. 001) and NS1 (p<. 004) (Fig.71).

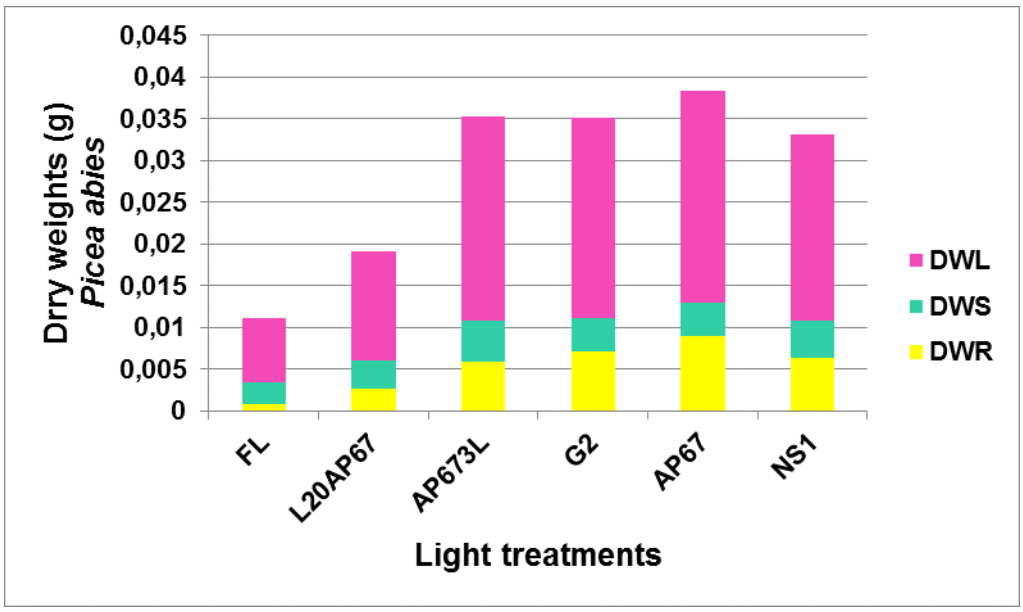


Figure 71. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Picea abies provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

Pinus nigra Arn.

FL light induced the lowest dry weight mass of leaves, shoots and roots and significant differences found with all LEDs (p<. 001) except from the L20AP67 light quality (Fig. 72). For the DWL of *P.nigra* seedlings AP67 LED showed the highest value of 0.025 g, following by the AP673L, G2, NS1, and L20AP67 that had average values of 0.0244 g, 0.0240 g, 0.022 and 0.013 g, respectively. AP673L benefited more the DWS with an average value of 0.0049 g and significant difference found along with the FL showed value of 0.0025 g also with the L20AP67 (p<. 001) that had a value of 0.0033 g. Following the average values for the DWS were 0.0044 g and 0.0040 g induced by the NS1, AP67 and G2 LEDs. FL light had significantly induced the lowest value for the DWR of 0.0008 g, while the highest was for the AP67 with 0.0089 g, following by the G2 with 0.0071 g, NS1 with 0.0063 g and the AP673L with 0.0058 g. Among LEDs, L20AP67 also showed lower DWR value of 0.0027 g and significant differences found with the AP67, G2 (p<. 001) and the NS1 (p<. 004) (Fig.72).

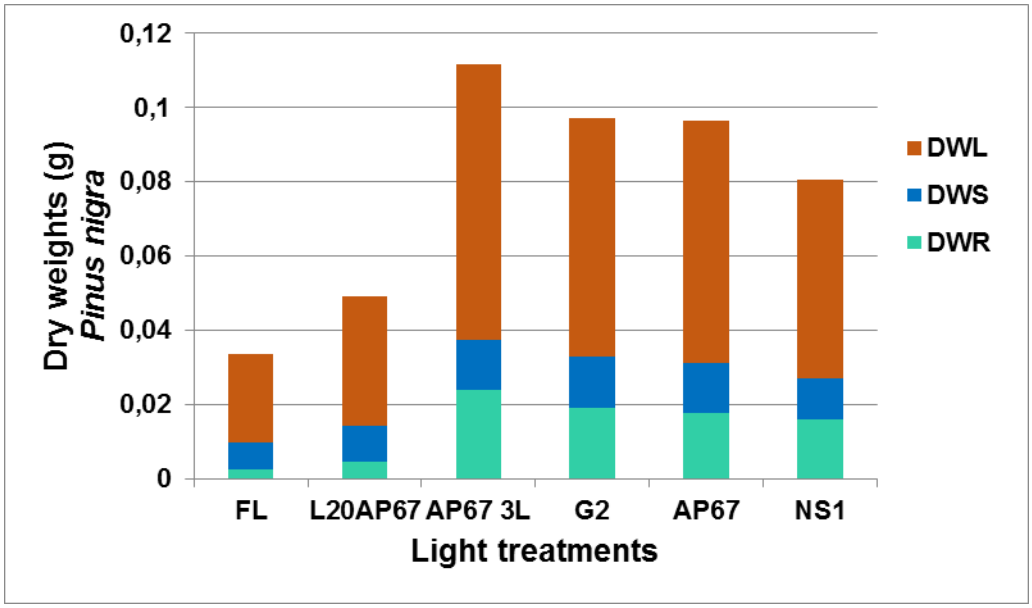


Figure 72. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Pinus nigra seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

Quercus ithaburensis ssp. *macrolepis*

Dry weight matter of seedlings showed significantly differences only for the DWR (p<. 005), where LEDs of NS1 and AP673L had the greatest effect with average values of 3.18 g and 2.9 g compared to the FL light that induced the lowest of 0.77 g (Fig.73). The rest of LEDs showed also greater average values than the FL, such as for the AP67 with 2.26 g, G2 with 2.23 g and L20AP67 with 1.22 g. LED lights also showed more beneficial effect for the dry weight accumulation in leaves and shoots compared to the FL that has the least. Greater impact both for the DWL and DWS was found under the L20AP67 with 0.523 g and 0.703 g, respectively; following by the AP673L with 0.520 g and 0.656 g., AP67 with 0.520 g and 0.583 g, NS1 with 0.486 g and 0.593 g, G2 with 0.440 g and 0.543 g. FL light had the lowest average values of 0.256 g and 0.290 g.

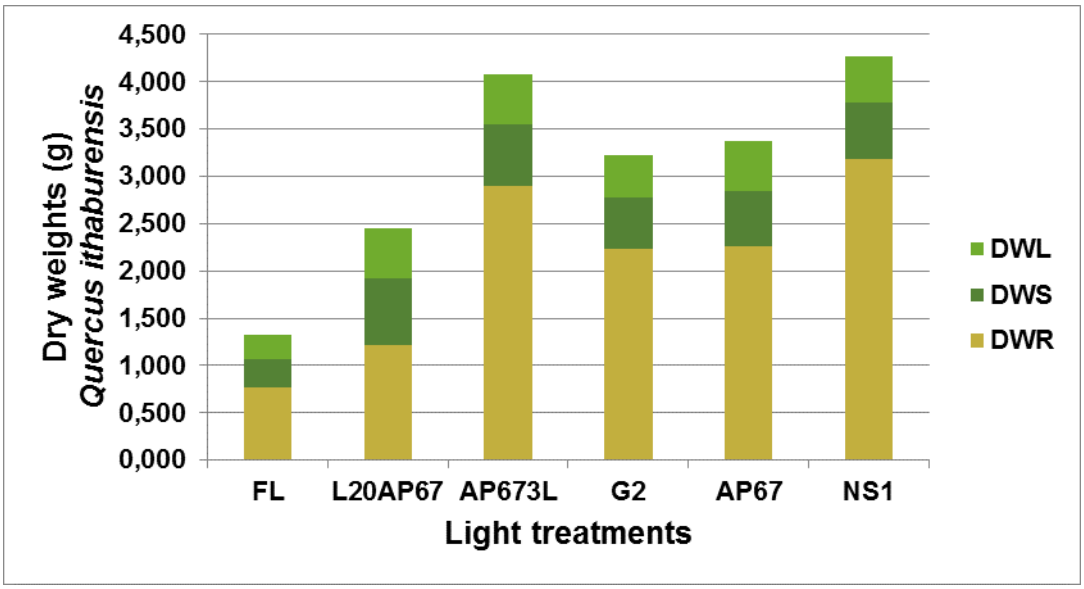


Figure 73. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

***Castanea sativa* Mill.**

Light treatments induced significant effect on DWS and DWR of *Castanea* seedlings (p<. 008). AP673L LED light induced significantly higher DWS of 1.315 g and DWR of 2.615 g compared to the FL light that showed an average of 0.53 g and 0.99 g, respectively (Fig.74). The rest of the lights also showed higher DWS and DWR than the FL. More specifically for the DWS, L20AP67, RGP, AP67, NS1 and G2 had average values of 1.032 g, 1,192 g, 1 g, 0.957 g and 0,815 g, respectively. Further for the DWR, AP67, RGP, NS1 and L20AP67 showed average values of 2.217 g, 1.897 g, 1.807 g and 1.795 g. FL light found to be the least beneficial light source for the DWL with 0.787 g, compared to the AP673L with1.412 g, AP67 with 1.195 g, NS1 with 1.035 g, RGP with 1.022 g, L20AP67 with 0.992 g and G2 with 0.980 g (Fig.74).

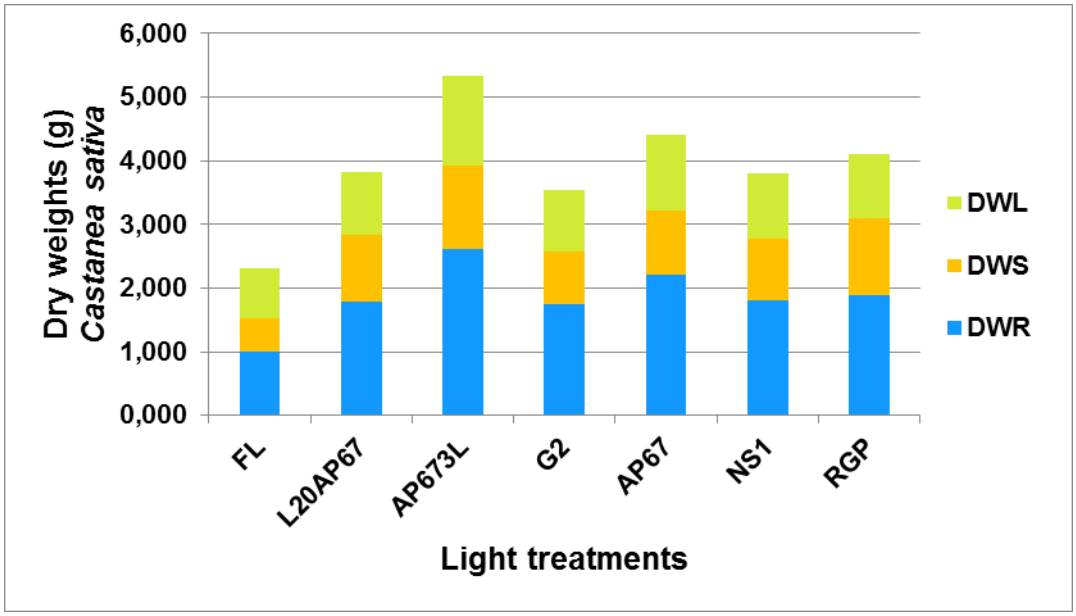


Figure 74. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Castanea sativa seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

***Myrtus communis* L.**

Different light qualities significantly affect the dry weight mass of *Myrtus* seedlings during their pre-cultivation especially for the LEDs AP673L and NS1 (p<. 000). NS1 and AP673L LEDs showed significantly higher DWL with 0.038 g compared to G2, L20AP67, RGP, FL and AP67 (P< .001) with average values of 0.010 g, 0.012 g,

0.013 g, 0.014 g and 0.021 g, respectively. AP673L induced significantly higher DWS with 0.005 g compared to G2, RGP (p<. 001), AP67 (p<. 002) and L20AP67 (p<. 003) with values between 0.002-0.003 g. Also both NS1 and AP673L light qualities differed significantly for the DWR that showed an average value of 0.012 g, three times higher than the DWR obtained under the rest of light treatments (p<. 001) (Fig.75).

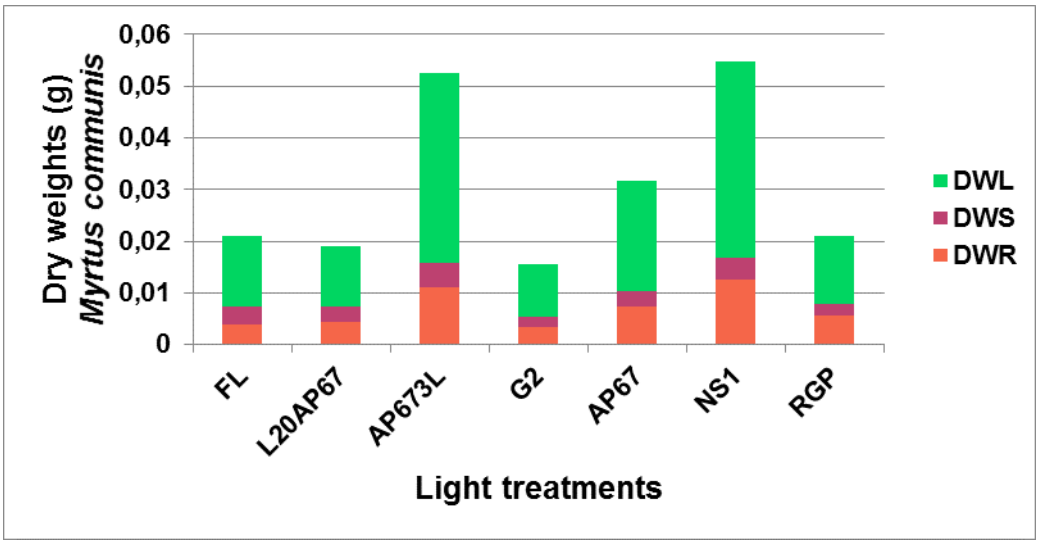


Figure 75. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Myrtus communis seedlings under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size at the end of the 3week experimental period.

Ocimum basilicum L.

According to the results there were significant differences between the light treatments for the DWL between the AP67 (p<. 003) light that showed higher value of 0.026 g and the FL light that had 0.014 g (Fig.76). The rest of the LEDs such as the G2, AP673L, L20AP67 and NS1 also showed higher DWL accumulation than the FL control light with average values of 0.021 g and 0.017 g. G2 LED light induced the highest value for the DWS of 0.0062 g compared to the FL (p < .001) with 0.0032 g, AP673L (p < .004), with 0.0035 g, NS1 (p < .007) with 0.0036 g and L20AP67 (p < .014) with 0.0029 g., while AP67 light showed an average DWS value of 0.0051 g (Fig.76). DWR of *O. basilicum* seedlings benefited under the LEDs, especially under the NS1 light that induced the heavier roots of 0.011 g compared to the rest of the treatments such as the FL, L20AP67, AP673L, G2 (p < .001), AP67 (p<. 009) with average values varied between 0.002 g- 0.008 g (Fig.76).

Furthermore, FL light induced lighter roots compared to the AP673L, G2 and AP67 lights ($p < .001$), while the L20AP67 differed significantly from the AP67 ($p < .001$) and G2 ($p<. 004$) (Fig.76).

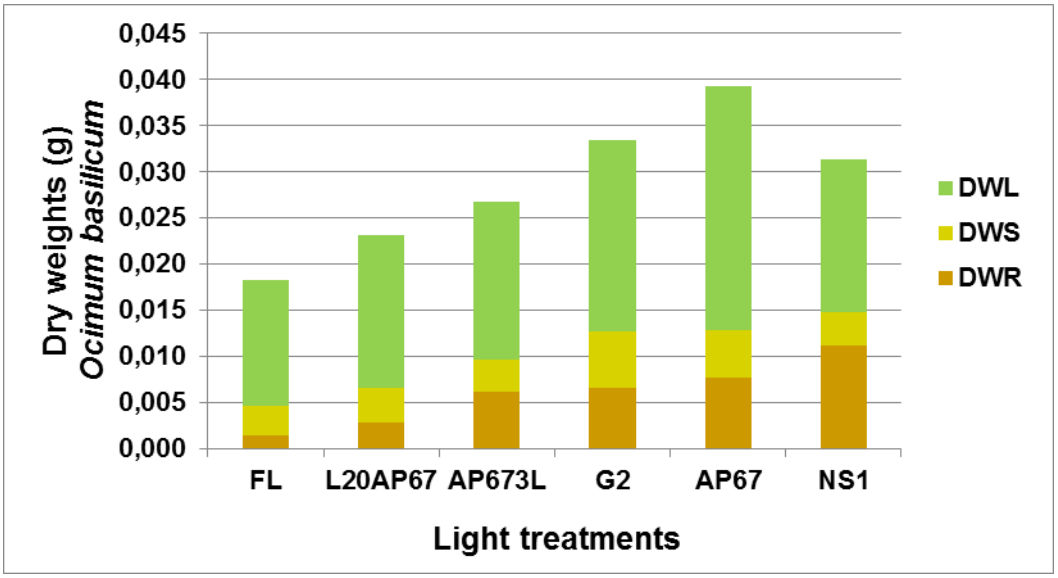


Figure 76. Dry weight of leaves (DWL), shoots (DWS) and roots (DWR) (g) of Ocimum basilicum LL seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

Ocimum basilicum RR hybrid

NS1 light induced significantly higher DWL of 0.016 g than the FL control light ($p < .001$) with an average value of 0.008 g. G2 and AP67 lights had average values of 0.013 g, while L20AP67 and AP673L had 0.010 g, respectively (Fig.77). As for the DWS G2 and AP67 lights showed significantly greater values of 0.0071 g and 0.0066 g than the FL ($p< .001$) with 0.0027 g, L20AP67 ($p< .001$) ($p< .006$), with 0.0038 g and AP673L ($p< .004$) with 0.0042 g lights. Also the FL light induced lighter shoots than the NS1 light ($p< .014$) that had an average DWS value of 0.0054 g (Fig.77). The NS1 light promoted greater DWR of 0.013 g compared to the FL, L20AP67, AP673L and G2 ($p<. 001$) that showed 0.002 g and 0.006 g, respectively (Fig.77).

Further LEDs of AP673L (p<.009), G2 (p<. 002) (p<. 009) and AP67 (p<. 001) (Fig.77) had significantly greater DWR than both FL and L20AP67.

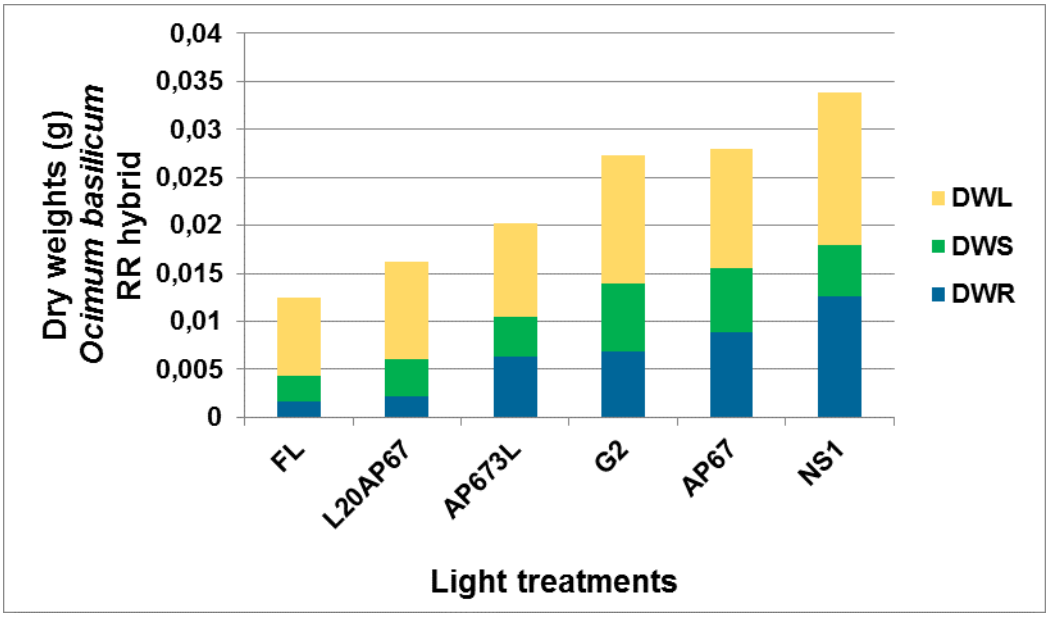


Figure 77. Dry weight of leaves (DWL), shoots (DWS) and roots (DWR) (g) of Ocimum basilicum RR hybrid seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat (soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

***Cornus sanguinea* L.**

C. sanguinea seedlings showed significant differences between the different light treatments for the dry weight mass (p< .001). Specifically, FL (p<.001) and L20AP67 (p<.001) (p<. 003) lights induced significantly lower DWL with average values of 0.015 g and 0.017 g compared to G2 and AP67 LED lights that showed average values of 0.029 g and 0.026 g, respectively (Fig.78). AP673L and NS1 LEDs showed similar values of 0.024 g and 0.021 g that are also higher than those obtained by FL and L20AP67. Regarding the DWS significantly lower values were found for the FL light of 0.0072 g compared to the G2 (p<. 001), AP67 (p<. 002) and AP673L (p<. 003) LEDs that showed DWS average values of .0140 g, 0.0128 g and 0.0125 g,

respectively (Fig.78). Additionally, AP673L showed significantly higher DWS allocation than the L20AP67 (p<. 005) that showed an average value of 0.0089 g. NS1 LED showed an average DWS value of 0.0106 g that was also higher than the values obtained under FL and L20AP67 lights, however was not significantly different. Significantly higher dry weight allocation to the roots was induced by the G2, AP67, AP673L and NS1 LEDs that showed average values of 0.024 g and 0.021 g compared to the lowest obtained under the FL and L20AP67 (p<. 001) with an average value of 0.005 g and 0.010 g, respectively (Fig.78).

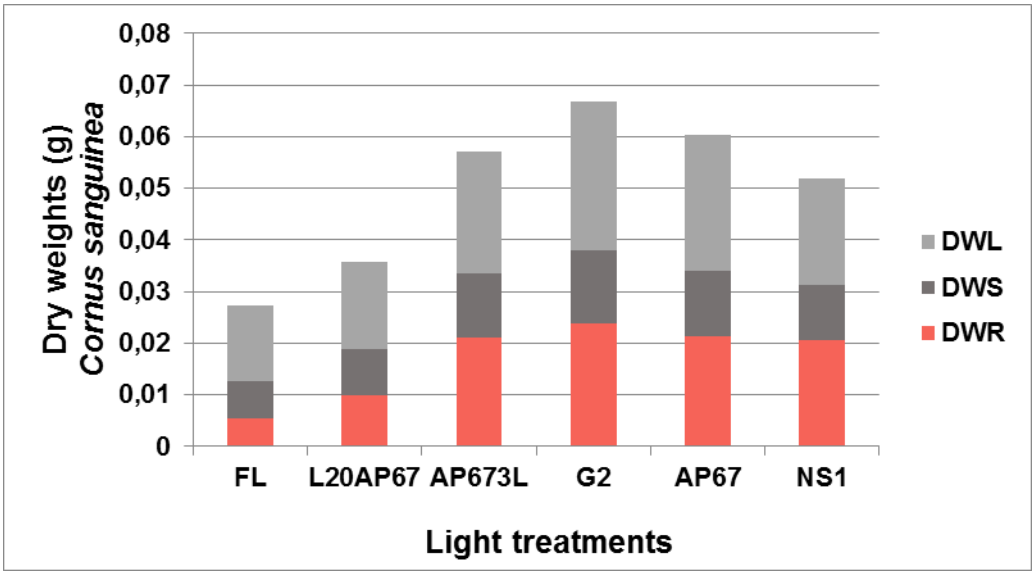


Figure 78. Dry weight of leaves (DWL), shoots (DWS) and roots (DWR) (g) of Cornus sanguinea seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

***Prunus avium* L.**

DWL of cherry seedlings was significantly higher for the AP67, NS1 with 0.138 g, AP673L and G2 with 0.130 g (p<. 001) compared to the FL light that induced the lowest average value of 0.041 g. Further L20AP67 LED also induced significantly lower DWL of 0.067 g compared to the AP67 (p<. 006) and NS1 (p<. 008) lights (Fig.79). Seedlings grown under the FL light showed significantly lower DWS allocation of 0.0186 g compared to LEDs of NS1, AP67 (p<. 001), G2 (p<. 004) and AP673L (p<. 017) that had average values of 0.074 g, 0.065 g, 0.061 g and 0.056 g, respectively. NS1 LED light also induced significantly higher DWS than the L20AP67 (p<. 004) that showed an average value of 0.031 g (Fig.79). DWR of cherry seedlings was found significantly lower for the FL control light that had an average value of 0.014 g compared to the NS1, AP67 (p<. 001), AP673L (p<. 019) and G2 LEDs that showed average values of 0.087 g, 0.075 g, 0.062 g and 0.060 g, respectively, while L20AP67 also showed significantly lower DWR of 0.025 g than the NS1 (p<. 001) and AP67 (p<. 012) (Fig.79).

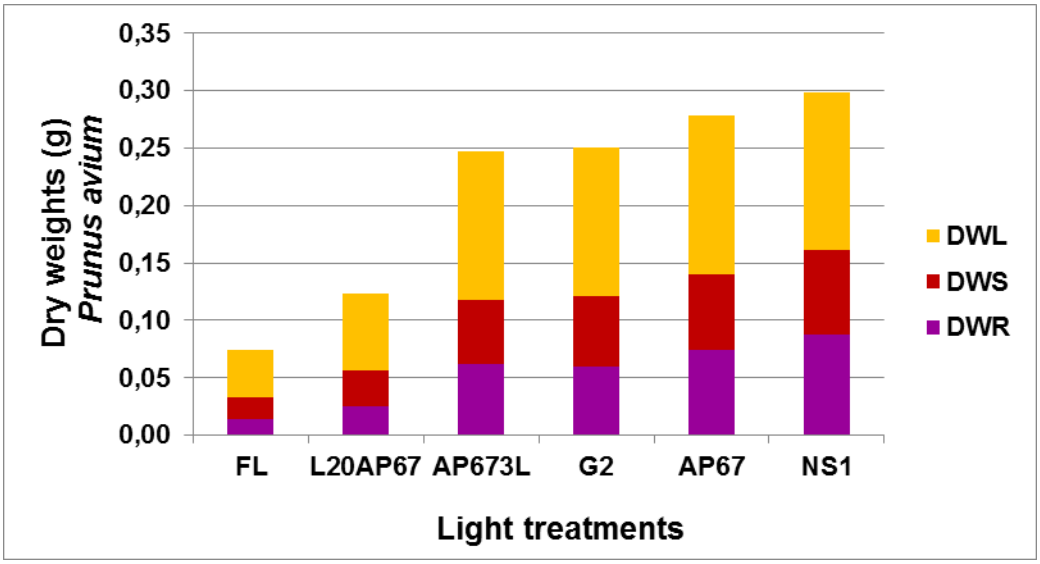


Figure 79. Dry weight of leaves (DWL), shoots (DWS) and roots (DWR) (g) of Prunus avium seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6week experimental period.

***Punica granatum* L.**

FL conventional light induced significantly lower DWL of 0.023 g compared to LEDs L20AP67, AP673L and NS1 (p<. 001) that showed average values of 0.076 g and 0.056 g, respectively. Further L20AP67 also found significantly greater for the DWL than G2 and AP67 LEDs that had average values of 0.042 g and 0.040 g (p < .001) (Fig.80). Regarding the DWS, L20AP67 LED (p<. 001) showed the highest accumulation of 0.0237 and significant differences found with all the light treatments. On the other hand, FL control light had the lowest average value of 0.0067 g compared to AP673L (p < .003), G2 (p<. 004) and NS1 (p<. 005) that showed average DWS values of 0.014 g, 0.0137 g and 0.0136 g, respectively (Fig.80). AP67 LED had an average DWS value of 0.0109 g. Control light also induced the lowest DWR of 0.005 g compared to L20AP67 (p<. 001), AP673L, NS1 (p<. 006) and G2 (p<. 013) that showed significantly greater average values of 0.017 g, 0.012 g and 0.011 g, respectively. Moreover L20AP67 obtained significantly greater DWR than the AP67 (p<. 001) that showed an average value of 0.008 g (Fig.80).

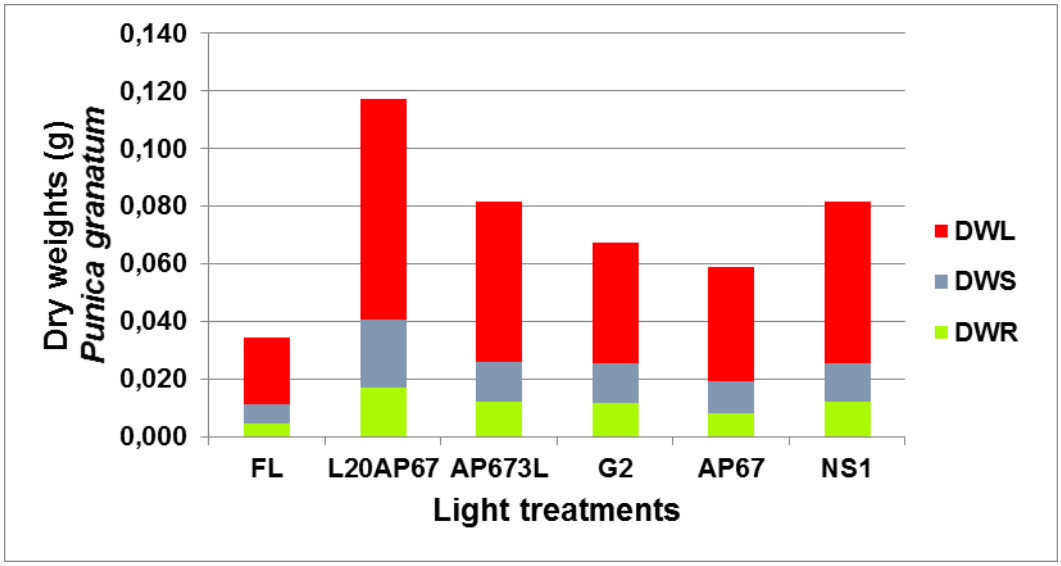


Figure 80. Dry weight of leaves (DWL), shoots (DWS) and roots (DWR) (g) of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6week experimental period.

3.3.10.4. R/S ratio

Pinus sylvestris L. (provenances Greece-Sweden)

FL and L20AP67 LED (p<. 001) lights obtained the lowest dry weight allocation to the roots with average values of 0.10 and 0.13 and significant differences found with the rest of LEDs that obtained higher, especially for the AP673L and NS1 with ratios of 0.34 and 0.31, following by the AP67 and G2 with ratios of 0.27 and 0.25, respectively (Fig.81).

As for the Swedish provenance R/S ratio obtained under the conventional FL light and L20AP67 LED were significantly the lowest (p<. 001) compared to the rest of LED treatments, especially for the NS1 with a ratio of 0.33 that also differed significantly with the ratio obtained under the G2 LED (p<. 005) that showed 0.24 (Fig.82). LEDs such as AP67, AP673L obtained the same allocation to the roots with a ratio of 0.31.

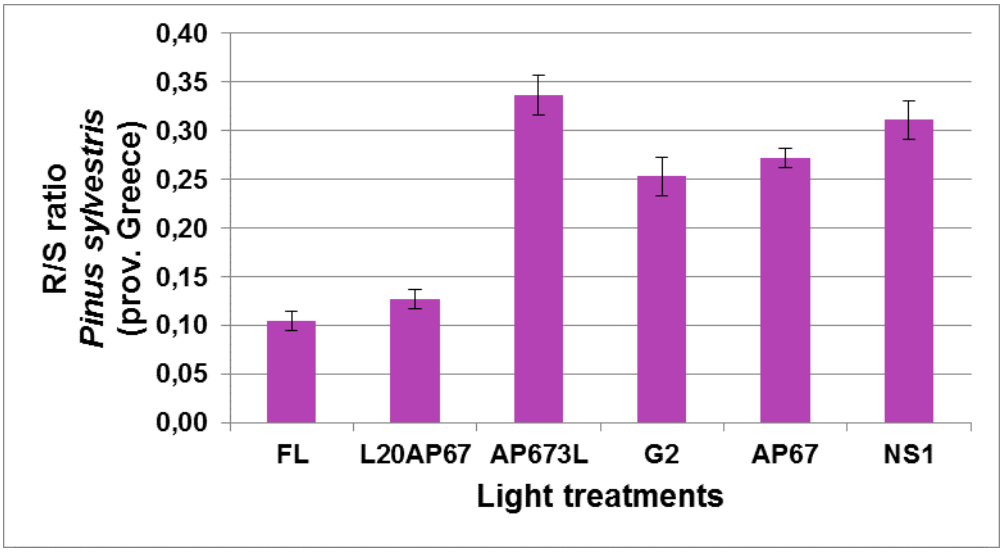


Figure 81. Root to shoot ratio (R/S) based on dry weight matter of Pinus sylvestris provenance Greece seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

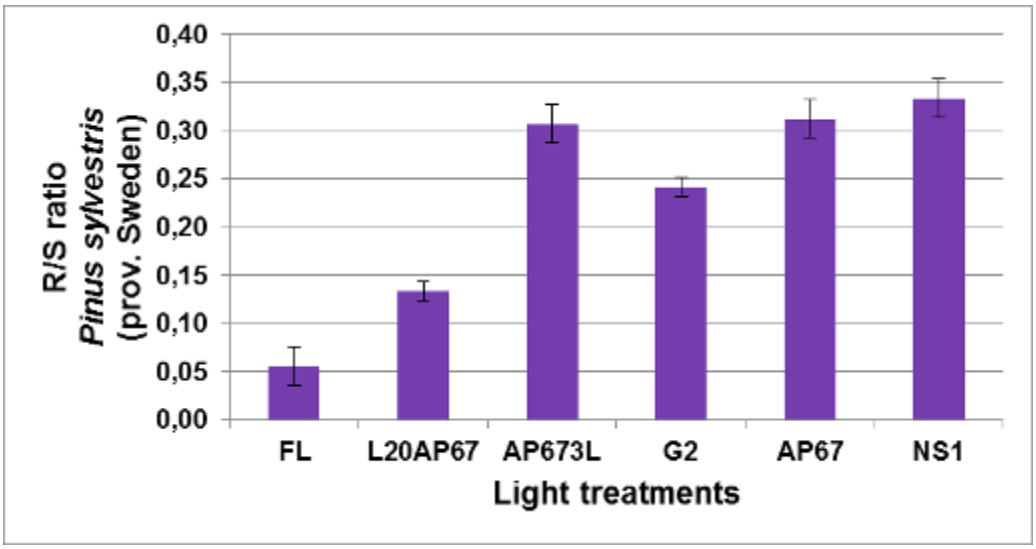


Figure 82. Root to shoot ratio (R/S) based on dry weight matter of Pinus sylvestris provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

***Picea abies* Karst.**

R/S ratio obtained of *Picea* seedlings was generally low; however LED lights had benefited more the above dry weight accumulation compared to the FL light that showed the least ratio of 0.08 and differed significantly with all LEDs (p<. 001) except from the L20AP67 that also had low ratio of 0.16 and differed significantly with the ratio of 0.30 obtained under the AP67 (p<. 001) illumination. The rest of LEDs showed ratios in descending order such as 0.25, 0.23 and 0.20 under the G2, NS1 and AP673L, respectively (Fig.83).

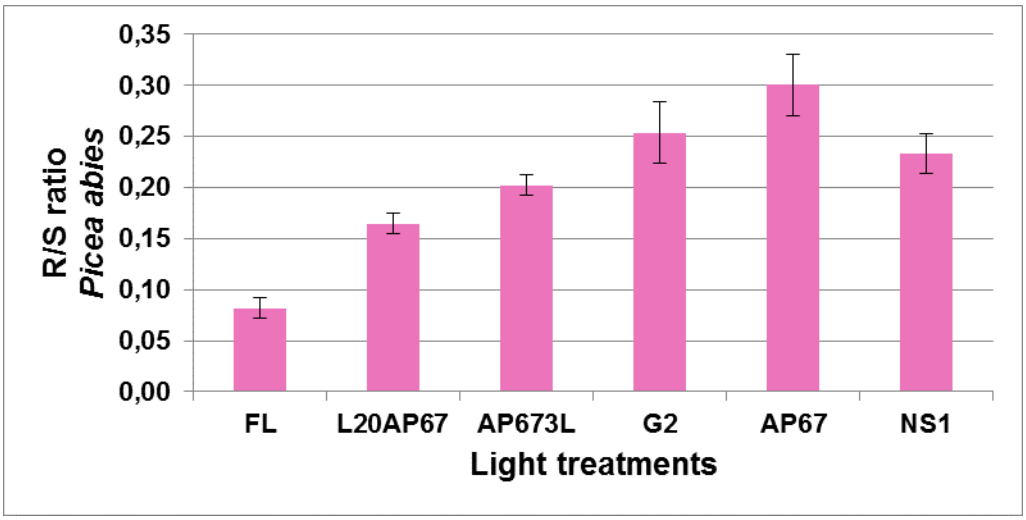


Figure 83. Root to shoot ratio (R/S) based on dry weight matter of Picea abies provenance Sweden seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

***Pinus nigra* Arn.**

R/S ratio of *P. nigra* obtained was low; however FL light had the least beneficial effect with a ratio of 0.08 and significant differences found with all LEDs (p<. 001) except from the L20AP67 (p<. 001) that also obtained low ratio of 0.16 than the highest obtained under the AP67 with 0.30 (Fig.84). Following the rest of LEDs such as the G2, NS1 and AP673L obtained similar R/S ratios of 0.25, 0.23 and 0.20, respectively.

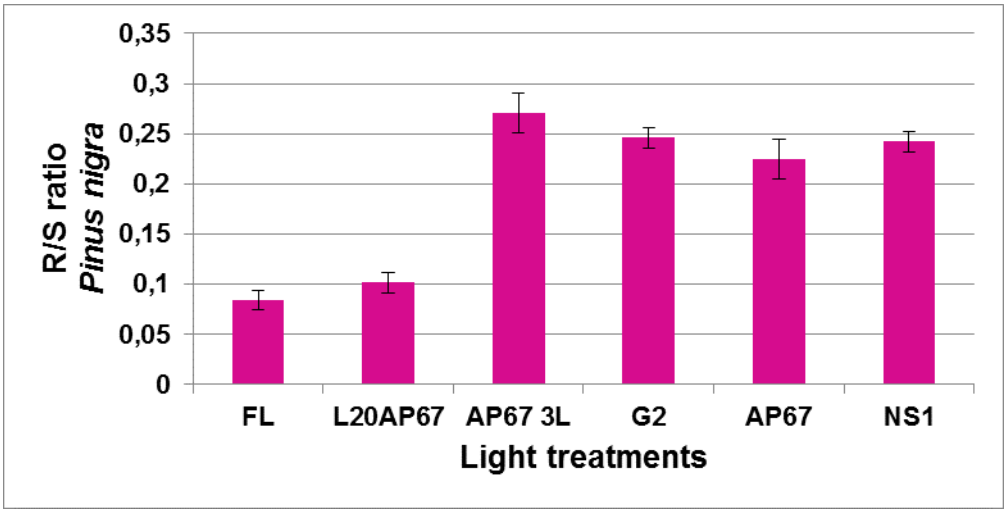


Figure 84. Root to shoot ratio (R/S) based on dry weight matter of Pinus nigra seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in stabilized peat soil substrate and in QPD104 VW mini-plug size at the end of the 5week experimental period.

Quercus ithaburensis* ssp. *macrolepis

R/S ratio of seedlings was significantly affected by the different illuminations (p<.001) (Fig.85). More specifically L20AP67 LED and FL conventional light showed the lowest R/S ratio than the rest of lights with 0.96 and 1.37, respectively. On the other hand significantly higher dry weight matter allocation in roots was found under the LEDs of NS1 (p<. 001), AP673L (p<. 010) and G2 (p<. 022) with 2.99, 2.46 and 2.31, respectively. Finally AP67 LED had a ratio of 1.98.

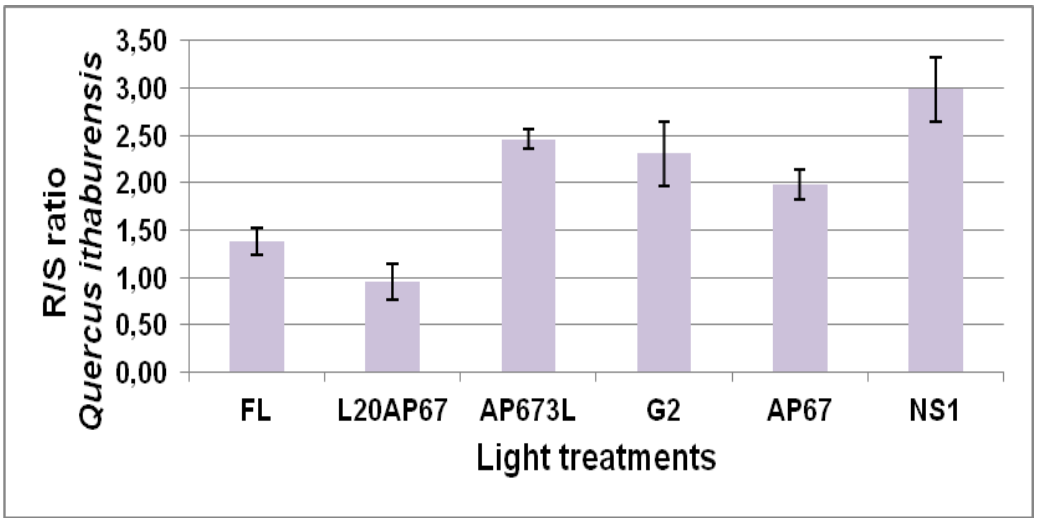


Figure 85. Root to shoot ratio (R/S) based on dry weight matter of Quercus ithaburensis seedlings under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments in enriched peat soil substrate and in DL48R mini-plug size at the end of the 4week experimental period.

Castanea sativa Mill.

No significant differences found between the light treatments for the R/S ratio of *Castanea*. However lower allocation to the roots was obtained under the FL light quality with a ratio of 0.79, while the highest was obtained under the AP67 LED with a ratio of 1 that is a more balanced allocation of dry weight mass both for the above and below parts of the plants. The rest of light qualities had ratios of 0.97, 0.96, 0.90, 0.87 and 0.86 obtained under AP673L, G2, RGP, NS1 and L20AP67, respectively (Fig.86).

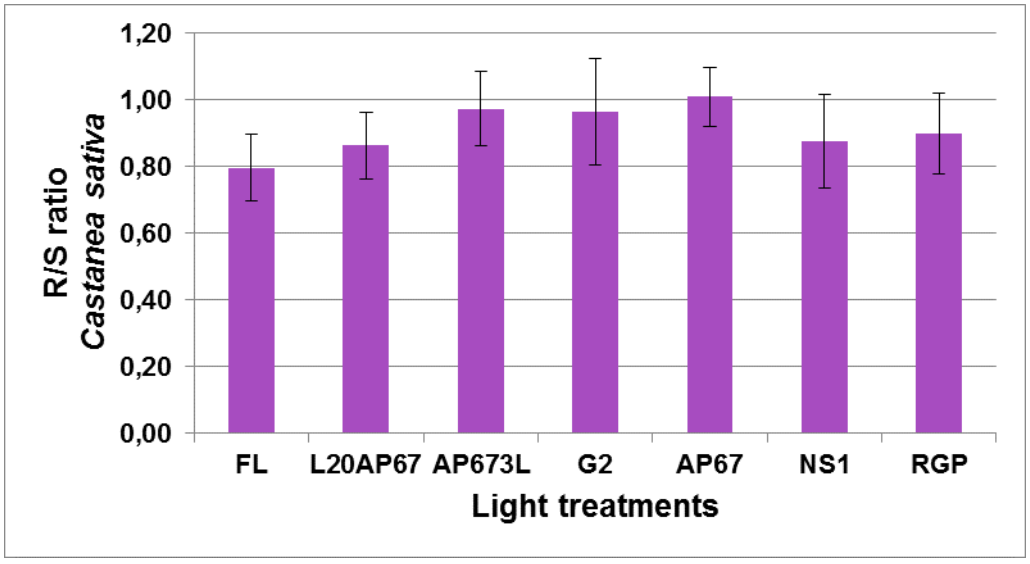


Figure 86. Root to shoot ratio (R/S) based on dry weight matter of Castanea sativa under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QP60/7R mini-plug size at the end of the 3week experimental period.

***Myrtus communis* L.**

Greater allocation to the roots was found under the RGP light environment with a ratio of 0.38 and significant difference found only with the ratio obtained under the FL (p<. 001) light which was the lowest of 0.21. The rest of LEDs also show greater R/S ratio than FL, specifically AP67 with 0.32, NS1 with 0.30, G2 and L20AP67 with 0.28 finally AP673L with 0.27 (Fig.87).

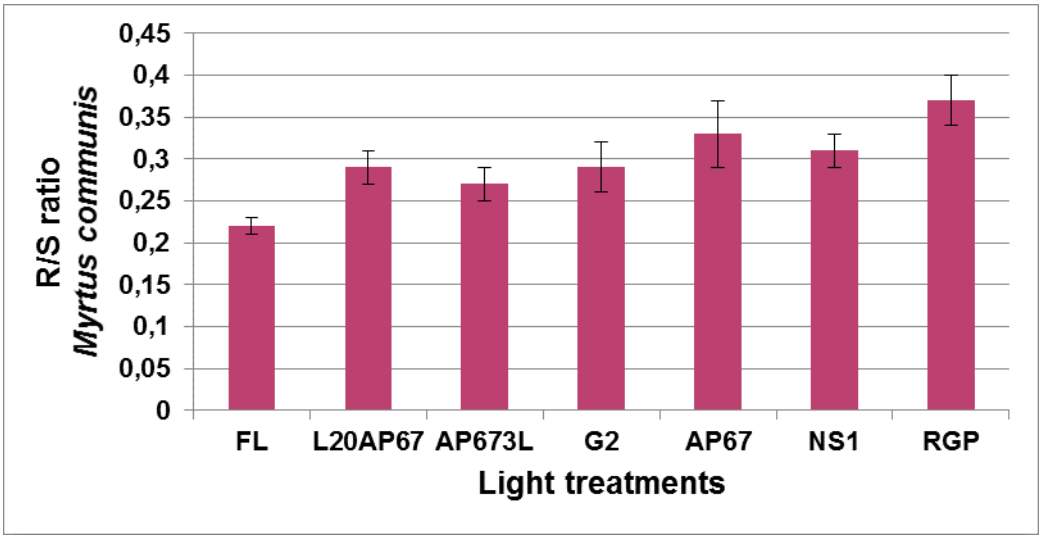


Figure 87. Root to shoot ratio (R/S) based on dry weight matter of Myrtus communis under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments in enriched peat soil substrate and in QPD104 VW mini-plug size at the end of the 3week experimental period.

***Ocimum basilicum* L.**

NS1 LED (p<. 001) light obtained R/S ratio of 0.57 that showed significantly greater allocation to the roots compared to the rest of lights such as the FL that showed the lowest of 0.09, following by the L20AP67 with a ratio of 0.14, G2 with 0.26 and AP673L with 0.29. Furthermore, the FL control light showed significantly lower R/S ratio compared to the AP67 light (p< .002) that obtained R/S ratio of 0.35 (Fig.88).

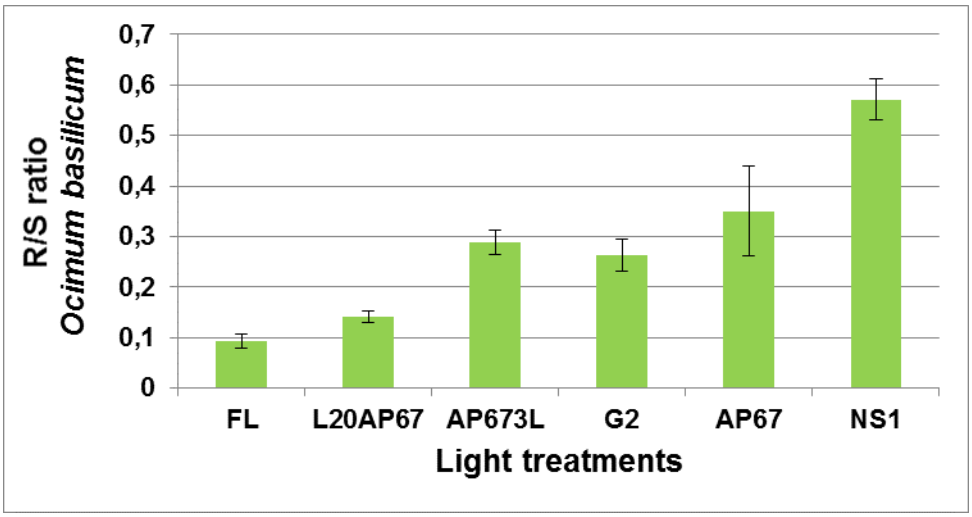


Figure 88. Root to shoot ratio (R/S) based on dry weight matter of Ocimum basilicum LL seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

Ocimum basilicum RR hybrid

According to the results the R/S ratio of the seedlings was higher under the NS1 light with 0.61 compared to the FL and L20AP67 ($p < .001$) that obtained 0.16 and the G2 light ($p < .016$) that showed R/S ratio of 0.34. Additionally, both AP673L and AP67 LEDs obtained significantly greater R/S ratio around 0.47 than the FL ($p < .001$) and L20AP67 ($p < .004$) (Fig.89).

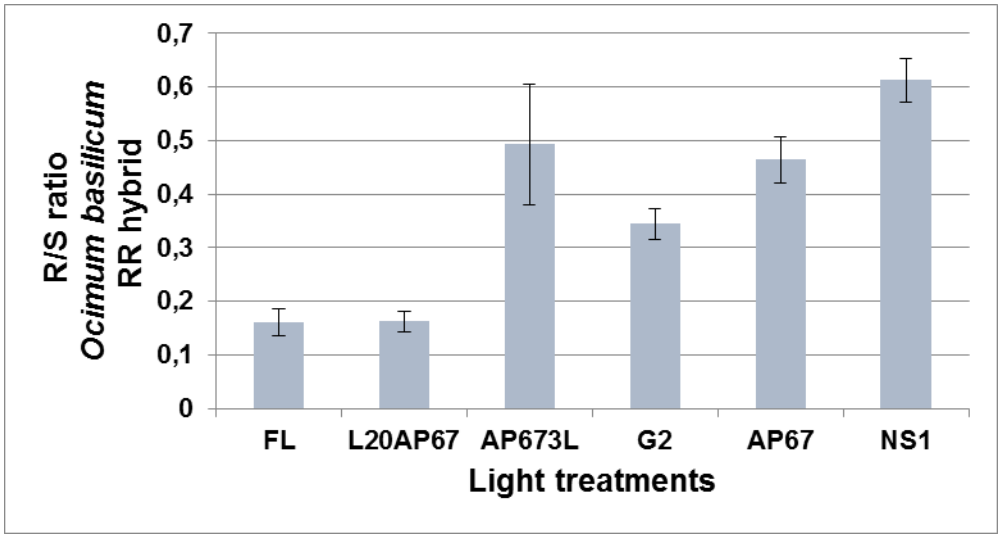


Figure 89. Root to shoot (R/S) ratio based on dry weight matter of Ocimum basilicum RR hybrid seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

***Cornus sanguinea* L.**

C. sanguinea seedlings showed significantly greater R/S ratio for LEDs such as the NS1, G2, AP673L and AP67 of 0.66, 0.56 and 0.55 respectively, compared to the lowest obtained under the FL (p< .001) conventional light with 0.24. L20AP67 light also showed significantly lower root allocation of 0.38 than NS1 (p<. 001), G2 (p<. 007), AP673L (p< .010) and AP67 lights (p< .016) (Fig.90).

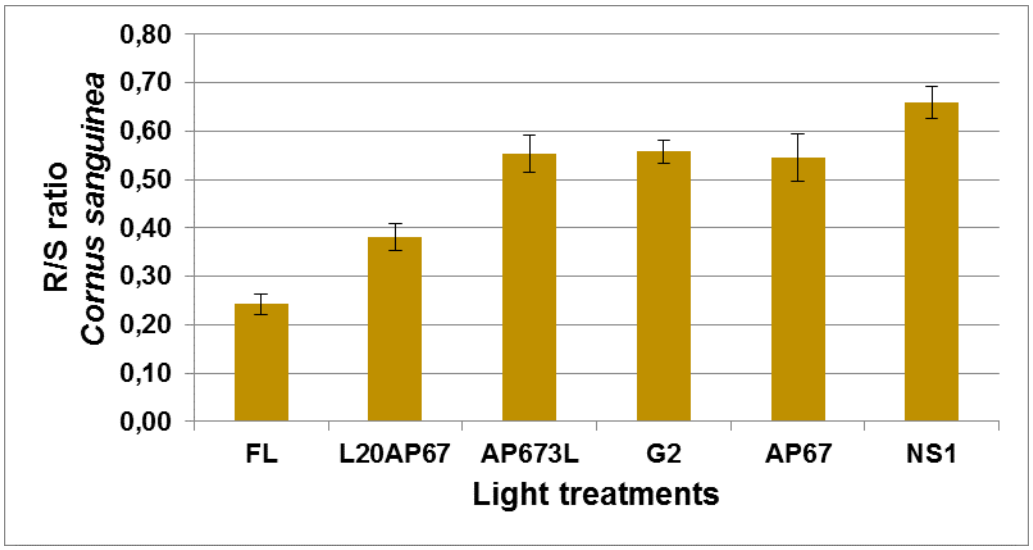


Figure 90. Root to shoot ratio (R/S) based on dry weight matter of Cornus sanguinea seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 4week experimental period.

***Prunus avium* L.**

Cherry seedlings had significantly lower dry weight allocation to the roots for the L20AP67 light with an average value of 0.22 compared to those seedlings grown under NS1 LED (p< .009) that had an average value of 0.42. The rest of the

treatments such as the AP67, AP673L, G2 and FL light showed similar R/S ratios of 0.33 and 0.26, respectively (Fig.91).

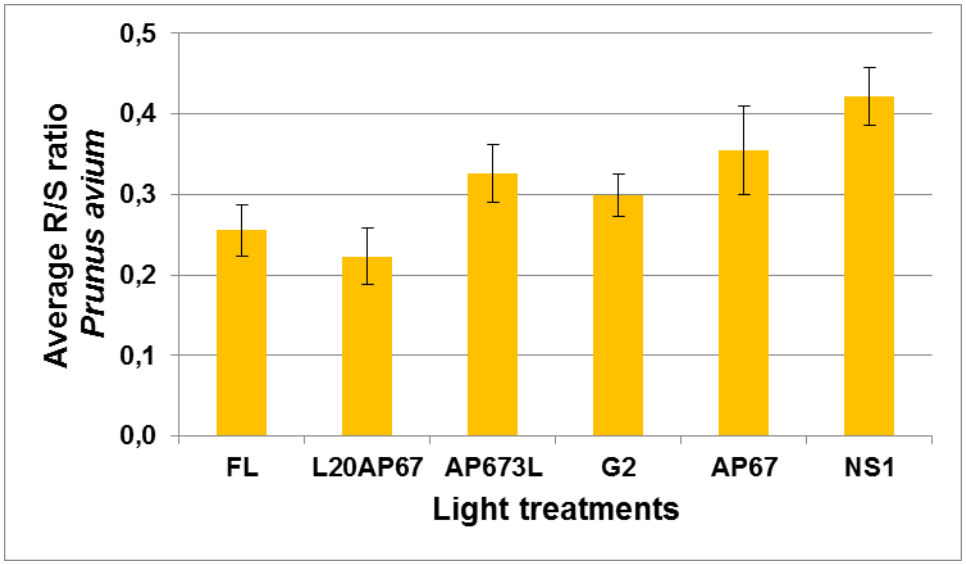


Figure 91. Root to shoot ratio (R/S) based on dry weight matter of Prunus avium seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in stabilized peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6 week experimental period.

***Punica granatum* L.**

No significant effect of the different light treatments was observed concerning the R/S ratio of pomegranate seedlings. The highest value was obtained under the G2 LED of 0.20, followed by NS1, L20AP67, AP673L, AP67 and FL showed similar average values around 0.17 (Fig.92).

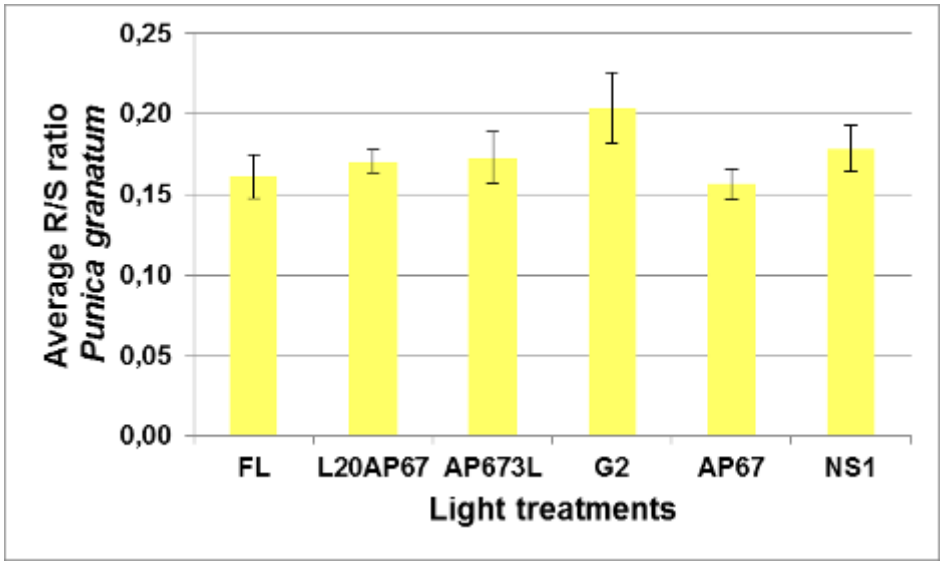


Figure 92. Root/shoot ratio (R/S) based on dry weight matter of Punica granatum seedlings under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments in enriched peat soil substrate and in QPD 104 RW mini-plug size at the end of the 6week experimental period.

3.3.10.5. Root Growth Potential (RGP) - New Root Length & New Root Dry Weight

***Pinus sylvestris* L. (provenances Greece-Sweden)**

According to our results the two provenances showed similar potential in the root growth. Specifically both of the provenances had no significant increase in the NRL after 15 days into the RGP bath however those pre-cultivated under G2 for the Greek provenance and L20AP67 for the Swedish provenance showed higher NRL of 3.21 cm and 3.45 cm, while the FL showed the lowest length of 2.5 cm (Fig.88) (Fig.93).

P. sylvestris seedlings of the Greek provenance showed no significant differences for the NRL after 31 days; however the longest obtained for the G2 pre-cultivation with an average value of 9 cm, following by the AP673L, L20AP67, AP67, NS1 with average values of 7.9 cm, 7.65 cm and 6.38 cm and the lowest for the FL with 5.5 cm (Fig. 94). Significant increase in the NRL was found for the Swedish provenance after 31 days into the RGP bath for the seedlings pre-cultivated under the L20AP67, G2 ($p<.001$) and AP673L ($p<.003$) with 10.28 cm, 9.87 cm and 9.41 cm compared to the FL with 5.15 cm (Fig.96).

For the NRDW no significant differences found either for the Greek provenance or the Swedish at the 15th day of RGP physiological test. However the highest for the Greek provenance was obtained for the NS1 pre-cultivated seedlings with an average value of 0.0037 g following by the G2, AP673L, AP67 and L20AP67 with 0.0031 g, 0.0022 g, 0.0018 g and 0.0015 g, while the lowest was for the FL with 0.0007 g (Fig.97). The highest NRDW for the Swedish provenance at the 15th day of the RGP test was under the G2 with an average value of 0.0042 g, following by the AP67, NS1, AP673L and L20AP67 with average values of 0.0029 g, 0.0026 g, 0.0021 g and 0.0015 g, while the lowest was found again for the FL light with an average of 0.0012 g (Fig.97).

At the end of the RGP test thus at the 31st day significant differences found for the NRDW of the *P. sylvestris* Greek provenance seedlings that pre-cultivated under the L20AP67 LED with an average value of 0.0195 g compared to the lowest obtained

under the pre-cultivation of FL (p<. 005) conventional light with almost the half weight of 0.0050 g. The rest of the lights such as the AP67, G2, AP673L and NS1 showed average values of 0.0092 g, 0.0069 g, 0.0060 g and 0.0058 g, respectively (Fig.98). For the Swedish provenance although there were no significant differences for the NRDW, the highest was for the seedlings pre-cultivated under the G2 with an average of 0.0058 g, following by the AP673L, NS1, AP67, L20AP67 with 0.0057 g, 0.0052 g, 0.0049 g, 0.0041 g and the lowest for the FL light with an average value of 0.0038 g (Fig.99).

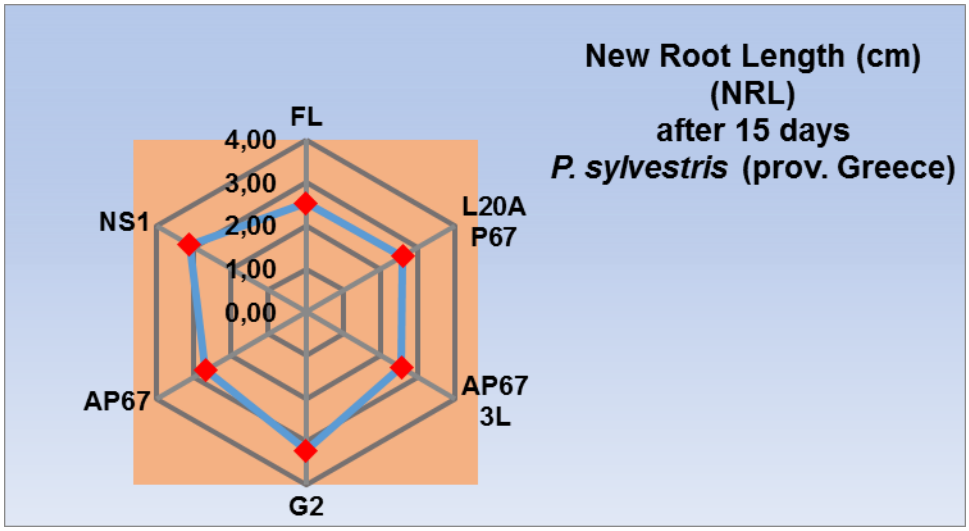


Figure 93. *New Root Length (cm) (NRL) of Pinus sylvestris provenance Greece pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.*

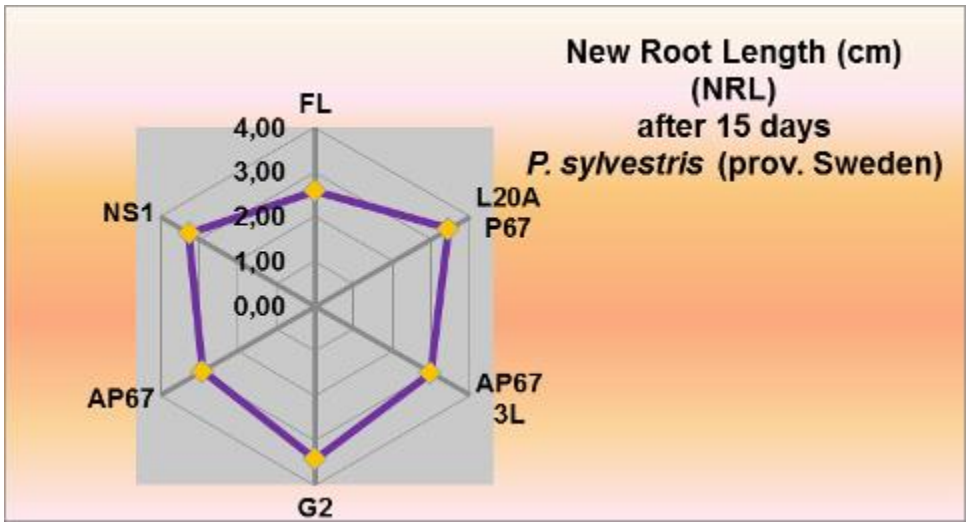


Figure 94. *New Root Length (cm) (NRL) of Pinus sylvestris provenance Sweden pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.*

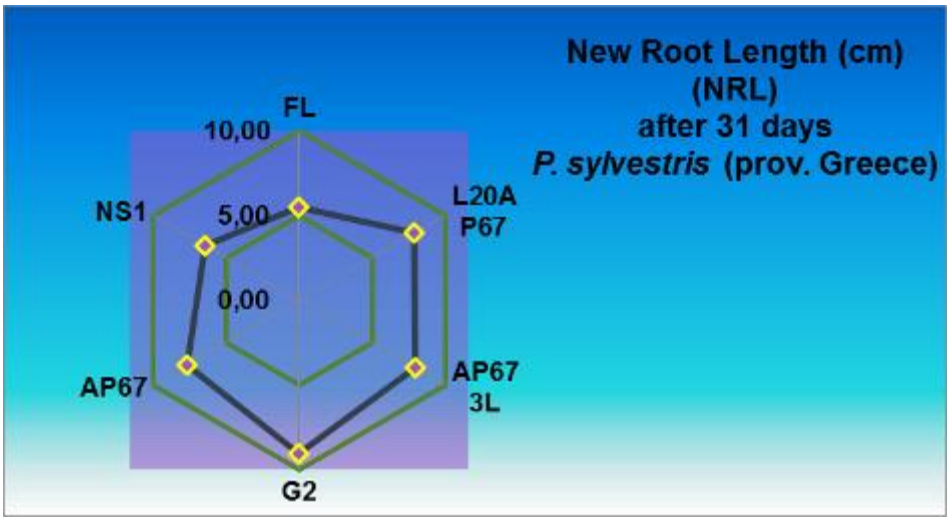


Figure 95. New Root Length (cm) (NRL) of Pinus sylvestris provenance Greece pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.

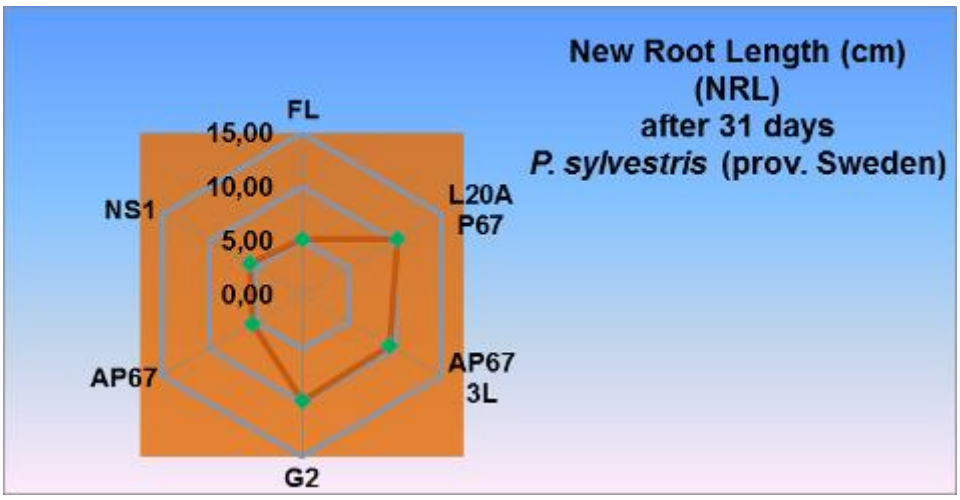


Figure 96. New Root Length (cm) (NRL) of Pinus sylvestris provenance Sweden pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.

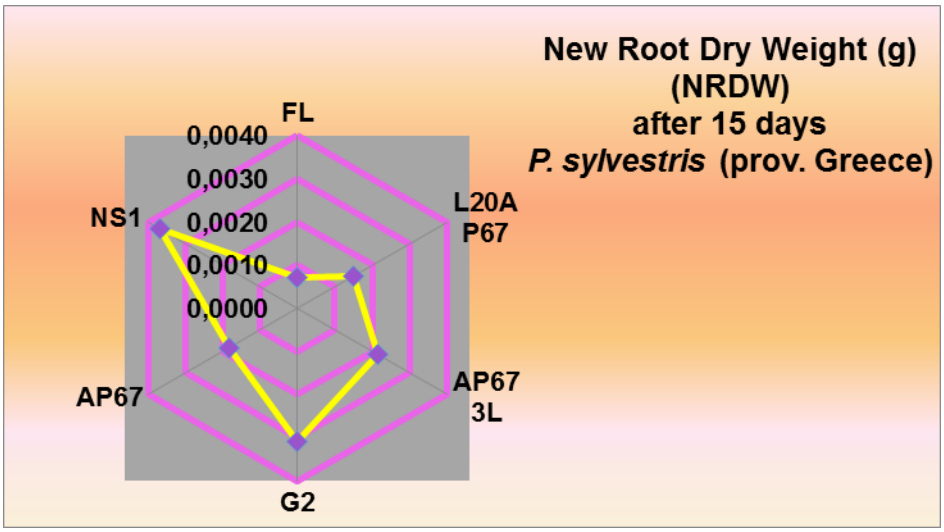


Figure 97. New Root Dry Weight (g) (NRDW) of Pinus sylvestris provenance Greece pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.

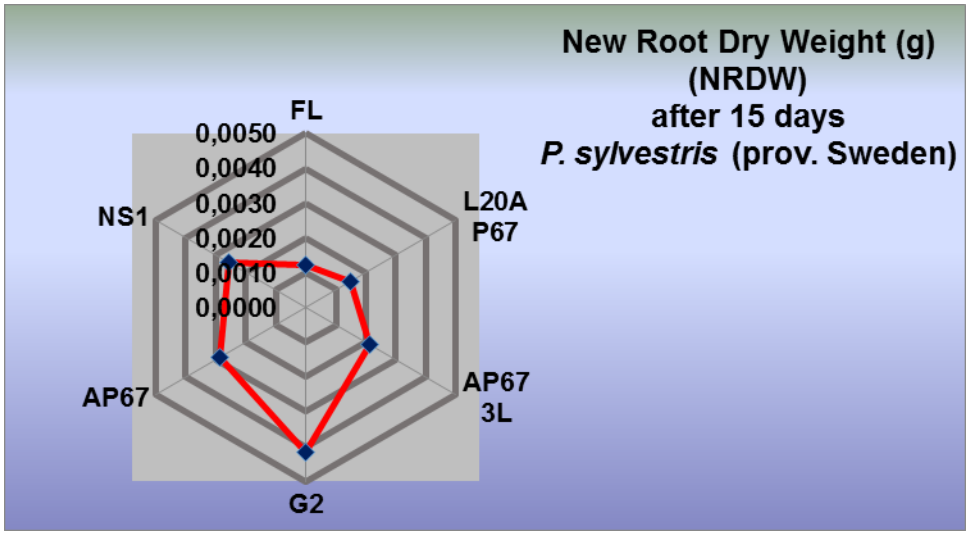


Figure 98. New Root Dry Weight (g) (NRDW) of Pinus sylvestris provenance Sweden pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.

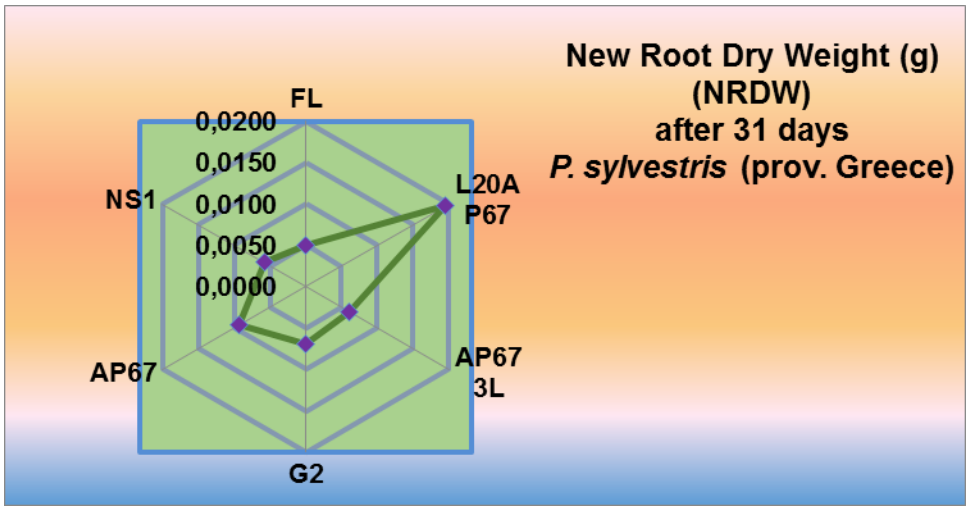


Figure 99. *New Root Dry Weight (g) (NRDW) of Pinus sylvestris provenance Greece pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.*

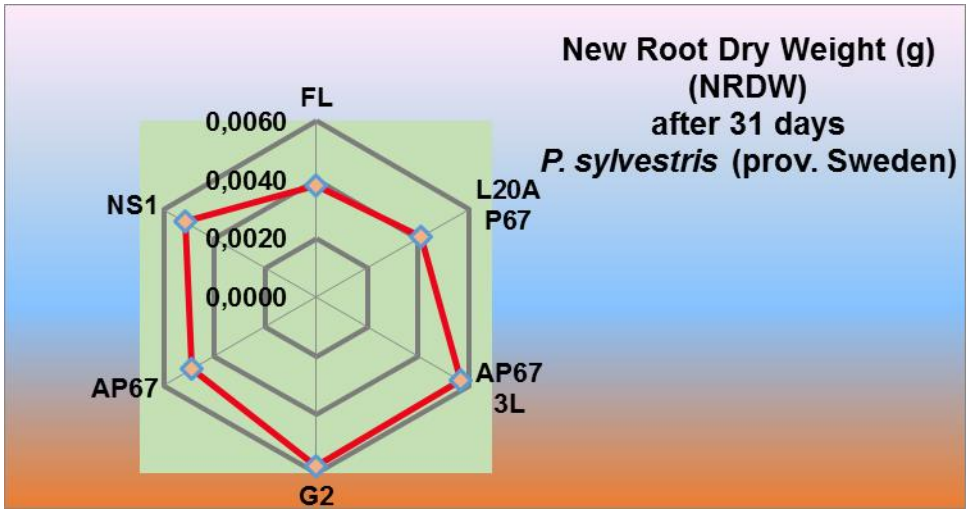


Figure 100. *New Root Dry Weight (g) (NRDW) of Pinus sylvestris provenance Sweden pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.*

***Myrtus communis* L.**

NS1 LED light significantly benefited the NRL with 7.53 cm and NRDW with 0.025 g of *Myrtus* seedlings after 31 days into the RGP bath compared to the rest of lights (p<. 001) (Fig. 101) (Fig.102). In contrast less beneficial effect found under the FL

and L20AP67 light qualities by means of the potential formation of new roots indicating possible lower field performance.

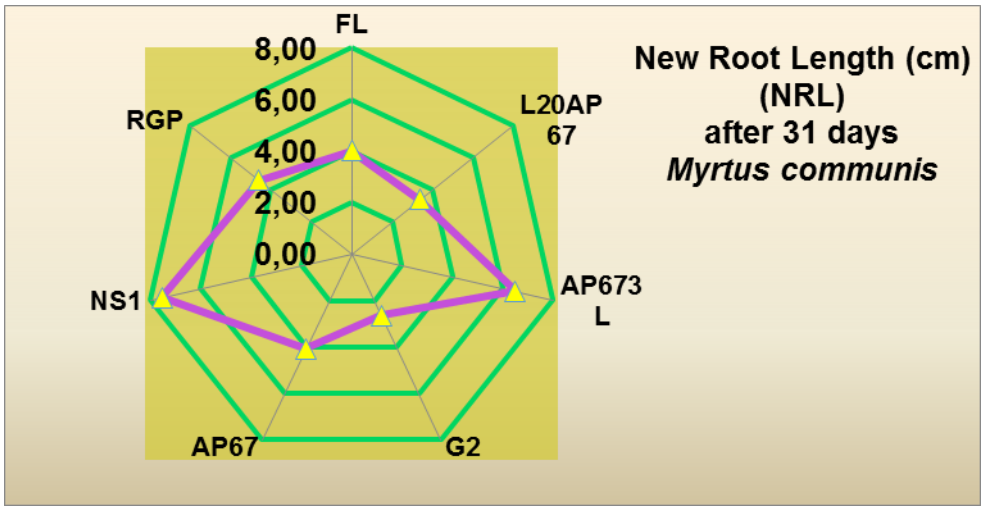


Figure 101. *New Root Length (cm) (NRL) of Myrtus communis pre-cultivated under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments and left grown for 31 days in RGP bath.*

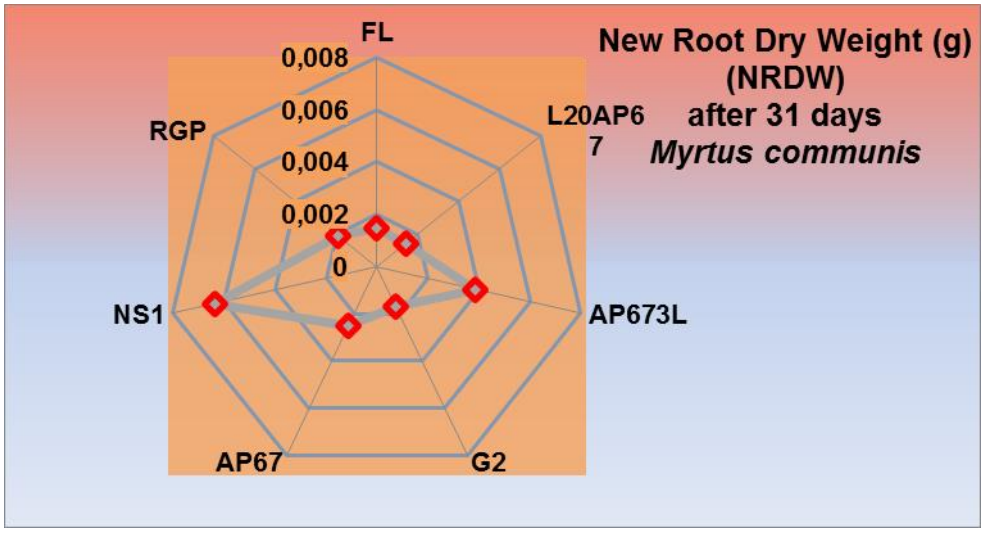


Figure 102. *New Root Dry Weight (g) (NRDW) of Myrtus communis pre-cultivated under FL, L20AP67, AP673L, G2, AP67, NS1 & RGP (FL & sodium lamps) light treatments and left grown for 31 days in RGP bath.*

***Ocimum basilicum* L.**

After 15 days of the RGP physiological treatment, there were significant differences for the NRL. L20AP67 pre-cultivation obtained significantly shorter new roots with an

average length of 8.32 cm compared to the AP673L (p<. 005) and G2 (p<. 007) that obtained average new root length of 17.08 cm and 16.85 cm, respectively. Control light had an average value of 13.63 cm, NS1 had 12.70 cm and AP67 had 11.15 cm (Fig.103). After 31 days of pre-cultivation, basil seedlings showed no significant effect on the length of new roots at any of the different light treatments. However, it was better predicted under the L20AP67 with 25.20 cm contrast to G2 with the lowest value of 19.30 cm. As for the rest of the treatments, FL and AP67 obtained 24.60 cm, while NS1 and AP673L obtained 20.25 cm (Fig.104).

In the first measurement of the new root dry weight, L20AP67 seedlings had significantly lighter roots of 0.004 g (p<. 017) than AP673L with 0.0166 g. The rest of treatments showed average values of 0.013 g, 0.012 g, 0.009 g and 0.008 g by the FL, G2, NS1 and AP67 pre-cultivation (Fig.105). Following at the 31st day significantly lighter new roots were found for the *O. basilicum* LL seedlings that were pre-cultivated under NS1 light treatment with 0.019 g compared to those pre-cultivated under L20AP67 (p<. 001) with 0.037 g, FL (p=.001) with 0.036 g and AP673L (p<. 019) with 0.032 g (Fig.106).

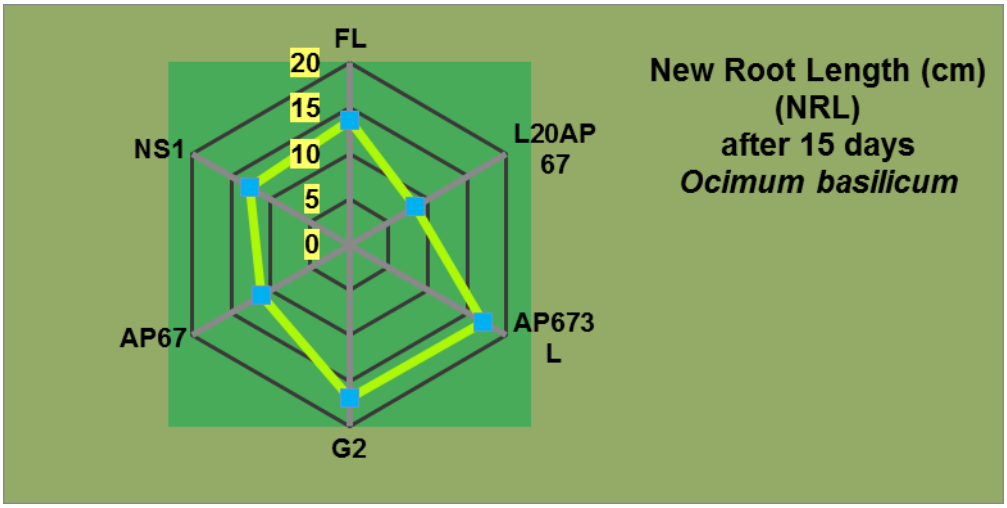


Figure 103. New Root Length (cm) (NRL) of Ocimum basilicum LL *pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.*

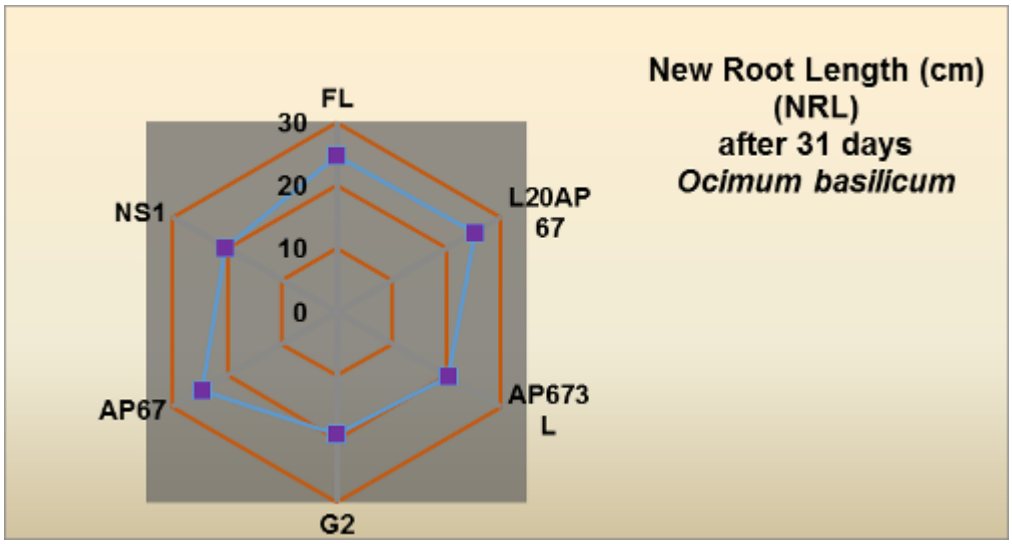


Figure 104. New Root Length (cm) (NRL) of Ocimum basilicum LL *pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.*

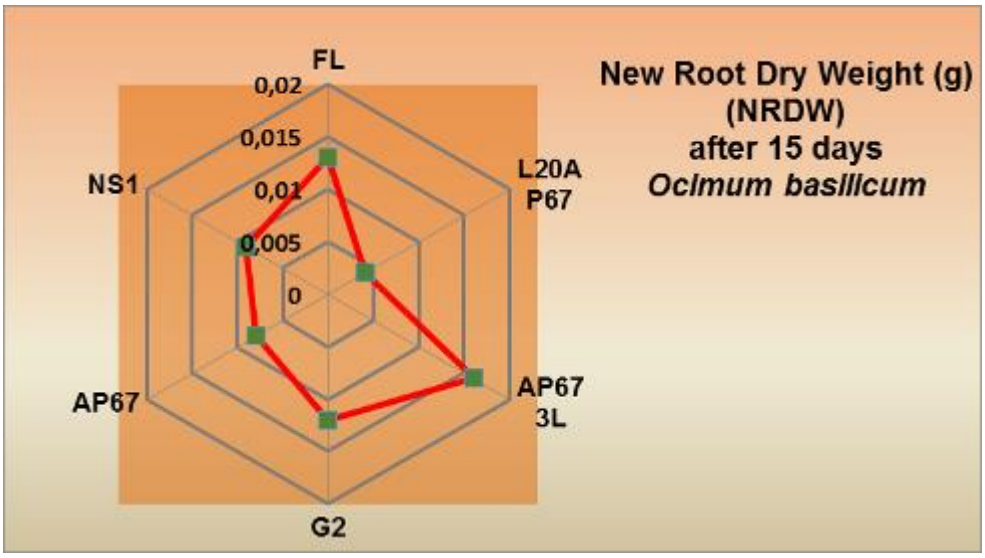


Figure 105. New Root Dry Weight (g) (NRDW) of Ocimum basilicum LL *pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.*

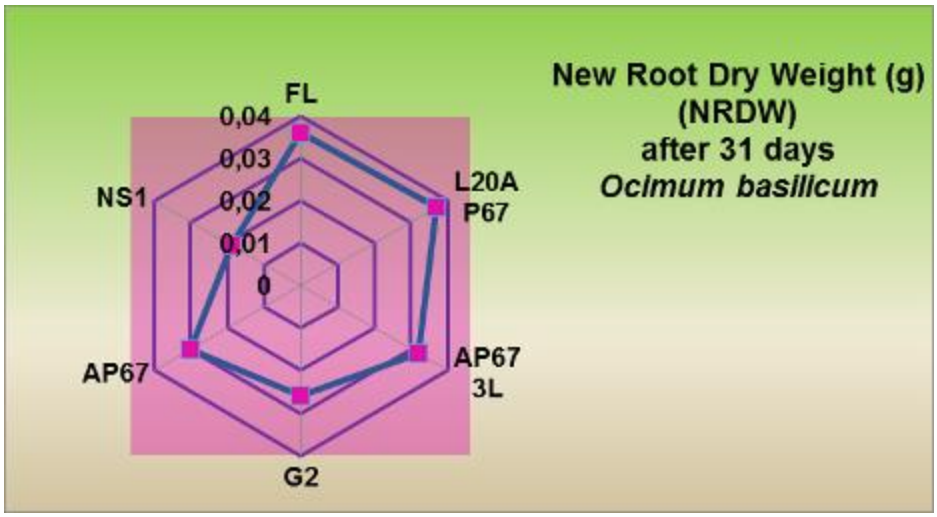


Figure 106. New Root Dry Weight (g) (NRDW) of Ocimum basilicum LL *pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.*

Ocimum basilicum RR hybrid

Results for the new root length for RR hybrid seedlings after 15 days, showed no significant differences between the treatments. However, AP673L had the greatest average value of 14.02 cm followed by NS1, G2, FL and L20AP67 that showed average values for the NRL of 13.55 cm, 11.53 cm, 10.72 cm, 8.45 cm and 8.27 cm respectively (Fig.107). However, after 31 days in the RGP bath, pre-cultivation of NS1 LED induced the shortest new roots of 12.97 cm compared to significantly longer obtained by AP67 ($p < .004$) that showed 23.23 cm. The rest of the light treatments pre-cultivation showed average values for the NRL in descending order of 21.87 cm, 21.27 cm, 17.8 cm and 17.35 cm by the FL, AP673L, G2 and L20AP67, respectively (Fig.108).

According to the results for the NRDW after 15 days into the RGP bath, no significant differences were found regardless the different light pre-cultivation; however heavier roots were obtained for the NS1 and AP673L of 0.011 g, following by the G2, AP67, FL and L20AP67 that had very low values range of 0.008 g- 0.004 g (Fig.109). At the end of the 31 days in the RGP bath, basil seedlings pre-cultivated under NS1 obtained significantly lighter roots of 0.009 g compared to G2, AP673L, L20AP67 ($p < .001$) and A6P67 ($p < .005$) LEDs that had average values of 0.030 g, 0.027 g, 0.026 g and 0.023 g, respectively. Moreover FL ($p < .002$) pre-cultivation also induced significantly lighter roots of 0.015 g than those for the G2 (Fig.110).

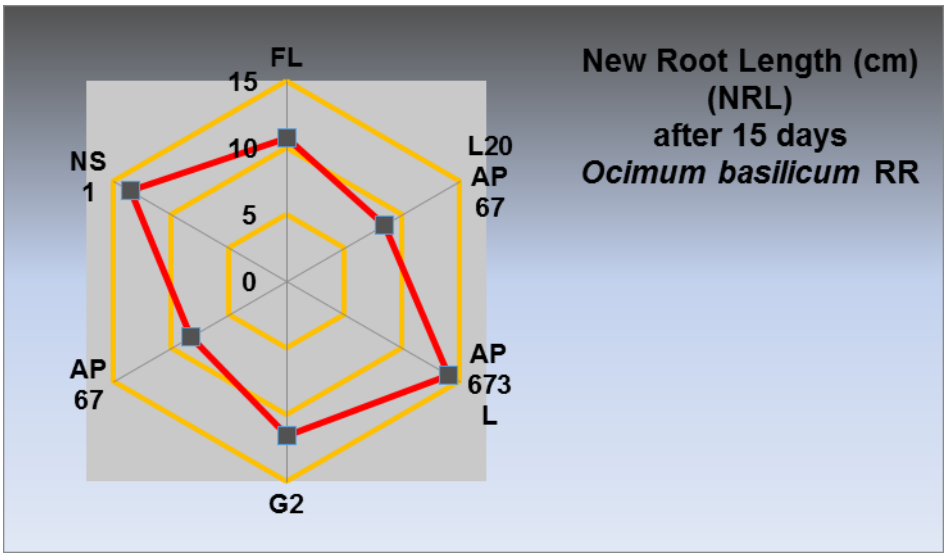


Figure 107. New Root Length (cm) (NRL) of Ocimum basilicum RR *pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.*

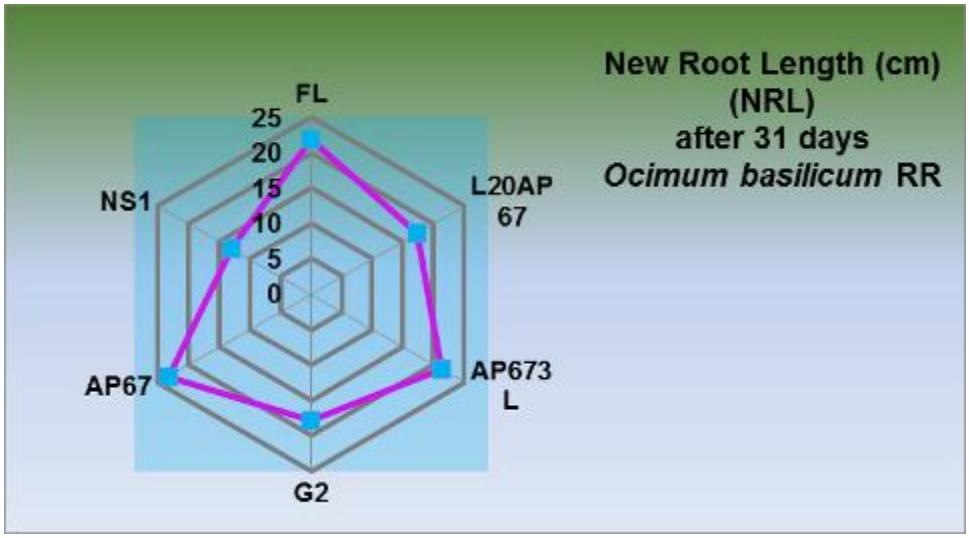


Figure 108. New Root Length (cm) (NRL) of Ocimum basilicum RR *pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.*

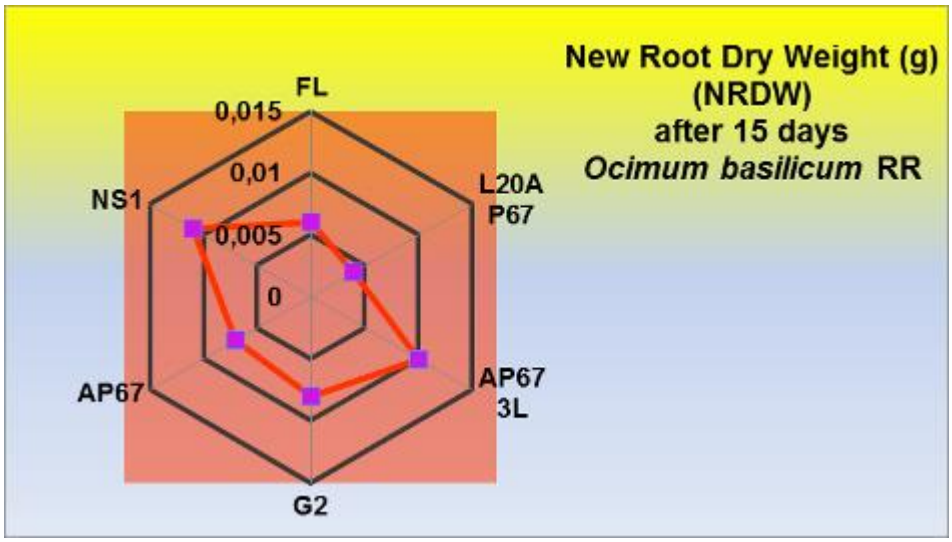


Figure 109. New Root Dry Weight (g) (NRDW) of Ocimum basilicum RR *pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.*

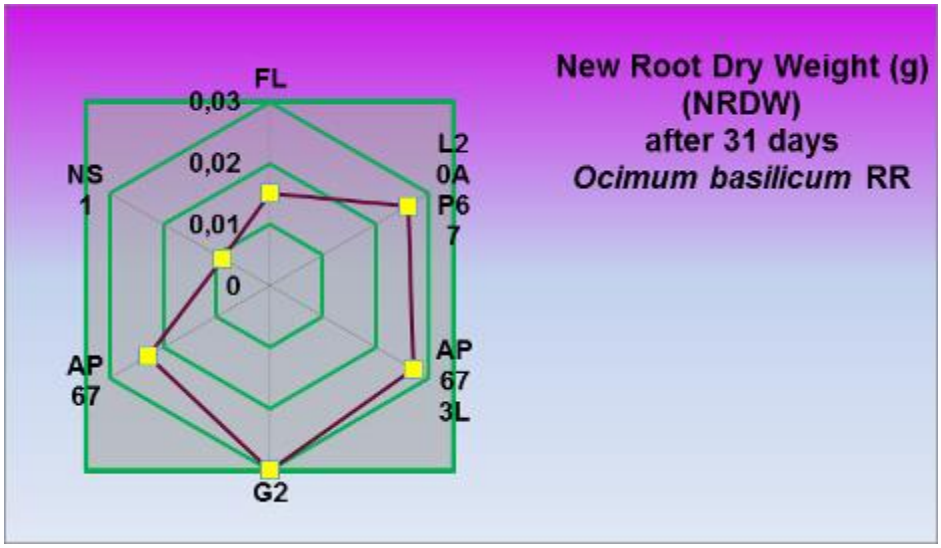


Figure 110. New Root Dry Weight (g) (NRDW) of Ocimum basilicum RR *pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.*

***Cornus sanguinea* L.**

Examining *C. sanguinea* seedlings we found that different light treatments at the pre-cultivation period into the growth chambers induced a significant effect ($p < .001$) on the formation of new roots. The seedlings that were pre-cultivated under NS1 LED had significantly longer new roots of 17.47 cm compared to those under AP673L with 8.54 cm, L20AP67 with 8.09 cm ($p< .001$), FL with 11.61 cm ($p< .007$) and G2 LED with 11.82 cm ($p< .010$). In addition, AP67 light with an average new root length of 15.62 cm induced the formation of longer roots than L20AP67 ($p< .001$) and AP673L lights ($p= .001$) (Fig.111).

The analysis of the new root dry weight showed significant heavier roots for the *Cornus* seedlings that were pre-cultivated under AP67 LED having an average value of 0.039 g, compared to those grown under L20AP67 ($p< .006$) with 0.017 g and AP673L ($p< .018$) with 0.020 g. Moreover, NS1 light promoted the formation of significantly heavier new roots of 0.039 g than L20AP67 ($p< .007$) . Finally, FL conventional light and G2 LED showed average NRDW values of 0.028 g and 0.030 g, respectively (Fig.112).

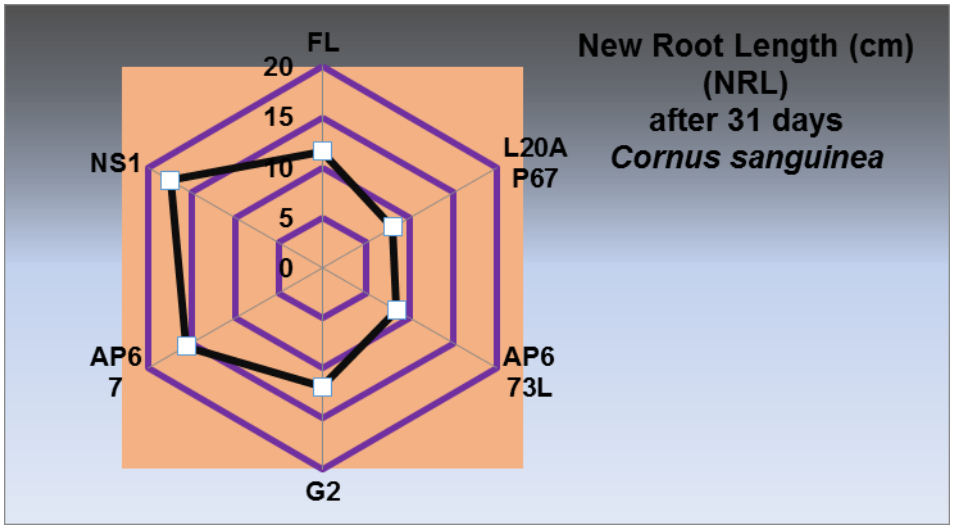


Figure 111. New Root Length (cm) (NRL) of Cornus sanguinea pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.

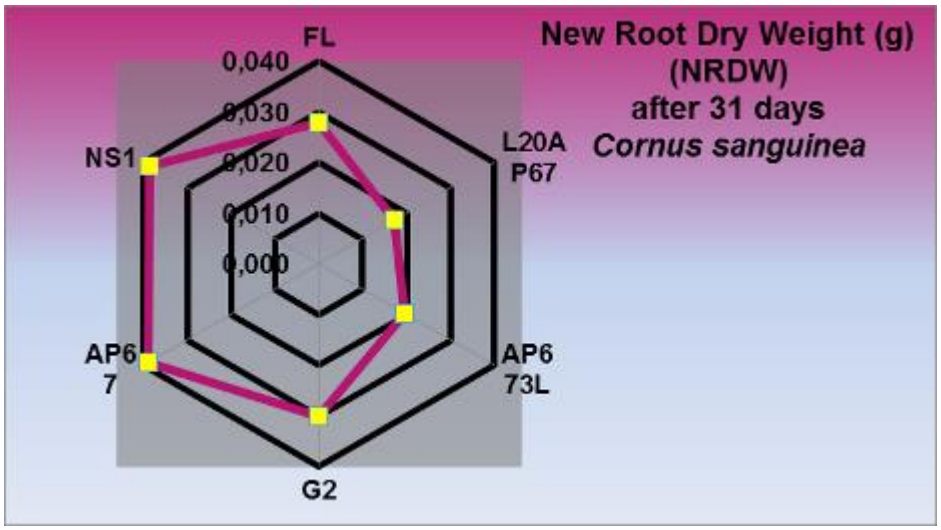


Figure 112. *New Root Dry Weight (g) (NRDW) of Cornus sanguinea pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.*

***Prunus avium* L.**

Cherry seedlings pre-cultivated under different light treatments showed no significant differences for the NRL after 31 days into the RGP bath; however shorter roots formed by the L20AP67 that obtained an average value of 16.04 cm, following by the AP673L, NS1, FL, AP67 and G2 that showed average NRL values of 18.9 cm, 21.03 cm, 23.66 cm, 25.7 cm and 25.85 cm, respectively (Fig.113). *Prunus* seedlings response was better under G2 LED that obtained significantly higher NRDW of 0.086 g than AP673L and L20AP67 that had average NRDW value of 0.044 g ($p < .001$). The rest of the lights such as the AP67, NS1 and FL showed average NRDW values of 0.073 g, 0.064 g and 0.061 (Fig.114).

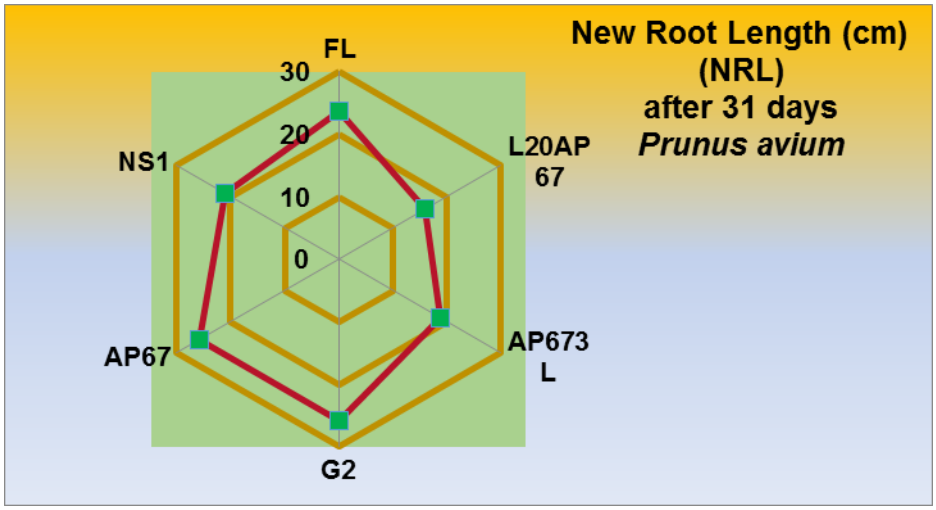


Figure 113. New Root Length (cm) (NRl) of Prunus avium pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.

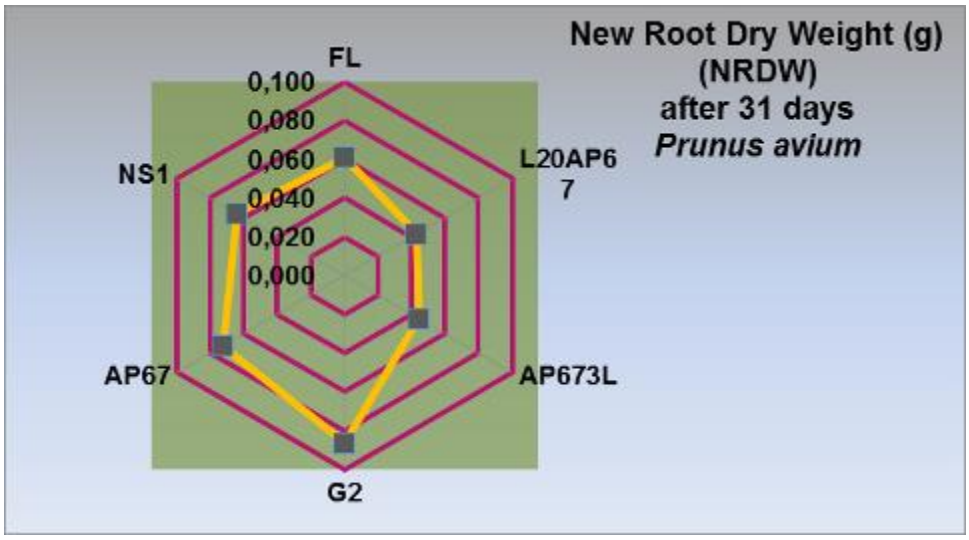


Figure 114. New Root Dry Weight (g) (NRDW) of Prunus avium pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.

***Punica granatum* L.**

In the first replicate of the RGP test no significant differences found for the NRL irrespective the light spectrum; However better results found for the NS1 that obtained of 4.91 cm, following by the FL, G2, AP63L, L20AP67 and AP67 that showed average values of 4.78 cm, 4.60 cm, 4.46 cm, 3.71 cm and 3.28 cm, respectively (Fig.115). Further at the second trial at 31st day of RGP test, seedlings

pre-cultivated under AP67 LED had significantly induced longer new roots of 18.81 cm compared to the rest of the treatments such as the G2, L20AP67, FL, AP673L ($p < .001$) and NS1 ($p < .002$) that had average values of 6.27 cm, 7.34 cm, 10.47 cm, 10.6 cm and 11.96 cm, respectively. In addition, G2 also induced the formation of shorter new roots compared to NS1 ($p < .016$) (Fig.116).

Results for the first 15 days in the RGP showed no significant effect on NRDW irrespective the light treatment pre-cultivation; actually there were too low average values ranged from 0.003 g- 0.0012 g (Fig.117). However at the end of the 31 days into the RGP bath, L20AP67 pre-cultivation of pomegranate seedlings showed significant NRDW increase of 0.015 g compared to those pre-cultivated under the NS1 ($p < .001$) and G2 ($p < .003$) lights that showed the lowest around 0.009 g. As for the AP67, FL and AP673L showed similar average values for the NRDW of 0.010 g (Fig.118).

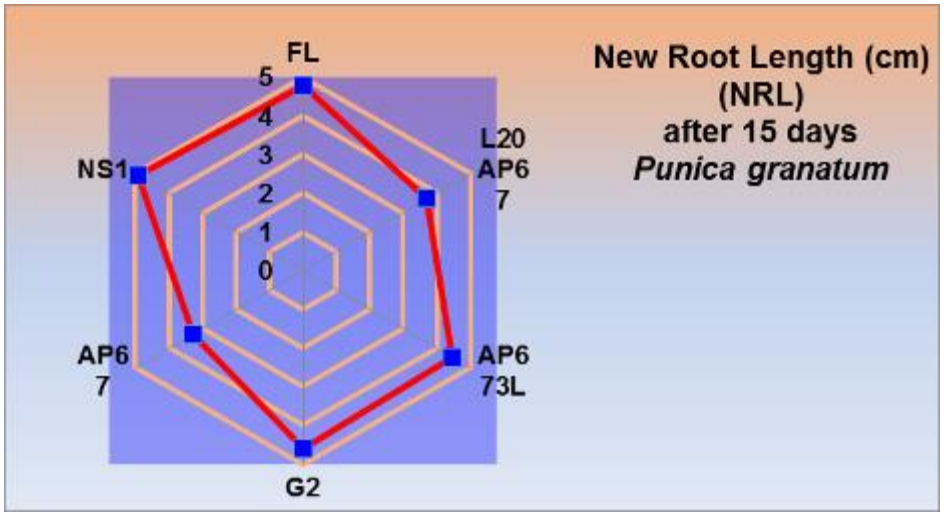


Figure 115. New Root Length (cm) (NRL) of Punica granatum pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.

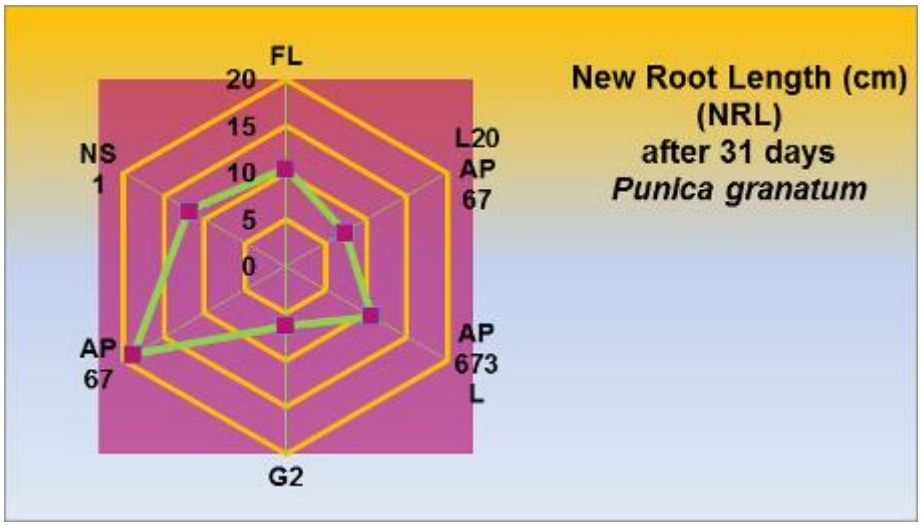


Figure 116. *New Root Length (cm) (NRL) of Punica granatum pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.*

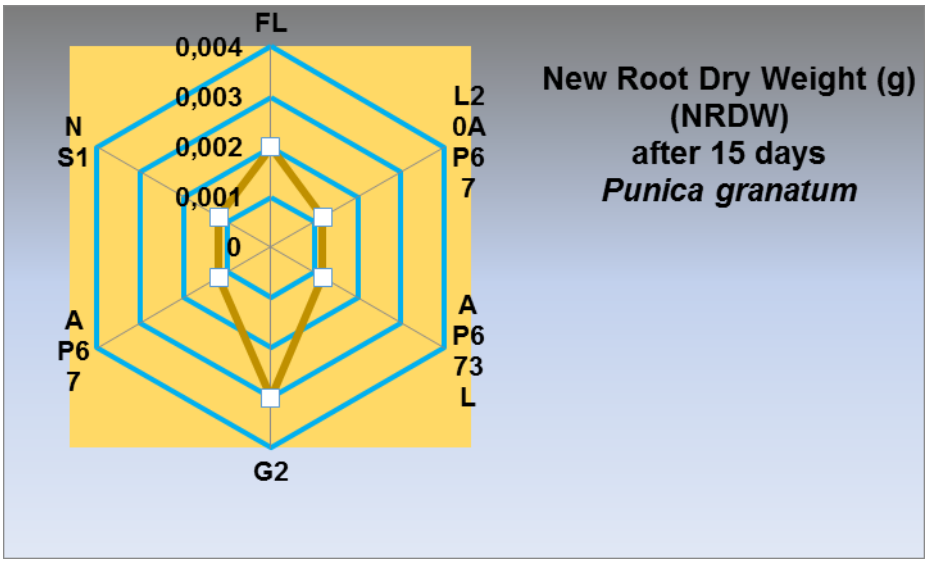


Figure 117. *New Root Dry Weight (g) (NRDW) of Punica granatum pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 15 days in RGP bath.*

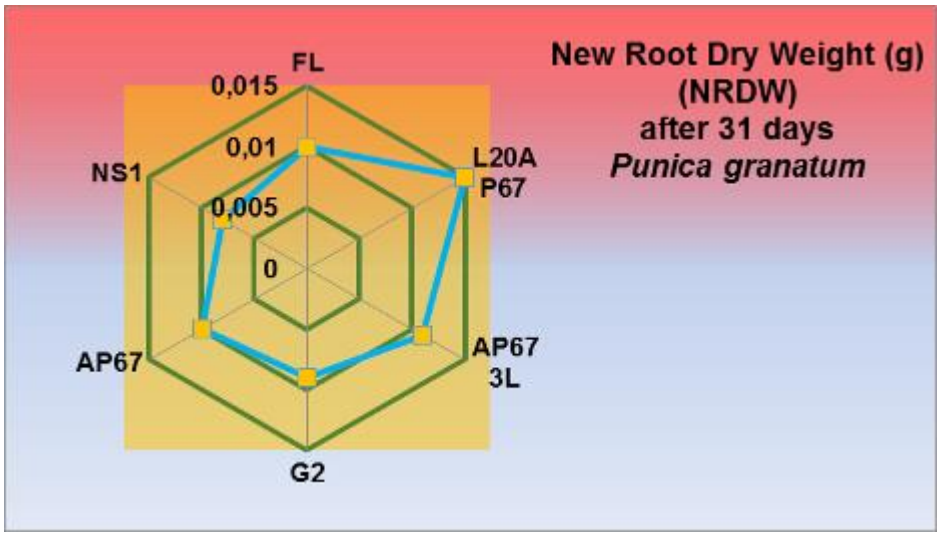


Figure 118. New Root Dry Weight (g) (NRDW) of Punica granatum pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 light treatments and left grown for 31 days in RGP bath.

3.3.11. Nursery performance

3.3.11.1. Seedling survival (%)

After 6 months at the nursery high percentage of survival was succeeded for all light treatments (Fig.119). Especially for the AP673L LED light that shown 100% survival.

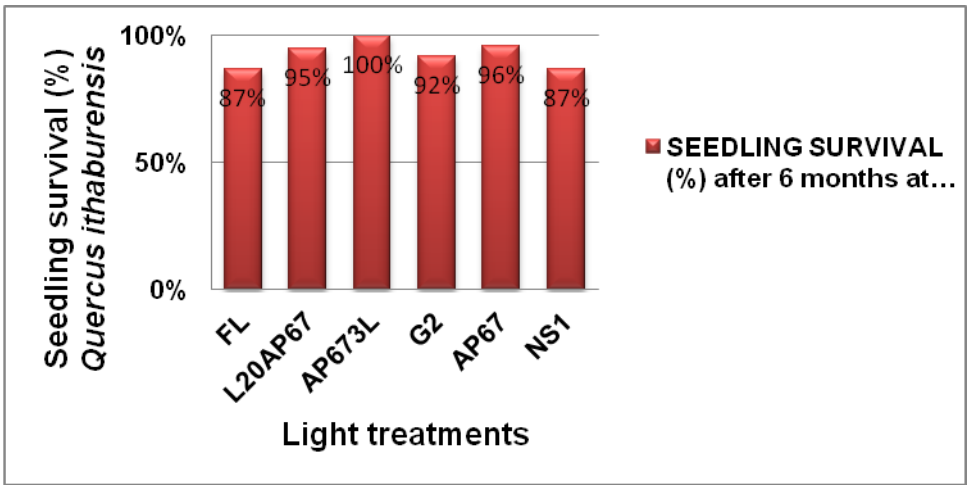


Figure 119. Seedling survival (%) of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.

3.3.11.2. Number of leaves

After a 6month period that *Q. ithaburensis* grown at Xalkidona nursery, significant differences found for the leaf formation (p<. 001). Seedlings pre-cultivated under the L20AP67 light formed 11 leaves in average that was differed significantly for those of NS1 and AP67 LEDs that had an average of 6 leaves. The rest of lights such as FL, AP673L and G2 had formed in average 9, 8 and 7 leaves (Fig.120).

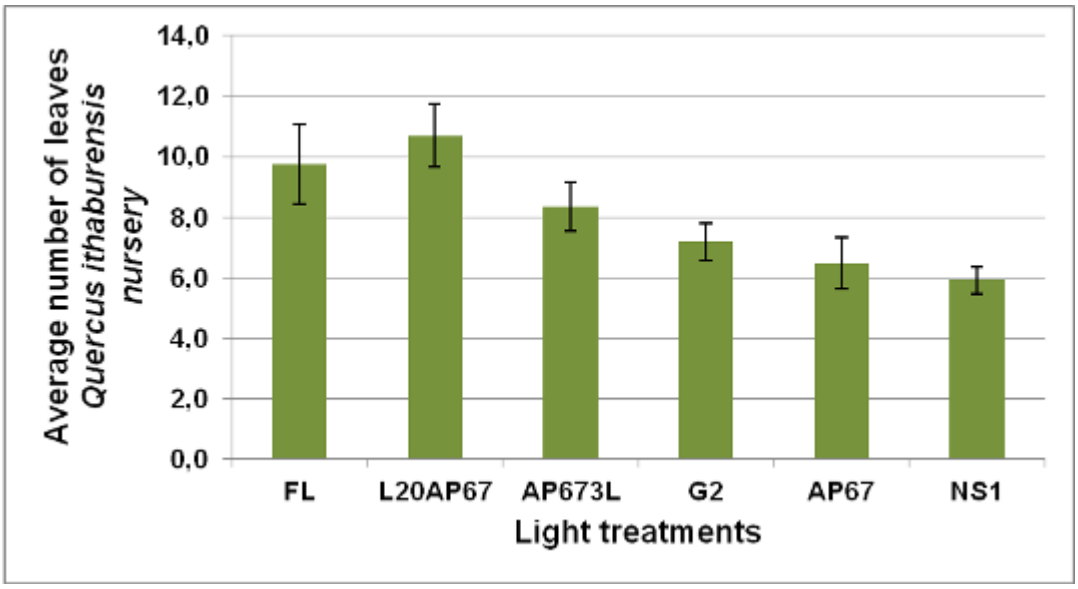


Figure 120. Average number of leaves of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.

3.3.12. Chlorophyll content (CCI)

Chlorophyll content of seedlings pre-cultivated under LED treatments showed higher average values compared to FL light. G2 LED pre-cultivation induced significantly higher CCI=13.4 compared to the CCI=8.4 obtained for the FL ($p<.003$). The rest of LEDs such as NS1, AP673L, L20AP67 and AP67 had average CCI values of 11.9, 10.7, 9.6, 9.3, respectively (Fig.121).

It should be mentioned that CCI of *Q. ithaburensis* seedlings into growth chambers (Fig.38) showed greater average values for the FL light (18.4) that seems to be reduced by ten times under solar radiation, while G2 LED still gain high CCI.

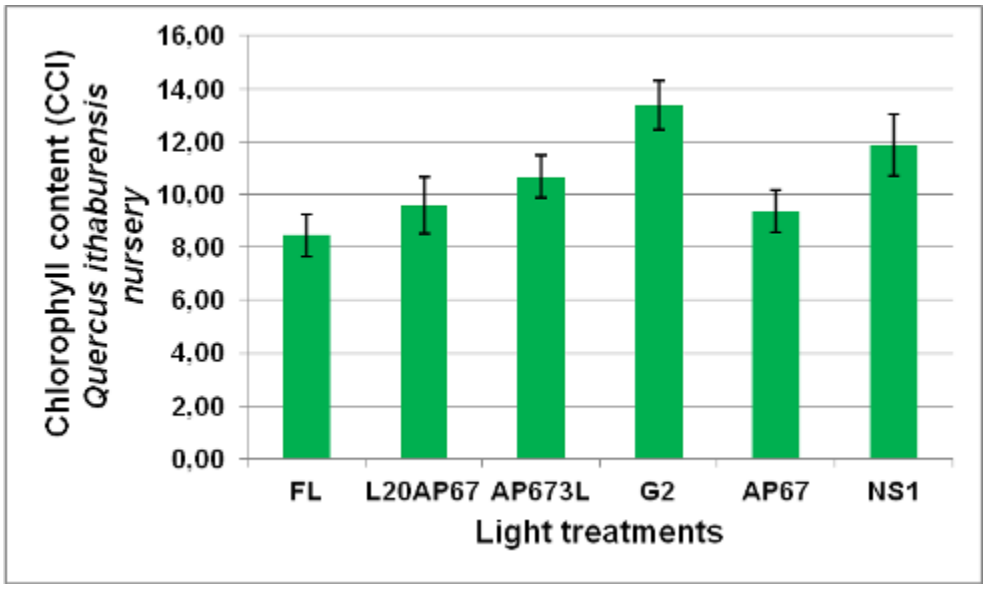


Figure 121. *Chlorophyll content (CCI) of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.*

3.3.13. Plant height

Significantly taller seedlings were found for those pre-cultivated under the FL and L20AP67 lights with average height of 20.9 cm and 19.3 cm. FL light seedlings were significantly taller (p<. 001) compared to the rest of LEDs such as AP673L, NS1, AP67 and G2 with 12.5 cm, 12.8 cm, 14 cm and 14.4 cm, respectively. L20AP67 also differed significantly with the LEDs of AP673L, NS1 (p<.001), and AP67 (p<. 004) (Fig. 122).

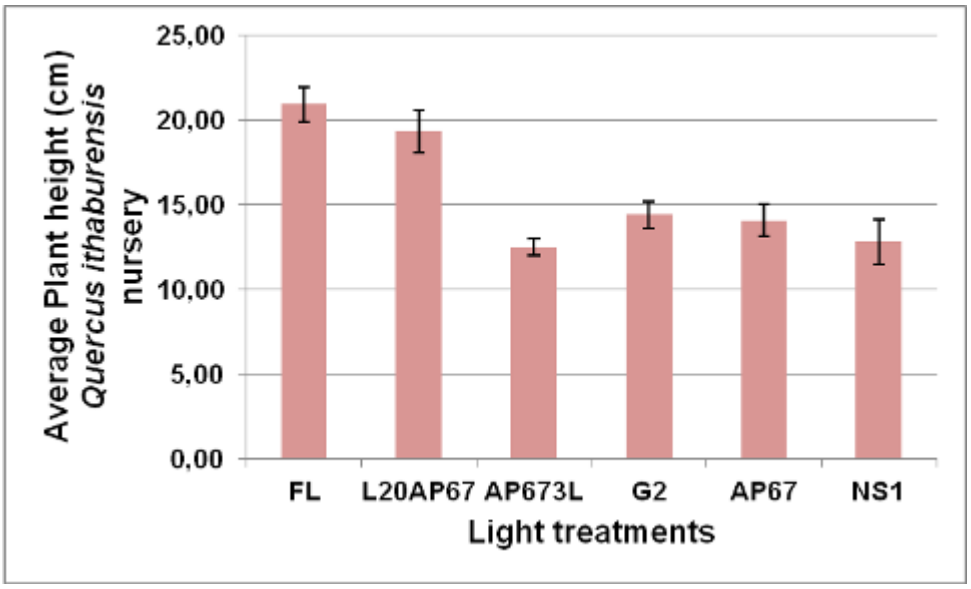


Figure 122. *Average Plant height (cm) of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.*

3.3.14. Shoot height (SH)

Likewise the plant height significant differences found for the SH between FL (p<.001), L20AP67 with average values of 19.7 cm, 18.34 cm with the rest of LEDs, such as the AP673L, NS1 (p<. 001), and AP67 (p<. 004) with 11.8 cm, 11.7, and 13.47 cm (Fig.123). G2 LED light had average SH value of 13.28 cm.

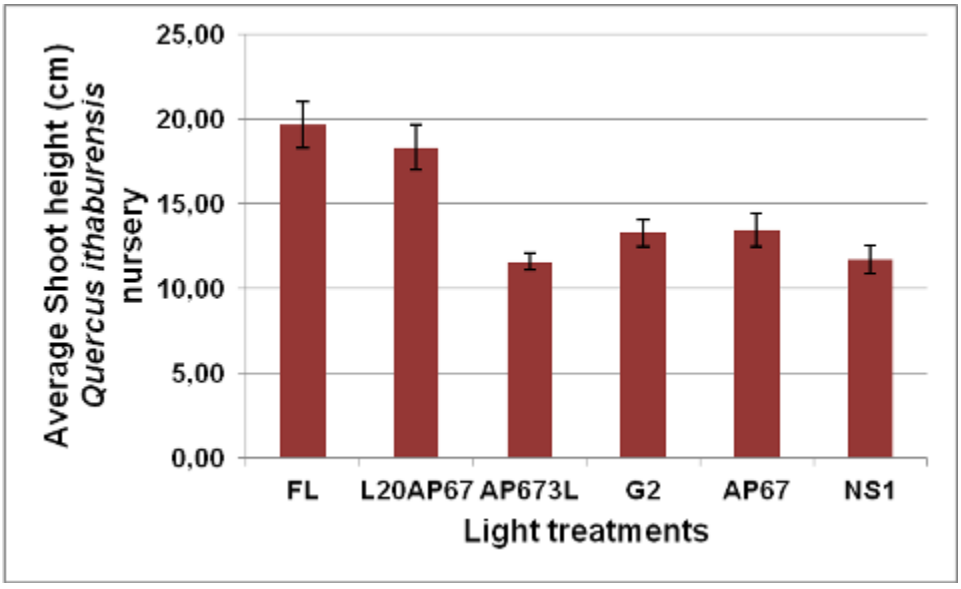


Figure 123. *Average Shoot height (SH) (cm) of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.*

3.3.15. Shoot Diameter (SD)

No significant differences found for the SD between the lights. However the largest was found for the G2 with 11.9 mm, following by the NS1, AP67, L20AP67, AP673L and the smallest for the FL with 11.8 mm, 11.7 mm, 11.63 mm, 11.58 mm and 10.5 mm, respectively (Fig. 124).

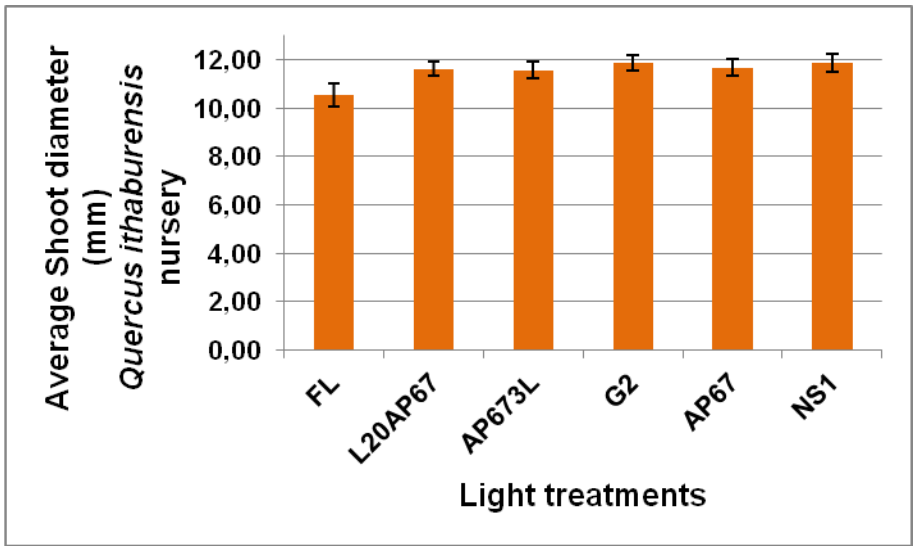


Figure 124. *Average Shoot Diameter (SD) (cm) of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.*

3.3.16. Root collar Diameter (RCD)

Significantly larger RCD found for the seedlings pre-cultivated under the L20AP67 LED with 14 mm only compared to the FL with 12 mm (p<. 010) light which presented the smallest of all lights (Fig.125). As for the rest of LEDs such as the AP673L, G2, AP67 and NS1 the average values were 13.8 mm, 13.78 mm, 13.36 mm and 13.26 mm.

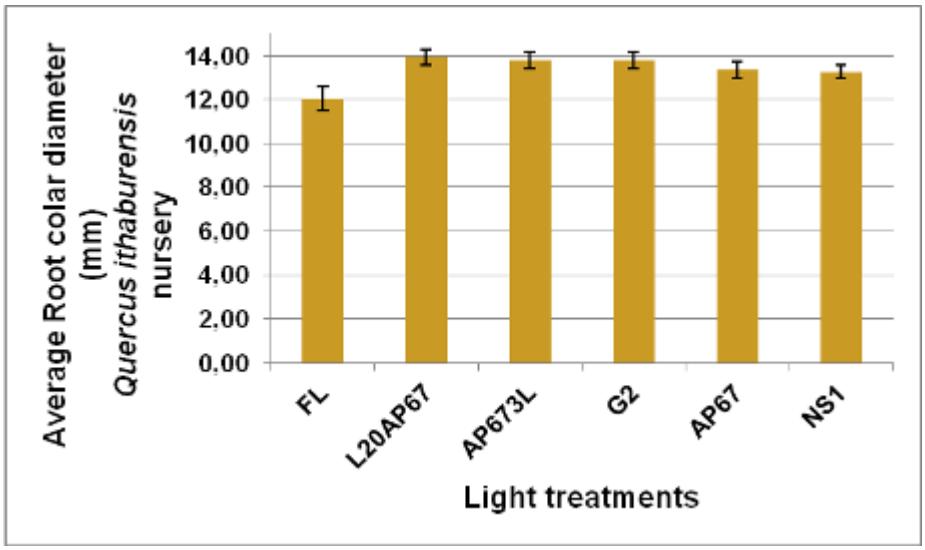


Figure 125. Average Root Collar Diameter (RCD) (cm) of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.

3.3.17. Dry weight

The pre-cultivation under LEDs had a significant effect on DWL (p<. 001) and DWR (p<. 002) of *Q. ithaburensis* seedlings after their 6 month growth under solar radiation (Fig.126). For the DWL, AP673L and NS1 LEDs showed significantly higher allocation compared to the other lights. AP673L and NS1 average values for the DWL of 0.663 g and 0.626 g that were significantly higher than the FL (p<. 001) and G2 (p<. 001) with 0.373 g and 0.456 g, respectively. The lowest average value for the DWL was for the FL light and significant differences found with all LEDs (p<. 001) except from the G2.

As for the DWR FL light also had the lowest average value of 2.833 g and differed significantly with all LEDs (p<. 001) except from the L20AP67 with average value of 5.266 g. The highest of all was for the NS1 with 6.773, following by the AP67 with 6.443 g, G2 with 6.270 g and AP673L with 5.523 g (Fig.126).

As for the DWS, FL light induced the lowest average value of 3.943 g, following by the L20AP67 with 6.893 g, AP673L with 7.026 g, G2 with 7.613 g, AP67 with 7.883 g and NS1 with 8.273 g, respectively.

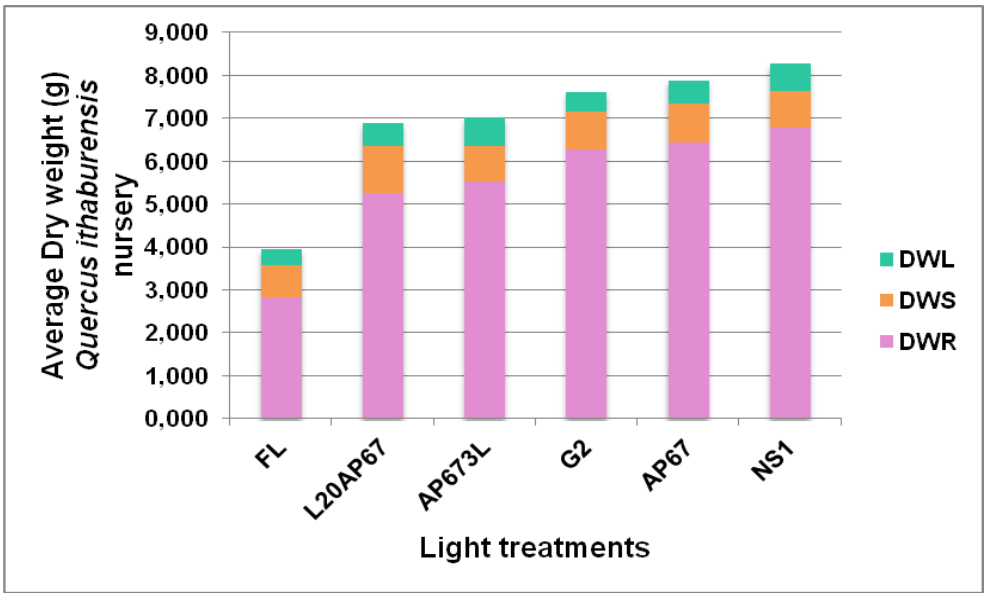


Figure 126. Dry weight of leaves (DWL), shoots (DWS) & roots (DWR) (g) of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.

3.3.18. R/S ratio

Significantly allocation to the roots than to the above parts of the plants were found for the pre-cultivated seedlings of G2, NS1, AP67 with ratios of 4.71, 4.49 and 4.46 compared to FL with a ratio of 2.55 which was the lowest. AP673L and L20AP67 had also greater ratio than FL with 3.66 and 3.26, respectively (Fig.127).

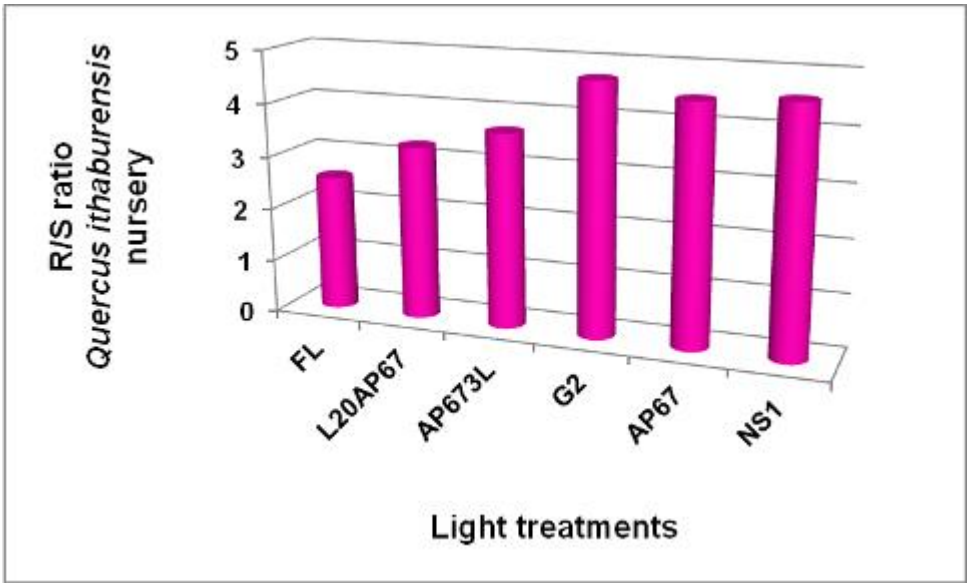


Figure 127. Root to Shoot ratio (R/S) based on dry weight of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery

3.3.19. Dickson's Quality Index (DQI)

Dickson's quality index (DQI) was a reliable predictor of seedling field performance for those pre-cultivated under LEDs than the FL light. Especially for the NS1 LED with DQI of 7.52 that differed significantly with the FL that has the lowest 1.86 (Fig.128). The rest of LEDs such as the G2, AP673L, AP67 and L20AP67 had values of 6.53, 6.31, 5.48 and 3.93, respectively.

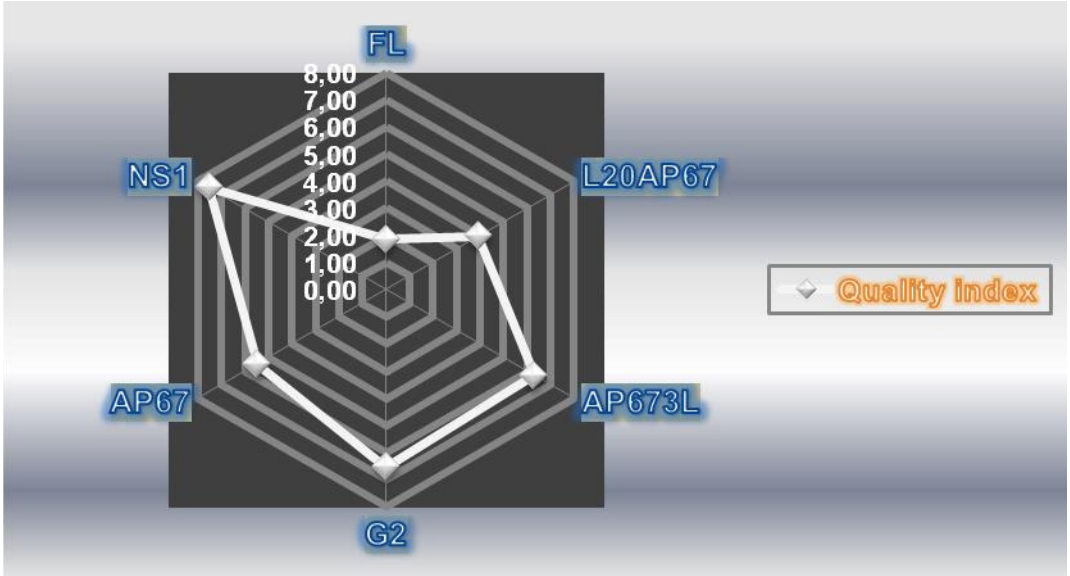


Figure 128. Dickson's Quality Index (DQI) of Quercus ithaburensis seedlings pre-cultivated under FL, L20AP67, AP673L, G2, AP67 & NS1 transplanted in QP24T/16 mini-plugs and grown for a 6 month growing period at Xalkidona Nursery.

3.4. Conclusion

Cultivation of the tested coniferous and broad-leaved species showed significant morphological and physiological adjustments after only one month of growing under different light qualities, reaffirming the reliance and the sensitivity of the seedlings to the inherent need of light especially in early developmental stages.

Since the environmental deviations are negligible under controlled conditions for the two different provenances of *P. sylvestris* experiment, while the only changing parameter is the light quality, we found that seedlings responded similarly by accelerating their height growth rate under L20AP67, FL and G2 lights and kept slower under NS1 and AP673L LEDs. *P. abies* seedlings showed also similar growth rate under different lights, but considering only the average values the highest was induced by the L20AP67 and the lowest for the NS1. Also *P. nigra* seedlings grew faster under both FL and L20AP67 and slower under NS1 light quality. From the onset of the differential light quality irradiation to the end of the experiment seedlings of *Q. ithaburensis* showed similar growth rate, considering the measurements held for each of the time intervals set (each week). However L20AP67 LED and FL conventional light triggered higher height increment throughout time than the slowest obtained under the NS1. *Castanea* seedlings showed significantly accelerated growth rate at the begging of time for those grown under L20AP67 LED than those grown under light qualities of NS1, FL and AP673L. Considering only the light quality effects height increment was more enhanced by L20AP67, RGP (FL& sodium lamps) and G2 throughout the indoor cultivation, than NS1 and FL*. M. communis* seedlings grew significantly faster under the effects of FL and AP673L lights and slower under both AP67 and G2 LEDs. Growth rate of *Ocimum basilicum* LL was lower under AP673L and NS1 LEDs, while L20AP67 and G2 seedlings grew faster for the first three weeks in the growth chamber. RR hybrid basil seedlings also showed lower height increment under NS1 light quality and favored under AP67 LED light. As far as the growth rate of *C. sanguinea* seedlings is concerned, it was higher under both FL and L20AP67 lights after the second week, while NS1 induced the lowest. *Prunus avium* results showed significant differences during the 3rd and 4th week explained by the significantly higher height increment induced by the AP67 LED compared to the

FL conventional light. Growth rate of pomegranate seedlings was benefited under L20AP67 light throughout the 6-week experimental period contrast to G2 LED.

Overall stimulation of leaf/needle development and colouring was species dependent regarding the different light qualities used into the growth chamber experiments. Thus both Scots pine provenances showed significantly more needles under the combined effects of AP67 and G2 LEDs compared to the FL and L20AP67 lights that induced 10 needles less. The needle colour however was the same during the whole experimental period. LEDs generally enhanced the formation of needles for Norway spruce, especially for the seedlings grown under the AP673L, AP67 and G2 by inducing twice fold increase than the FL conventional light. Also the needle colour was unaffected by the different light qualities. *P. nigra* seedlings showed no significant differences either for the needle number or colour irrespective the light spectrum; nonetheless greater number of needles was obtained under the G2 LED light effect than the FL control light. *Q.ithaburensis* seedlings better response by forming significantly faster more leaves under the AP67 compared to the G2 and FL lights, while *Castanea* seedlings inducing faster leaf expansion under both L20AP67 and G2 LEDs. Different lights induced no significant effect on the leaf colour for both of the *Q.ithaburensis* and *Castanea* seedlings over time. NS1 LED combined effects significantly increase the leaf number of *M. communis* compared to the L20AP67, RGP (FL & sodium lamps) and FL lights, while seedlings maintained the same light green colour of leaves over time. Both basil varieties showed significant differences for the number of leaves only at the first week of the experimental period. At that point, seedlings grown under the FL and L20AP67 formed less leaves than the rest of the LEDs. Besides AP673L LED light induced the formation of more leaves for both of basil varieties. Also, both LL and RR hybrid basil varieties were not affected by the different light treatments relatively to the leaf colour, thus maintaining throughout the experimental period, light green and purple colour, respectively. Leaf formation of *C. sanguinea* seedlings was not affected by the different light treatments over time; however both G2 and AP67 LEDs promoted greater leaf development than the FL and L20AP67 lights. Further seedlings grown under FL and L20AP67 maintained a dark green colour while the rest of the treatments turned reddish after four weeks in the growth chamber. Wild cherry seedlings showed similar leaf formation irrespective the light spectrum although L20AP67 LED light induced greater number of leaves at the final harvest. Also seedlings grown under the FL and

L20AP67 had dark green leaves during the whole experimental period, while those grown under the rest of the LEDs turned from dark green to light green at the final two weeks of the indoor experiment. *P. granatum* leaf development was faster during the first two weeks of the experiment under G2 and L20AP67 compared to FL, AP673L and AP67 at the first two weeks and at the final week NS1 and L20AP67 LED lights enhanced leaf formation by inducing fifteen leaves compared to the FL that showed the lowest formation of ten leaves. Pomegranate seedlings maintained a light green colour for the 6-week experimental period under all light treatments except from the NS1 LED that induced reddish leaves after two weeks in the growth chamber.

It is well known that light causes also alternations in the stomata movements of plants. Unfortunately there are few studies about the effects of light quality on this subject, especially for the parameters examined in this study. Stomatal opening and closure is affected by light for instance Talbott *et al.*, (2006) observed that blue and red light stimulated the stomata opening, whereas green light inhibited the opening. Also it is determined that an increase in light intensity, decrease the epidermis cell number and increase the stomata number, index and size (Fernandez and Mujica, 1973). Schoch *et al.* (1984) reported that blue and far-red light reduced the stomatal index while red light increased this index. Kim *et al*. (2004) showed that blue and red light increased the stomata size and decreased the stomata number. Further according to Lee *et al.* (2007) found that white light increased the stomata number and size, while blue light reduced the mentioned parameters. In our case LED lights had combined effects due to different percentages covering different bands of the light spectrum introducing a continuous light spectrum than monochromatic as mentioned in the previous references above. Nevertheless G2 LED has far higher percentage of 64.4% covering the red band (600-700 nm) and the lowest in blue (400-500 nm) of 7.7%, among all lights and found inducing significantly higher number of stomata compared to the FL light for both *Q. ithaburensis* and *Castanea* seedlings. As for the number of epidermal cells created of *Q. ithaburensis* seedlings, no significant differences found; however higher number was observed for the G2 and NS1 LEDs and lowest for the FL conventional light. *Castanea* seedlings showed significantly higher number of epidermal cells under NS1 and AP67 LEDs contrast to the FL that showed the lowest. It should be mentioned that NS1 LED spectrum has

the highest percentage of 38.9% in blue-green region (500-600 nm) than the rest of lights.

Contribution of the abaxial surface of leaves was similar for all light qualities investigated, demonstrating that the hypostomatous character of the *Quercus* leaves was unaffected by light quality. Stomatal density (SD) was significantly higher on leaves grown in the presence of G2 LED light (488 stomata /mm^2). This higher SD was not due to an increased production of stomata, as stomatal index (SI) (17.8%) was not substantially affected by the G2 light. Instead, this higher SD was due to the highest cell density (CD) (2723) (i.e. a lower epidermal cell size) on leaves that developed, compared to the rest of lights. Lowest average values for SD, SI and CD were found for the FL light, 254 stomata/ mm^2, 12.42% and 1986, respectively. Hypostomatous character of *Castanea* leaves under G2 light with 251 stomata/mm^2, showed significantly higher SD compared to the L20AP67 and FL light that induced 137 stomata/mm^2 and 139 stomata/mm^2, respectively. Furthermore G2 LED also induced significantly higher SI with 16.03% compared to the L20AP67 with 10.6%. FL light quality induced significantly lower CD of 1140 compared to NS1 and AP67 LED lights that had 1714 and 1688.7, respectively. Also the rest of the light qualities showed higher values of CD than the FL conventional light.

The tested light spectrum caused different effects on the leaf area of the seedlings during the indoor phase experiments and it was species-dependent. Thus *Q. ithaburensis* and *C. sativa* seedlings showed no significant differences for the leaf expansion. Although higher leaf area was observed for *Q. ithaburensis* seedlings, grown under L20AP67 and for the *Castanea* under the AP673L light quality. In contrast leaf area of *Ocimum basilicum* LL was greater under FL light compared to the rest of the treatments. For RR hybrid basil, FL and L20AP67 promoted significantly larger leaf area than AP673L and AP67 LEDs. On the other hand *C. sanguinea, P. avium* and *P. granatum* seedlings obtained larger leaf expansion under the effect of AP67 and L20AP67 LED light qualities.

Moreover different light irradiations did not induce significant changes either in the chlorophyll content index CCI or the chlorophyll fluorescence values of *Q. ithaburensis*. However FL, G2 and L20AP67 lights had greater CCI and quantum yields of PSII than the rest of the lights. In non-stressed leaves of numerous species, Fv/Fm ranges from 0.78 to 0.86 which has been shown in two species of *Quercus* (Rodríguez-Calcerrada *et al.* 2008). For *Castanea* seedlings significant higher CCI

was found under L20AP67 compared to NS1; but lights such a G2 and FL still also had high CCI. Likewise the quantum yields obtained for *Quercus* no significant differences found also for the *Castanea*; although the values found were less than the optimal value of 0.832 for Fv/Fmax*,* typical of well-functioning photosynthetic apparatus (Björkman and Demmig, 1987), however, this should not be a surprise as the parameter varies with species and environmental conditions (Bjorkman and Demmig 1987, Cha-um *et al*., 2010). Thus in *Castanea* case higher F_v/F_m found for the L20AP67 and the FL lights with 0.80 and 0.74, respectively.

According to Naidu *et al.*, 1984, suppression of photosynthesis is due to reduction of Chl levels, particularly chla, which is directly involved in determination of photosynthetic activity (Sestak, 1996). Our experiment results are evidence of the former conclusion that showed no significant differences for the chla content however higher values were found for the FL, RGP and AP673L light qualities that also induced higher growth rate levels compared to the rest of LEDs that had lower relatively growth rate and chla content values. *M. communis* showed significantly higher chlb content under the FL light compared to AP67 and NS1 LEDs, while AP673L likewise chla showed the highest chlb content among LEDs. On the other hand, Chl content is one of the most important factors to estimate dry matter production (Ghosh *et al*., 2004). In our case higher Chl content induced by the FL and RGP (FL & sodium lamps) does not match with higher dry weight accumulation, actually those light qualities obtained the least dry mass compared to the AP673L and NS1 LEDs that showed the highest by far. Further Heo *et al.*, 2010 did not observe any difference in chlorophyll a and b and total carotenoid content under the combination of blue and red LEDs in *Dieffenbachia amoena* and *Ficus elastica.* Consistent to those findings common myrtle seedlings showed similar carotenoid content under the different lights; although higher synthesis was found once again for the FL, RGP (FL & sodium lamps) and AP673L lights. *P. granatum* seedlings that grown under the FL light showed significantly higher chla, chlb and carotenoid synthesis than the rest of LEDs; however LEDs induced significantly higher total dry weight accumulation.

Plants typically respond to environmental stressors by inducing antioxidant production as a defense mechanism. High light treatments also resulted in increased contents of phenolic compounds and antioxidant activity with no adverse effect on growth or yield (Oh *et al.,* 2009). Also at the present time, several researchers have

reported observing beneficial effects on secondary metabolism in vegetables such as the production of polyphenols or antioxidant properties under controlled environments (Dupont *et al*., 2000; Llorach *et al.,* 2008). According to several authors (Kim *et al.* 2013; Ouzounis *et al*., 2014), when blue light is engaged the content of total phenolic compounds of many plants such as tomatoes, roses, chrysanthemus and campanulas is increased. Likewise our results showed that NS1 LED light that is high in blue-green spectrum compared to the rest of lights (20.2% in blue and 38.9% blue-green) induced significantly higher total phenol content compared to the rest of the lights for the *M. communis*, *O. basilicum* LL., basil RR hybrid and *P. granatum* seedlings.

High antioxidant capacity has been attributed to members of the flavonoid family, particularly anthocyanins. Anthocyanins are a key indicator of commercial quality for many fruits, vegetables, and ornamentals. As such, treatments that can increase the accumulation of anthocyanins are of great interest and importance in agriculture (Gould, 2004). Thus according to our results cultivation of common myrtle and pomegranate seedlings under LEDs such as the L20AP67, AP67, G2 and NS1 showed significantly higher anthocyanin content than the FL conventional light. Further pomegranate seedlings grown under G2 and AP67 illuminations contained significantly more flavonoids in their tissues than the rest of the treatments, especially FL light that induced the lowest content. Consequently it could be assumed that LED lights in our experiments triggered biochemical defense; thus the seedlings of the tested species could potentially become more stress tolerant in open-field conditions.

Shoot height (SH) was similar under different lights for the Scots pine Greek provenance seedlings but that was not the case for the root length (RL), where LEDs especially G2 quality induced significant increase compared to the FL and L20AP67 lights. L20AP67 light significantly promoted the SH of Scot pine Sweden provenance than the NS1 light quality, while RL was benefited by far under AP673L, NS1 and AP67 LEDs compared to the FL and L20AP67. Norway spruce seedlings grown under the L20AP67 light were significantly taller than those grown under NS1 which induced significantly longer roots compared to the conventional FL light that showed the least beneficial effect. FL and L20AP67 lights significantly promoted the shoot development of *P. nigra* seedlings compared to the LEDs such as the AP673L, AP67 and NS1 that significantly increased the root system length. Morphological

parameters such as SH and RL of *Q. ithaburensis* seedlings showed no significant differences under light treatments. However, L20AP67 and FL light promoted the SH while AP673L formed more compact seedlings but with greater root development. SH of *Castanea* seedlings also were taller under L20AP67 and found with significantly greater root development when compared to the FL and RGP (FL & sodium lamps). Furthermore *M. communis* seedlings were significantly taller under the effects of AP673L and FL lights compared to those grown under the RGP (FL & sodium lamps), G2 and AP67 lights that appeared more compact. RL length was significantly increased by the effects of both AP673L and NS1 qualities than the G2. LL basil's seedlings were significantly shorter under the effect of both AP673L and NS1 LEDs compared to the rest of the treatments. Also AP673L promoted the root development, while NS1 induced the less beneficial effect. G2 LED light significantly promoted the shoot development of RR hybrid basil seedlings compared to L20AP67 and AP673L lights and NS1 LED obtained longer roots than the rest of the treatments. On the other hand *C. sanguinea* seedlings were better adapted under the FL light by means of significantly higher shoots than those grown under AP67 and NS1 LEDs that induced more compact seedlings although with greater root development. Wild cherry seedlings showed similar shoot development regarding the different lights; however NS1 LED light induced significant two fold increase in the root development compared to the rest of lights especially to the FL that induced the shortest root system. Finally morphological attributes of pomegranate seedlings were better promoted under the L20AP67 LED light quality.

Root architectural analysis allows a formal description of root systems and has important ecological applications since architecture reflects root plasticity responses to environmental heterogeneity and edaphic constraints to plant productivity and determines the function of roots in mechanical support of the shoots (Fitter and Stickland 1991; Lynch, 1995; McPhee, 2005). Densely root systems were formed by means of greater number of lateral roots occupied per cm of the primary root length of *Q. ithaburensis* seedlings grown under LED lights than the FL. Root density was similar for LED treatments, being higher for the lights of L20AP67, AP67 and NS1. Further LED lights, especially G2 and NS1 induced double number of FOLR of seedlings than the FL light. For oak seedlings, the large number of primary first order lateral roots and the high root system fibrosity (root system with a relatively high root surface area and with a large number of root apices) are considered parameters that

improve field survival and early growth of seedlings (Schultz and Thompson, 1997; Wilson *et al.*, 2007). Root fibrosity is a relative index of root branchiness. A fibrous root system has a relatively high root surface area with a large number of root apices (Ruelhe and Kormanik 1986). NS1 and AP67 cultivation light treatments modify the root system fibrosity of *Q. ithaburensis* seedlings by inducing significantly greater number of FOLR with diameter >1 mm compared to FL conventional light and L20AP67 LED. Therefore that attribute might enhance the potential to improve seedling quality. *Castanea sativa* seedlings showed denser root system under the effect of LEDs than the FL light, especially benefited by the G2 LED light. Also the number of FOLR remained significantly higher under all LED treatments inducing threefold increase compared to the conventional light sources such as FL and RGP light environment (FL & sodium lamps). Further AP673L and NS1 LEDs obtained significantly higher number of FOLR with diameter >1 mm and categorized to the highest fibrosity class compared to the seedlings grown under the FL light that characterized with the least fibrous root system.

The use of combined effects of monochromatic LED lights supplemented to conventional light sources such as fluorescent or high pressure lamps that stimulated the dry weight mass of several species were investigated by several authors (Johkan *et al*., 2010; Yorio *et al.*, 2011; Lie *et al.,* 2012). Those studies also revealed the higher dry matter accumulation of plants grown under the effects of LED lights. In this study the LED lights with continuous spectrum showed a predominately dry weight mass accumulation via the conventional light sources. Further R/S dry weight ratio also was better predicted under LED lights than the FL. An increase in R/S ratio is predicted to be a better strategy for maintaining growth under water- limiting conditions, because it can increase water and nutrient absorption and return carbon and nutrient contents to a more favorable balance for storage (Vilela *et al*., 2003).

Despite the fact that the two provenances of Scots pine are defining by the same genetic components but by different geographic origins were positively affected by the same LED qualities such as AP67 and AP673L that induced significantly increase in total dry weight accumulation compared to the FL and L20AP67 lights. The same was observed for *P. abies* and *P. nigra* that are coniferous species found in diverse natural geographic distributions thus with so different physiological requirements although AP67 and AP673L LEDs induced significantly increase of the total dry weight accumulation of seedlings compared to the FL conventional light. Likewise the

R/S ratio obtained was significantly higher under the AP673L and NS1 LEDs both for the Scots pine provenances and under the AP67 LED for the *P. abies* and *P. nigra* seedlings compared to the FL light indicating higher dry weight mass allocation to the roots. Dry weight matter of *Q. ithaburensis* seedlings was far better predicted under LED lights compared to the FL. Significant increase almost four fold was found for the DWR under the combined effects of NS1 and AP673L LEDs compared to FL light. Further DWL and DWS also were greater under LEDs than the FL light. As for the *Castanea* seedlings AP673L combined effects were significantly increased twice the DWS and DWR compared to FL light and also have the greater impact of all lights in the DWL. As a consequence higher allocation to the roots by means of higher R/S ratio was obtained under LED cultivation compared to FL light both for the *Q. ithaburensis* and the *Castanea* seedlings. Dry weight accumulation of *M. communis* seedlings was by far better promoted under the AP673L and NS1 LEDs, especially for the DWR that was three times greater compared to the rest of light treatments. R/S ratio was also better predicted under LEDs and RGP lights (FL & sodium lamps) than the conventional FL light. Higher total dry weight was obtained under AP67, G2 and NS1 LEDs both for the two basil varieties, while the lowest obtained under FL conventional light and L20AP67 LED. As for the R/S ratio obtained of both basil varieties was significantly higher under LEDs especially benefited for the NS1 LED, compared to the FL conventional light. *C. sanguinea* seedlings showed significantly low total dry weight mass under the effect of the FL light compared to the highest obtained under LEDs such as the AP673L, G2 and AP67, while R/S ratio was better predicted under all LEDs especially for the NS1 compared to the FL light. Also total dry weight mass of wild cherry seedlings was better predicted under AP673L, G2, AP67 and NS1 than the FL and L20AP67. Also wild cherry seedlings showed significantly greater dry weight allocation to the roots under the NS1 LED in contrast to the lowest obtained under the L20AP67. Finally *P. granatum* seedlings obtained significantly higher total dry weight mass under the effect of LEDs especially for the L20AP67, while the lowest was found for the FL conventional light. Pomegranate seedlings showed no significant differences for the R/S ratio, however greater dry weight allocation to the below ground parts of the seedlings than the above was found for LEDs than the FL light.

The production of large deep root system during the wet season before the onset of drought is essential for summer survival under Mediterranean conditions (Padilla and

Pugnaire, 2007). Generally Root Growth Potential physiological test showed better results for the seedlings of all the tested species that pre-cultivated under LEDs than the conventional FL light by means of the new formation of longer and heavier roots. Pre- cultivation under different light qualities did not induce significant effect on the NRL of both Scots pine provenances at least at the 15th day into the RGP bath, however longer roots were found for the G2 and L20AP67 for the Greek and Swedish provenance, respectively. At the 31st day into the RGP bath significantly longer new roots were obtained for the Swedish provenance of the seedlings pre-cultivated under L20AP67, G2 and AP673L than the FL light while for the Greek provenance longer new roots were found for the G2 LED than the FL. The new dry weight of roots for both provenances showed no significant differences irrespective the different light pre-cultivation; however at the final harvest significantly heavier new roots were obtained for the Greek provenance of seedlings that pre-cultivated under the L20AP67 compared to the FL, and heavier under the G2 for the Swedish. Higher field performance was better predicted for *M. communis* seedlings that pre-cultivated under NS1 LED by means of significantly longer and heavier roots obtained compared to the rest of the lights especially for the FL and L20AP67 that showed the least potential performance. *Ocimum basilicum* LL showed no significant differences for the new root length. However, pre-cultivation under NS1 light induced the formation of lighter new roots compared to FL, L20AP67 and AP673L treatments. NS1 LED also induced the shortest and lightest new roots for the RR hybrid basil, while FL, AP673L and AP67 promoted longer roots and AP673L and G2 obtained heavier new roots. *Cornus sanguinea,* NRL and NRDW was better predicted under NS1 and AP67 compared to L20AP67 and AP673L. Wild cherry seedlings formed longer new roots under AP67 and G2 compared to L20AP67, while heavier new roots were formed under G2 compared to L20AP67 and AP673L. Finally pre-cultivation of Pomegranate seedlings under AP67 obtained significantly longer new roots compared to the rest of the treatments, while NRDW was significantly favored under L20AP67 compared to FL, G2 and NS1 treatments.

Performance of *Q.ithaburensis* seedlings after a 6 month period at the Chalkidona nursery revealed more encouraging seedling quality traits for those pre-cultivated under LED lights compared to FL conventional light. Seedling survival was high for all treatments especially for the AP673L showed 100% success. Significantly greater number of leaves was formed for the L20AP67 treatment that it was in agreement

with indoor findings about the higher leaf area obtained by that light. Chlorophyll content of seedlings that pre-cultivated under G2 LED light quality showed significantly higher CCI compared to those of the FL treatment. However during the indoor experiment CCI was higher for the FL light but that was not the case when those seedlings kept for a long period under solar irradiation, while G2 still showed high CCI during the indoor experiment and during the nursery phase and that could be evidence of a more stable cultivation under LEDs. Seedlings that pre-cultivated under the FL and L20AP67 lights were significantly taller compared to the rest of light treatments by means of higher values for the plant height and the shoot height and that is also in agreement with the indoor cultivation findings where those two lights induced taller seedlings, while AP673L and NS1 induced more compact seedlings.

Numerous studies show larger shoot diameter seedlings tend to survive better than small shoot diameter seedlings (Mexal *et al.,* 2008; South *et al.,* 2005; Oliet *et al.,* 2009b; Morrissey *et al*., 2010). Also shoot diameter is closely related with root morphological characters particularly number of FOLR (Corpuz, 2012). While it is possible that large diameter seedlings inherently have a more fibrous root system (Carandang, 1994). Seedlings of *Q.ithaburensis* showed similar values for the shoot diameter for all pre-cultivation lights however LED lights such as G2 and NS1 had greater SD than the FL. In conjunction with the findings, about the greater number of FOLR formed under both G2 and NS1 LEDs support the latter statement. In addition root collar diameter of LED pre-cultivated seedlings also is larger than those of the FL light.

Pre-cultivation under different light treatments also had a significant effect on dry weight accumulation of seedlings that continued to be observed during the six month period at the nursery. LED lights such as AP673L and NS1 obtained significantly higher dry weight of leaves compared to FL and G2 light qualities. NS1 LED light showed a threefold increase in dry weight of roots compared to the FL light likewise the previous findings for DWR at the end of the growth chamber experiment. Furthermore NS1 LED light doubling the dry weight accumulation of the shoots compared to FL. Root/shoot ratios showed values around 4, which were relatively higher than other Mediterranean oak seedlings (Ksontini *et al*., 1998; Tsakaldimi *et al*., 2005). The relative allocation of resources to roots or shoots has been considered a key factor in plant strategies regarding water use and is very important for seedling

performance and survival in the field (South, 2000). Thus G2, NS1 and AP67 LEDs showed significantly higher root allocation compared to the FL.

The DQI is considered an index of morphological development to predict seedling field performance (Dickson et al., 1960) and has been successfully used in several species (Roller, 1976; Ritchie, 1984; Hunt, 1990; Luis *et al*., 2004; Marques *et al*., 2006). Thus mainly due to higher root dry weight accumulation of *Q. ithaburensis* seedlings that pre-cultivated under NS1, significantly higher Dickson Quality Index was obtained, specifically four times higher compared to those grown under the effect of FL light. The rest of LEDs also showed higher DQI than the FL.

These results confirms that many recognized advantages of LED lights as the mainly artificially light source for indoor cultivation of plants via conventional light sources like Fluorescent lamps appear to hold true for the production of high quality seedlings of forest and shrubs-tree species. Thus LED lights with continuous spectrum induced desirable seedling attributes such as more rapid early growth, greater number of leaves accompanied with higher number of stomata and epidermal cells, stomata index and cell density, larger leaf area containing higher chlorophyll content and photosynthetic yield performance, higher antioxidant capacity, compact seedlings with greater root development with favorable root architecture, fibrosity and higher dry biomass that is especially noteworthy. All these attributes are further considered by the enhanced nursery performance of seedlings, especially from the Dickson's quality index that can also reliably predict outplanting survival of seedlings of certain species. Nevertheless, further research is warranted to realize the full effects of LED lights on the morphology and physiology of forest tree species that cultivated in order to obtain high quality planting stock material.

3.4.1. Better indication of optimal light qualities suggested for each of the tested species:

- ***Pinus sylvestris* L.*:*** **AP67, AP673L, G2**
- ***Picea abies* Karst.*:*** **AP67, AP673L**
- ***Pinus nigra* Arn.*:*** **AP67, AP673L**
- ***Quercus ithaburensis* var. macrolepis*:*** **NS1, AP673L**
- ***Castanea sativa* Mill*:*** **AP673L, G2**
- ***Myrtus communis* L.*:*** **AP673L, NS1**
- ***Ocimum basilicum* L.*:*** **AP67, G2, NS1**
- ***Ocimum basilicum* RR hybrid:** **AP67, G2, NS1**
- ***Cornus sanguinea* L. :** **AP673L, G2, AP67**
- ***Prunus avium* L.*:*** **AP673L, G2, AP67**
- ***Punica granatum* L.:** **L20AP67, AP67, NS1**

3.4.2. In a few words…pros and cons of the light treatments tested

L20AP67: induce faster height growth rate, larger leaf area, caused dark-green leaf colour, high Fv/Fmax values, high anthocyanin content, higher shoot height, shorter roots, low dry weight accumulation, greater new root length, heavier new roots,

AP673L: slower height growth rate, caused reddish leaf colour for broad-leaved species, high chla, chlb and carotenoid synthesis, lower flavonoid content, compact seedlings, greater root length, higher root fibrosity, higher DWR, heavier new roots, higher total dry weight after harvesting from the nursery

G2: generally caused higher leaf formation, induce higher stomata number in broad-leaved species, high CCI, high Fv/Fmax values, high anthocyanin content, higher flavonoid content, greater root length, high DWS, greater new root length, heavier new roots, greater shoot diameter,

AP67: generally caused higher leaf formation, induce higher epidermal cell number in broad-leaved species, high anthocyanin content, higher flavonoid content, denser root system, high DWR, greater new root length,

NS1: slower height growth rate, caused reddish leaf colour for broad-leaved species, induce higher epidermal cell number in broad-leaved species, higher phenol content, high anthocyanin content, compact seedlings, greater root length, higher root fibrosity, higher DWR, high R/S ratio, greater shoot diameter, higher total dry weight after harvesting from the nursery, highest Dickson Quality Index obtained

FL: faster height growth rate, caused dark-green leaf colour, spindly shoot form, lower stomata and epidermal cell number, high CCI, high Fv/Fmax values, higher chla, chlb and carotenoid synthesis, lowest anthocyanin and flavonoid content, higher shoot height, shorter roots, low root density, low root fibrosity, lowest dry weight accumulation, lighter new roots, smaller shoot diameter, lowest total dry weight mass after harvesting from the nursery, lowest Dickson Quality Index obtained

RGP (FL & sodium lamps): high chla, chlb and carotenoid synthesis, low root density, low root fibrosity, low dry weight accumulation,

4. INPUT OF DALARNA UNIVERSITY

4.1. Introduction

4.1.1. Year-round production of seedlings in Northern Europe

One of the main advantages of the Zephyr concept is the possibility of producing high quality seedlings on a year-round basis. In northern Europe however, this production has to still be planned and adapted to the outdoor conditions in order to make the most out of it. Taking this into account, a sowing plan for northern Europe was designed and tested. The plan consists of 7 batches, 3 of which should occur during the summer months (Figure 1, green path); and 4 batches should occur during the cold months (Figure 1, blue path). Different experiments, shown in Figure 1, were conducted to find the optimal conditions in each path and develop the growth protocols presented in this report.

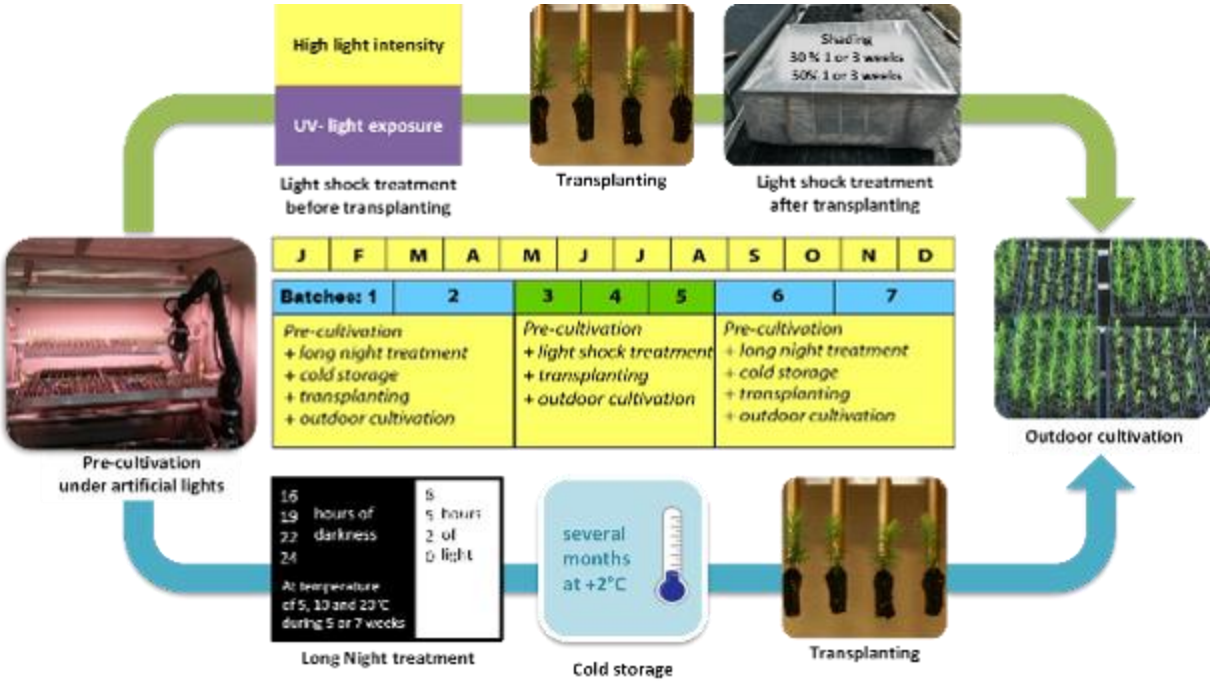


Figure 1: Sowing plan for northern Europe with 7 batches and the possible treatment paths studied depending on the time of the year and weather outdoors.

4.1.1.1. Long night (LN) treatment

As already reported in D3.2, Norway spruce seedlings are normally LN treated during 5 weeks with a daily photoperiod of 8 hours and a night period of 16 hours at an ambient temperature of 20°C. To test if this treatment could also be applicable for young pre-

cultivated Norway spruce seedlings, the same procedure was applied. As the LED light source AP67 in the tube version had proven to be favorable, this light source was also chosen during the 5 weeks of pre-cultivation of Norway spruce seedlings. After pre-cultivation, LN treatment was conducted during 5 weeks under the same conditions as described for conventionally grown Norway spruce seedlings. The treatment showed to be very effective and after the gene test the result showed that the seedlings were cold tolerant and ready for cold storage.

Pre-cultivated Scots pine did not react as Norway spruce on the described LN treatment. In general Scots pine has shown to be more difficult to induce cold hardiness compared with Norway spruce. This can be due to the history of how these species found its way into Sweden after the latest glacial period. Scots pine found its way mainly from central Europe while Norway spruce was coming to Sweden from the very north parts of Europe and therefore genetically different in there adaptation to a cold climate.

4.1.1.2. Light shock treatment

Direct transplanting from pre-cultivation under artificial light to full exposure of sunlight on open land can lead to a light shock for the young seedlings. This can result for example in severe needle damages that will affect the seedlings development negatively.

Photosynthesis proceeds with an optimal rate only within a narrow irradiance range, often lower than solar radiation under natural conditions. Consequently, photoinhibition is one of the most important regulatory mechanisms in photosynthesis! The extent to which this absorbed light is not fully used for photosynthesis is set by P_{max} = light-saturated rate of photosynthesis in normal air. At low light (< 100 µmol/m^2·s), both sun and shade leaves use more than 80% of absorbed light for photosynthesis. Once P_{max} has been reached, all additional light is in excess. Shade plants have a lower P_{max} than sun plants, so they experience more excess light at a given photon irradiance above saturation.

Even the most hardy sun plant will reach P_{max} at less than full sunlight. At that level (1000 µmol/m^2·s) ~ 25% of absorbed energy is used in driving photosynthesis, but at full sunlight (2000 µmol/m^2·s) as little as 10%. Additional stresses such as drought, nutrient limitation or temperature extremes can lead to a reduction in P_{max} and thus increase the probability that plants will be exposed to excess light. Further, in juvenile and senescing plants, the regulation of photosynthetic apparatus functioning is not operating optimally in comparison with mature leaves. Hence, juvenile plants are less efficient in the utilization of the absorbed light, and therefore, prone to photoinhibition by radiation fluxes that usually do not harm mature plants.

4.1.2. Light intensity

As it can be seen in Figure 2, for the case of the LED lamps used, the light output in the PAR region follows a linear relationship to the electrical energy input.

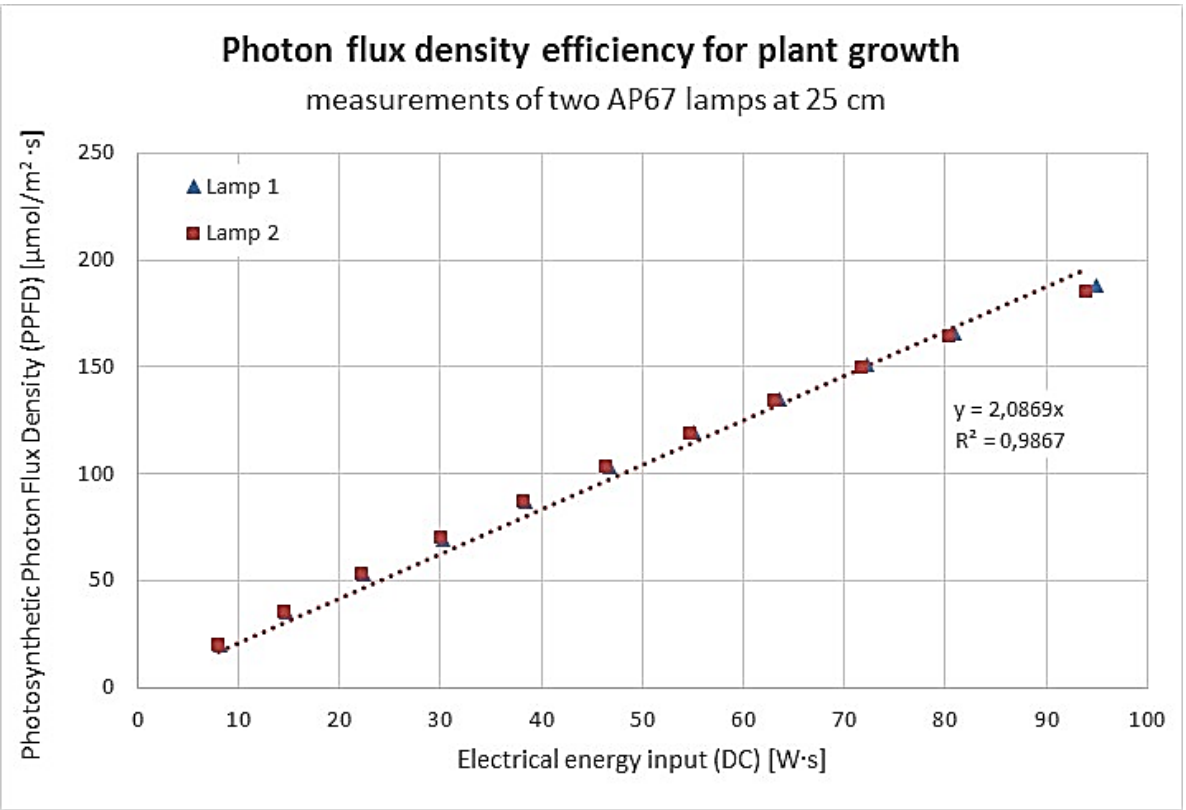


Figure 2: Photon flux density efficiency for plant growth measured 25 cm directly below two AP67 lamps.

In an autonomous system such as the Zephyr incubator, it is important to save energy wherever possible. Decreasing the light intensity is a very fast and efficient way to reduce the electricity consumption. However, the light intensity has a direct and strong impact on the development and growth of the seedlings. For this reason, any adjustment should be evaluated also from a biological point of view. Although it is true that half the amount of light requires half the amount of energy, depending on the level, it might not necessarily produce half as good seedlings.

The aim of this study was to explore the biological effects of different light intensities when growing seedlings of *Picea abies* and *Pinus sylvestris* and possibly find an optimal point between energy consumption and seedling quality.

4.1.3. Forest field trial (final inventory)

In order to test the performance in field conditions for seedlings pre-cultivated under artificial light sources, a forest field trial was designed as described already in D3.2. For this deliverable, the field trial was re-visited and the seedlings were measured again. This

document presents a final inventory of the seedlings status after two years growing under real conditions.

4.2. Materials and methods

All the studies in this section were carried out at the Forestry department of the Dalarna University, Sweden. The biological material used for all the experiments was the following:

- Norway spruce (*Picea abies L.*) seeds collected from the provenance of Vitebsk in Belarus (Lat. 55°, Long. 30°). The germination rate was 99.0% and the germination energy was 96.5%.

- Scots pine (*Pinus sylvestris*) seeds collected from a seed orchard with the provenance of Gotthardsberg in Sweden (Lat. 59°, Long. 16°). The germination rate and germination energy were both of 99.8%.

4.2.1. Long-night (LN) treatment

To test if cold tolerance also could be induced in Scots pine seedlings, a broad experimental design, which was introduced in D3.2, was conducted. This included variations in the duration of the treatment and photoperiod as well as different temperatures during LN preparation. All treatments were pre-cultivated under AP67-tube spectrum that has proven to be favorable during pre-cultivation of Scots pine. Light intensity was as for Norway spruce set at 100 µmol/m^2·s.

As it was previously described, after 5 weeks of pre-cultivation, the seedlings were treated for 5 or 7 weeks under shorter photoperiods (0, 2, 5, 8 hours of light) at different temperatures (5ºC, 10 ºC, 20 ºC). Then several measurements and gene tests were applied to determine the degree of cold tolerance (see description in D3.2). Afterwards they were cool stored at +2ºC for several months and then their vitality was measured.

4.2.2. Light Shock

All seedlings for this trial were pre-cultivated under one AP67-bar respectively at 100 µmol/m2·s for 4 weeks. The light intensity was adjusted to 100 µmol/m^2·s. After this period, the seedlings were randomly divided in the following conditions for 1 week:

- Treatment 1, Control: continuing growing for 5 weeks under AP67-bar at 100 µmol/m2·s.

- Treatment 2, High light intensity: continuing growing for 4 weeks under AP67-bar, but at 300 µmol/m^2·s using 2 AP67-bars during the last week.

- Treatment 3, UV-a 30min: continuing growing for 5 weeks under AP67-bar at 100 µmol/m^2·s, but the trays are placed under UV-A light source for 30min/day during the last week.

- Treatment 4, UV-a 60min: continuing growing for 5 weeks under AP67-bar at 100 µmol/m^2·s, but the trays are placed under UV-A light source for 60min/day during the last week.

After this 5 week period, seedlings were transplanted to a suitable container and transferred outdoors. To exclude the effect of changing humidity, temperature and/or irrigation, seedlings were sufficiently watered before, during and after the transplantation. Further, if the transplantation day is very sunny and warm, the seedlings were moved outdoors only in the afternoon, not during the sunniest/warmest hours in mid-day.

From each of the indoor treatments, one group of seedlings were transplanted directly to full sunlight without any shading. A second set was placed outdoors under a 30% shade cloth for one week, another set under 30% shade for three weeks. A fourth group of seedlings was under 50% shade for one week, and the fifth lot was under 50% shade for three weeks.

Pre cultivation

- Treatment 1

Pre-cultivation at 100 µmol/m^{2}·s under 1 AP67-bar during 5 weeks

- Treatment 2

Pre-cultivation at 100 µmol/m^{2}·s under 1 AP67-bar during 4 weeks

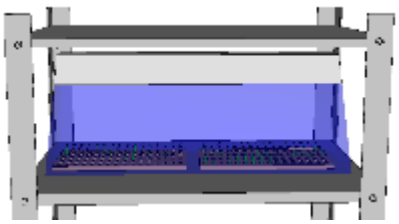
Then intensity is raised up to 300 µmol/m^{2}·s for the 5th week, by adding a second AP67-bar.

- Treatment 3

Pre-cultivation at 100 µmol/m^{2}·s under AP67-bar during 5 weeks

During the 5th week the trays are placed under UV light source for 30 min/day

UV-light source shelf (no AP67 lamp here, only UV)



- Treatment 4

Pre-cultivation at 100 µmol/m^{2}·s under AP67-bar during 5 weeks

During the 5th week the trays are placed under UV light source for 60 min/day

Figure 3: Experimental setup for pre-cultivation phase in light shock experiment

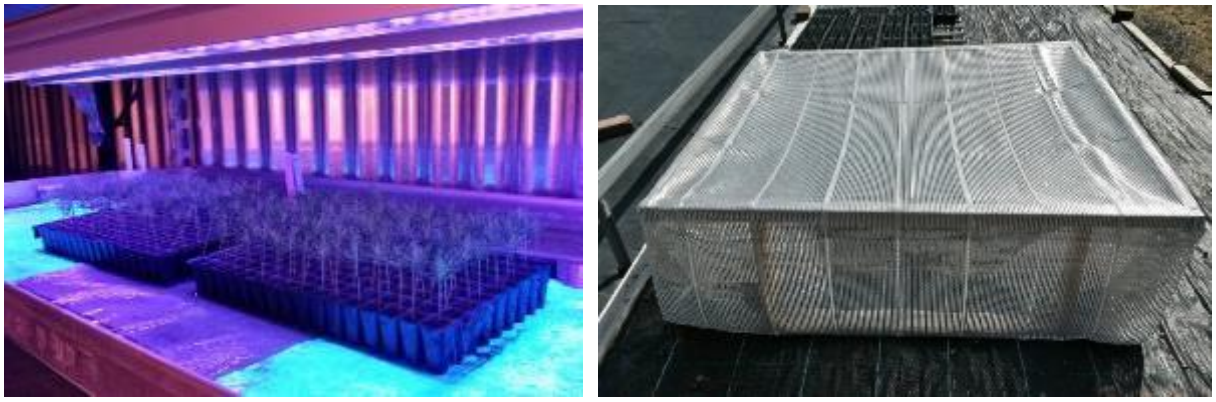


Figure 4: Light shock treatments: UV-A exposure before transplanting (left), shading cloth for transplanting (right)

4.2.2.1. Gas exchange and Chlorophyll Fluorescence

In order to obtain a better understanding of the seedlings development and performance in the field a portable photosynthesis system CIRAS-3 (PP-Systems, USA) equipped with a cuvette fitted for coniferous plants was used. Along with CO_2 assimilation, simultaneous measurement of the chlorophyll fluorescence (Chl F) was applied using a portable chlorophyll fluorometer FMS 2 (Hansatech Instruments, UK).

The overground part of dark adapted seedlings was placed in the cuvette and after 10 min in the dark to measure the respiration rate and Chl F (Fv/Fm). This was followed by 5min periods at different light intensities ranging from 50 to 2000 µmol/m^2·s in order to measure the CO_2 assimilation rate. These values were used to generate light curves and approximate the maximum photosynthetic efficiency of the seedlings. The gas exchange results were expressed as µmol of CO_2 assimilated per dry mass of the needles (kg).

Figure 5: A tailor-made PLC3 conifer cuvette allows the integration of the CIRAS-3 open gas exchange system with the FMS2 pulse modulated chlorophyll flourometer

4.2.3. Light Intensity

Three independent sowings of *Picea abies* and *Pinus sylvestris* were made for this experiment. The germination was done at 80% and 20°C for one week under AP67 spectra with a photoperiod of 16 hours. After the germination phase, the humidity was reduced to 60% while the other conditions remained the same and the seedlings were pre-cultivated during 4 more weeks before being measured.

The studied factor was the light intensity which for this experiment was doubled between each level starting at 50 µmol/m^2·s, then 100 µmol/m^2·s, 200 µmol/m^2·s and finally 400 µmol/m^2·s. As shown in Figure 2, each time the intensity doubles, the LED lamps consume twice as much energy as in the previous level. In order to find the relationship between the light increase and the biological response, 25 random seedlings from each treatment were sampled and measured in every sowing.

4.2.4. Forest field trial

As described in D3.2, a forest field trial was designed in order to test the performance in field conditions for seedlings pre-cultivated under the artificial light sources. After a vegetation period on open land, 3 replicates of 3 seedlings from each light treatment were transplanted to a clear-cut area. The trial was divided in two plots, one for Scots pine and one for Norway spruce seedlings which were planted in two scarified rows each as shown in Figure 6. The plot was revisited during autumn the two years following the planting in order to measure the performance of the seedlings in the field (Figure 7 and Figure 8).

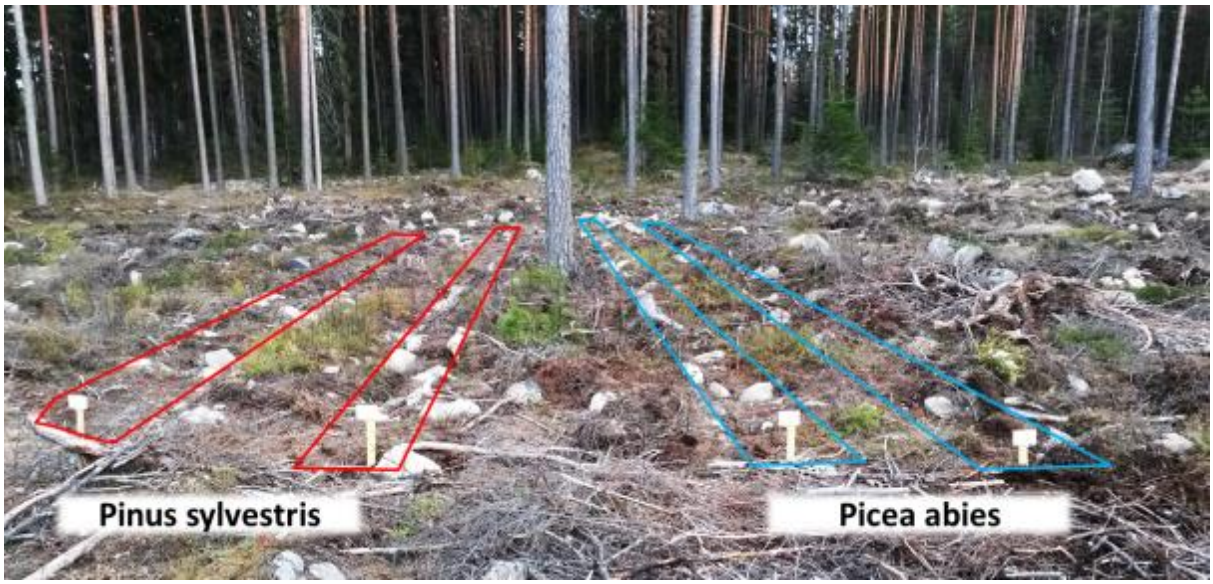


Figure 6: Plot distribution in the forest field trial at planting (autumn 2013)

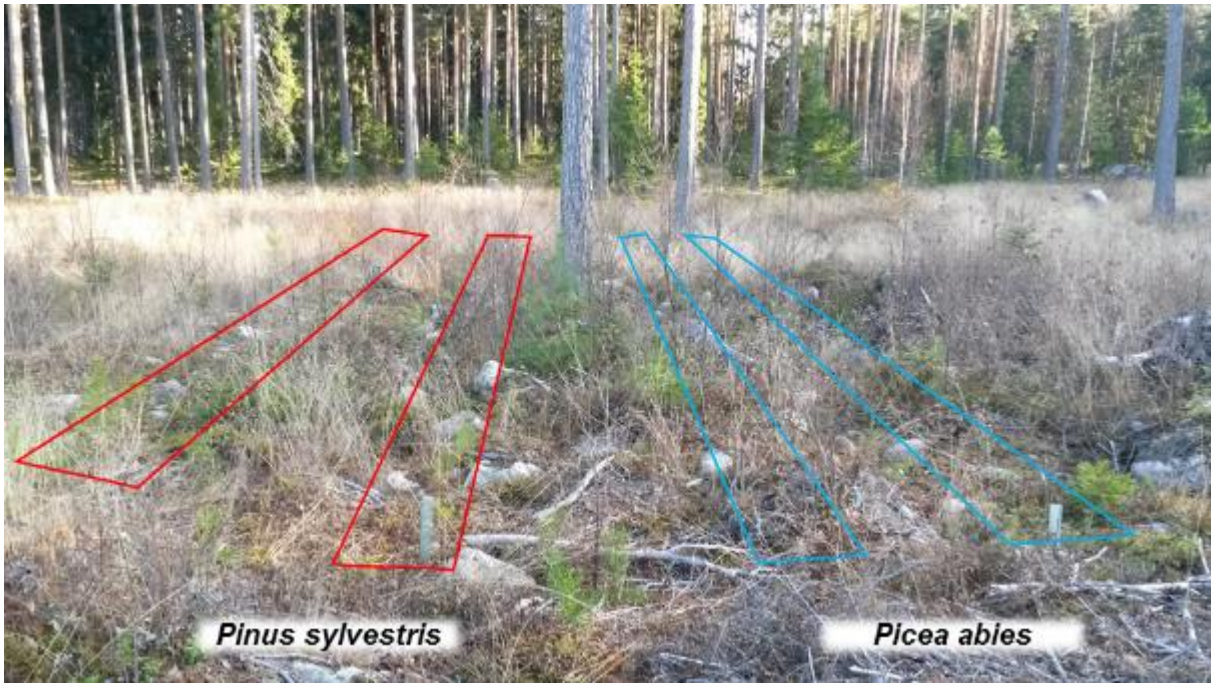


Figure 7: Plot distribution in the forest field trial after two years showing also the growing grass and other competing vegetation (autumn 2015)

Figure 8: Scots pine seedlings after 2 vegetation periods growing in the field trial (autumn 2015)

4.3. Results and discussions

The following results represent a summary of the outcomes that lead to the growth protocols presented in the final section. For more details and in order to have a complete overview of all the results please also consult D3.2. Unless otherwise specified, all the bar charts represent sample average with the error bars corresponding to the standard error.

4.3.1. Long night treatments

4.3.1.1. Gene tests after LN treatment

One of the key parameters that was used in order to determine the cold tolerance was the gene test ColdNSure. As described in the previous deliverable, this method provides a fast and reliable way of testing the seedlings for cold hardiness. It is important to clarify that the test was developed for older seedlings (one full vegetation period, instead of only 5 weeks), that had been cultivated under sunlight and cold harden in natural conditions. For this reason the results of the gene tests were used more in an exploratory phase and then validated with morphological and physiological tests. After these, we believe that the results of the gene tests are still valid to a certain degree and definitively give an insight to the stage of tolerance that the seedlings will have.

Figure 9 summarizes all the gene test results obtained for the various treatments applied to Scots pine seedlings. In general, the greatest factor influencing the cold tolerance induction seems to be the temperature at which the seedlings are treated (see Figure 10). There seems to be also an interaction between the photoperiod and the temperature but not so much with the duration. Complete dark or very short photoperiods during the LN treatment (0 or 2 hours of light per day) showed low levels of seedlings survival. The cold hardiness process requires a great energy input from the plants and it appears as if they were not receiving enough light to cope with their needs.

Species	LN -treatment (duration weeks)	Photoperiod for LN											
		0 hrs light 24-hrs dark			2 hrs light 22-hrs dark			5 hrs light 19-hrs dark			8 hrs light 16-hrs dark		
Scots Pine *Pinus sylvestris*	5 weeks	5°C	10°C	20°C	5°C	10°C	20°C	5°C	10°C	20°C	5°C	10°C	20°C
		1	1	1	1	1	2	3	2	1	2	2	1
	7 weeks	5°C	10°C	20°C	5°C	10°C	20°C	5°C	10°C	20°C	5°C	10°C	20°C
		2	2	*	2	2	2	3	2	1	2	2	1

**very low survival*

NSure defines four stages of cold tolerance based on the activity profile of the indicators:

0	Cold sensitive	The indicator profiles match the profiles of lots that are actively growing and no sign of cold tolerance development could be recognized.
1	Developing cold tolerance	Early signs of frost tolerance development can be recognized.
2	Developing cold tolerance	Frost tolerance level approaches full cold tolerance.
3	Cold tolerant	The indicator profiles match the profiles of lots that have ceased growth and that are fully tolerant, ready for lifting and storage.

Figure 9: Results of the genes tests of the different LN treatments together with the interpretation of the different "NSure stages"

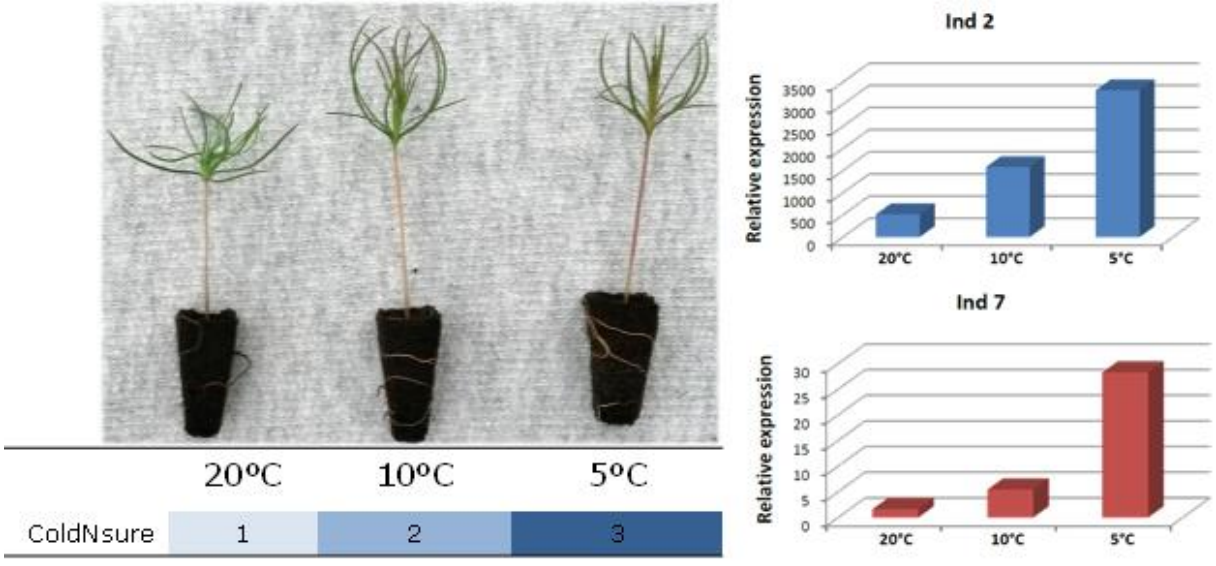


Figure 10: Long night treatment results for Pinus sylvestris (5 hours photoperiod for 5 weeks) showing the effect of the temperature on reaching gene expression level for cold tolerance

4.3.1.2. Growth during Long night treatment

To validate the results obtained from the gene tests, the morphological properties of the seedlings were measured. One of the first things that happens in nature as the plants start preparing for the winter is that they stop growing. Thus, a stop in the growth can give already a hint that the LN treatment applied is having some effect and that the seedlings are reacting to it. In contrast, if the seedlings continue growing during the treatment one could expect them to be not fully cold harden when taken into storage.

In Figure 11 shows the average shoot diameter of *Pinus sylvestris* seedlings after being LN treated under a photoperiod of 5 or 8 hours at different temperatures (5°C, 10°C or 20°C) during 5 or 7 weeks. Similarly Figure 12 presents the average shoot dry weight and Figure 13 the number of needles. These three figures show a similar trend of increasing seedling size with rising LN-treatment temperature. This would mean that even during the LN treatment the seedlings continued growing instead of using those resources to prepare for the cold. This agrees with the results from the gene tests where lower temperatures gave higher cold tolerances.

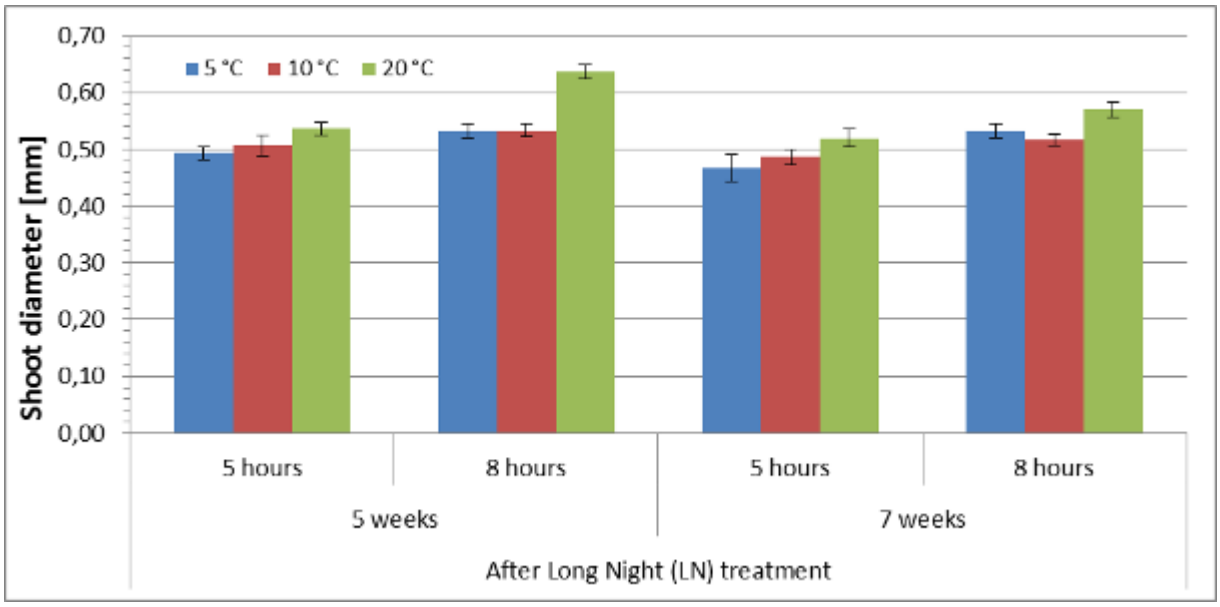


Figure 11: Shoot diameter of Pinus sylvestris seedlings after being LN treated in a photoperiod of 5 or 8 hours at different temperatures (5°C, 10°C or 20°C) during 5 or 7 weeks

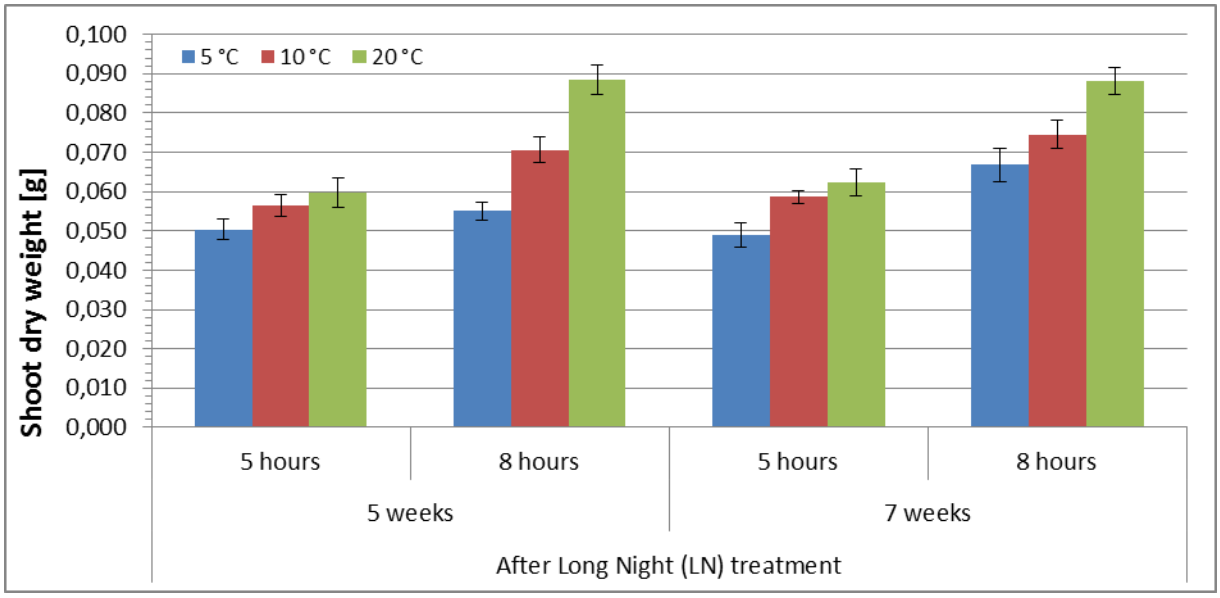


Figure 12: Shoot dry weight of Pinus sylvestris seedlings after being LN treated in a photoperiod of 5 or 8 hours at different temperatures (5°C, 10°C or 20°C) during 5 or 7 weeks

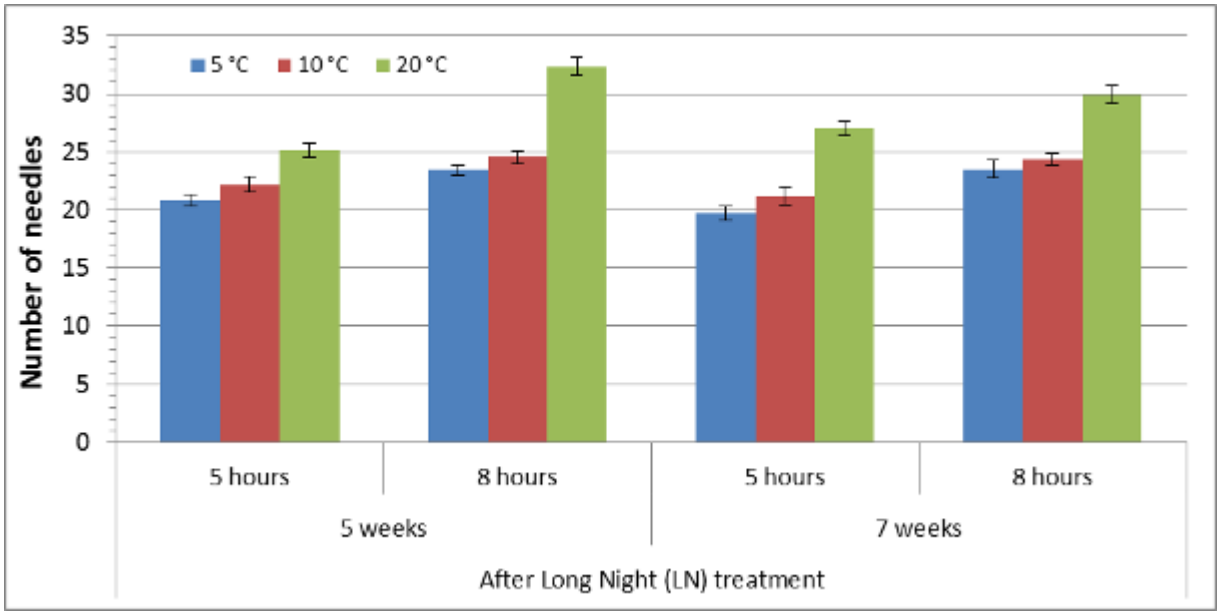


Figure 13: Number of needles of Pinus sylvestris seedlings after being LN treated in a photoperiod of 5 or 8 hours at different temperatures (5°C, 10°C or 20°C) during 5 or 7 weeks

4.3.1.3. Vitality after cool storage

Finally, to verify the previous results, the seedlings were cool stored in dark at +2°C for several months and then their vitality was measured using the *Root Growth Capacity* test (RGC) as it was explained in D3.2.

Figure 14 shows the average RGC results for *Pinus sylvestris* seedlings that were LN treated under a photoperiod of 5 hours at different temperatures (5°C, 10°C or 20°C) during 5 or 7 weeks and then cool stored in dark at 2°C for 9 months. The chart shows the opposite trend as the previous figures, with significantly higher values for the seedlings that were treated at colder (5°C) temperatures. These also agrees with the ColdNSure results since the seedlings with the higher gene expressions stopped growing first during the LN treatment and were more vital after the cool storage.

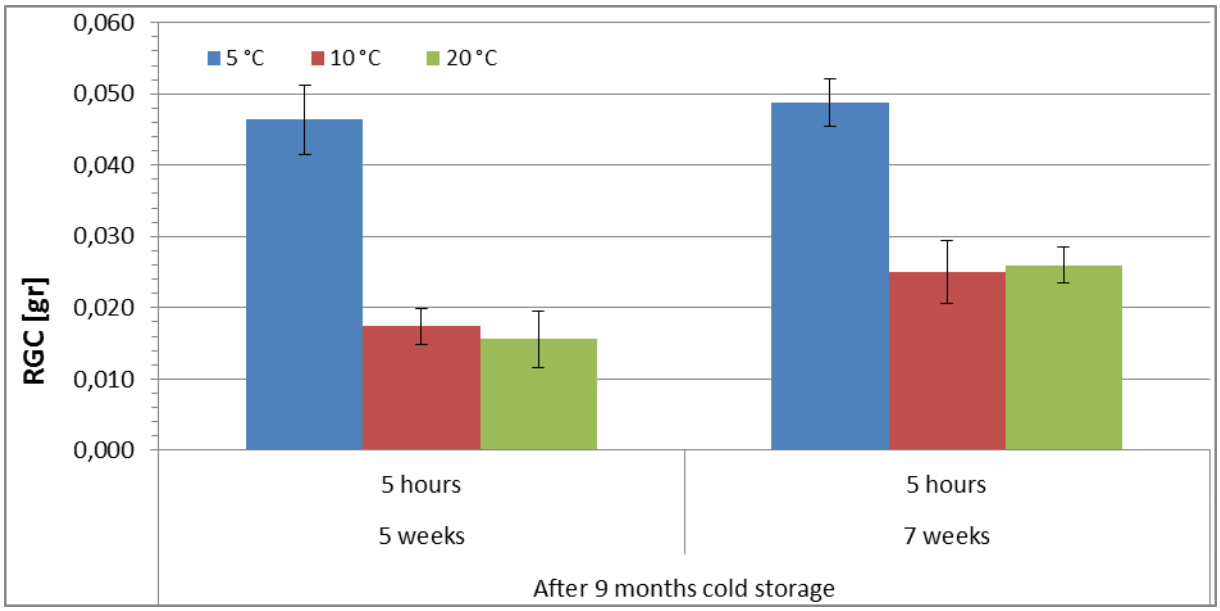


Figure 14: Root growth capacity (RGC) of Pinus sylvestris seedlings that were LN treated under a photoperiod of 5 hours at different temperatures (5°C, 10°C or 20°C) during 5 or 7 weeks and then cool stored in dark at 2°C for 9 months

4.3.2. Light shock treatment

4.3.2.1. Weather conditions at open land

The sunlight and UV-light levels during the first 5 weeks after transplanting to open land are shown in Figure 15. Together with the ambient temperature during this period and the effect of the shading clothes during the first week are also shown.

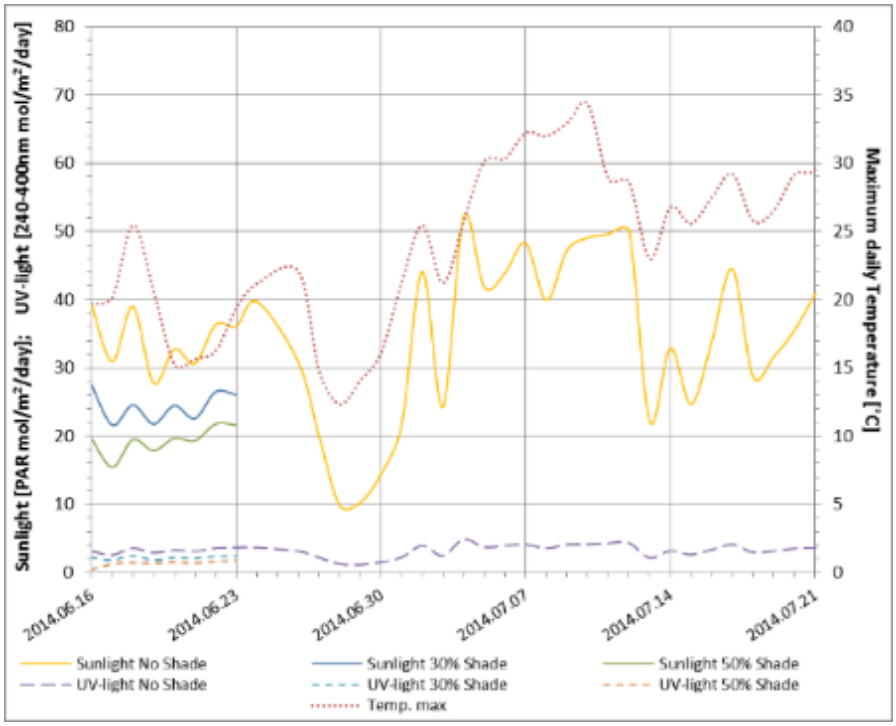


Figure 15: Outdoor conditions during light shock experiment showing the daily temperature, sunlight and UV-light levels displaying also the effects of the shading cloths during the first week after transplanting.

4.3.2.2. Visual damage on the needles of *Picea abies*

Soon after transplanting it was possible to notice some visual damage on the needles of *Picea abies* seedlings (Figure 16 and Figure 17). Especially those seedlings transplanted directly to full sunlight without any shading presented the largest damages.

One can also observe that from those seedlings, the ones that had been treated with UV-a light for 30 minutes daily during the week before transplanting seemed more damaged than the others. It could have been that the way the UV light was applied had not been effective in making them more resistant, but had only stressed them. This way, when they were transplanted to direct sunlight, the seedlings had not developed any protection but were even weaker than the others.

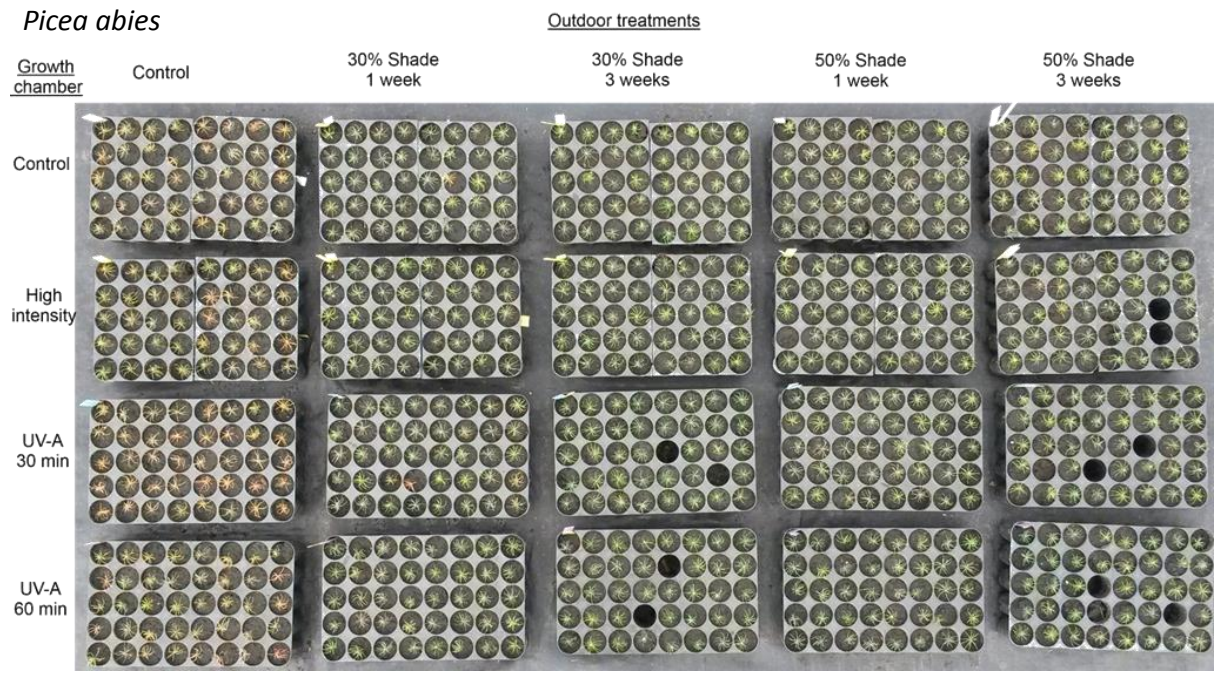


Figure 16: Visual damage on the needles of Picea abies, top view

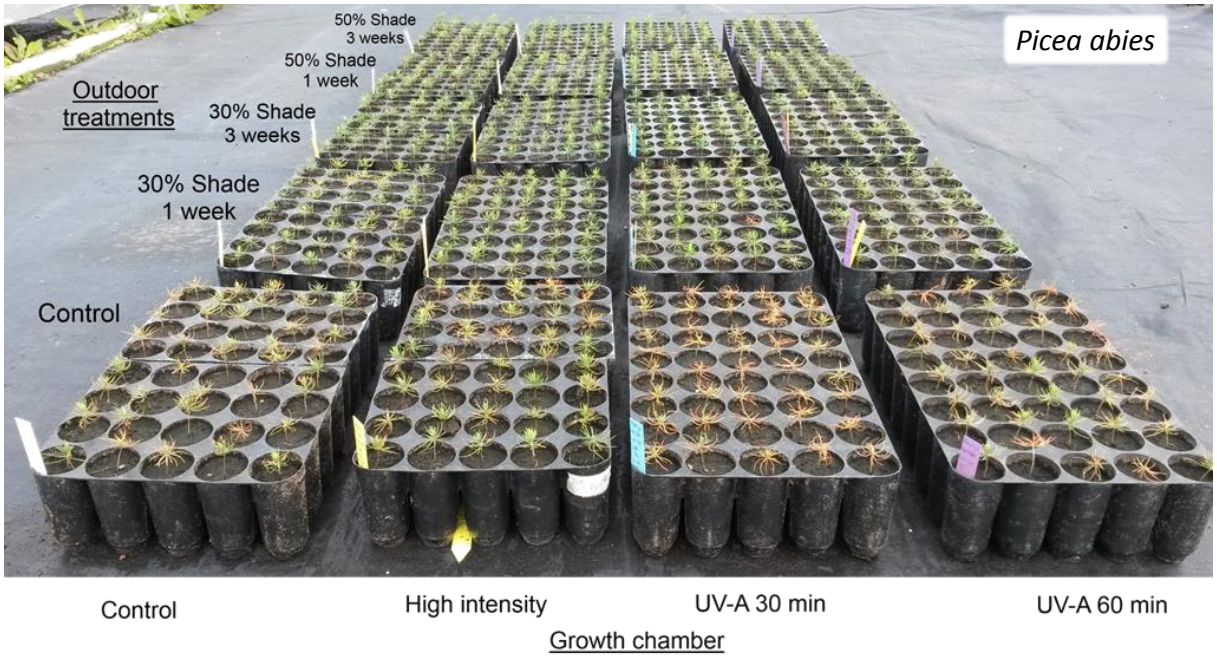


Figure 17: Visual damage on the needles of Picea abies, lateral view

4.3.2.3. Status of the seedlings after one vegetation period in open land

As already observed in previous experiments (see D3.2), the seedlings of *Pinus sylvestris* adapted very quickly to the outdoor conditions and suffered little stress from the light shock. At the end of the vegetation period there were no significant difference between the treated and the control seedlings (Figure 18, 19, and 20). In fact, some of the results from the

previous experiments (presented in D3.2) suggested that leaving the seedlings too long under the shading cloth could actually affect them negatively.

Being a shade tolerant species, Norway spruce seedlings are more affected by the change in conditions. They suffered from a light shock when being transplanted after the five weeks of pre-cultivation. While the indoor treatments applied directly inside the growth chamber presented little or no improvement, it was possible to see a different tendency between the seedlings that had been under a shading cloth and those that had not (Figure 21, 22, and 23).

From the results of the experiment, reducing the sunlight intensity by 30% using a shading cloth gave positive results for the *Picea abies* seedlings. Using a 50% shading cloth did not give significantly better results as neither did applying it for 3 weeks instead of for just 1 week.

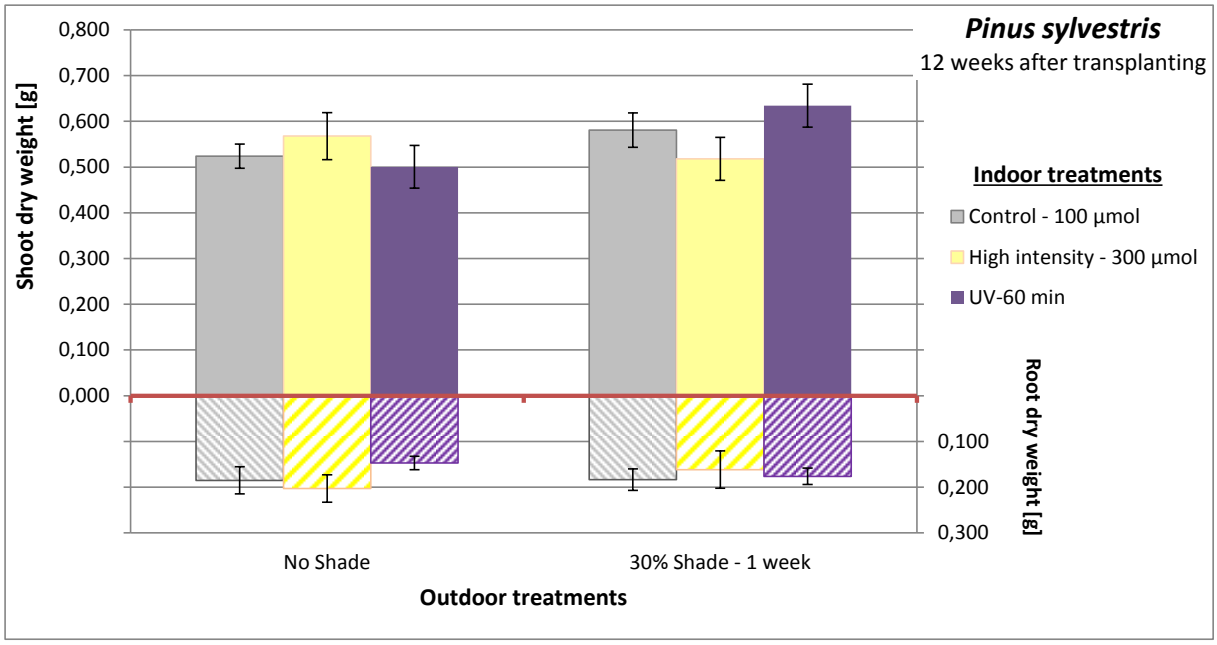


Figure 18: Shoot and root dry weights of Pinus sylvestris seedlings comparing some of the indoor and some of the outdoor light shock treatments

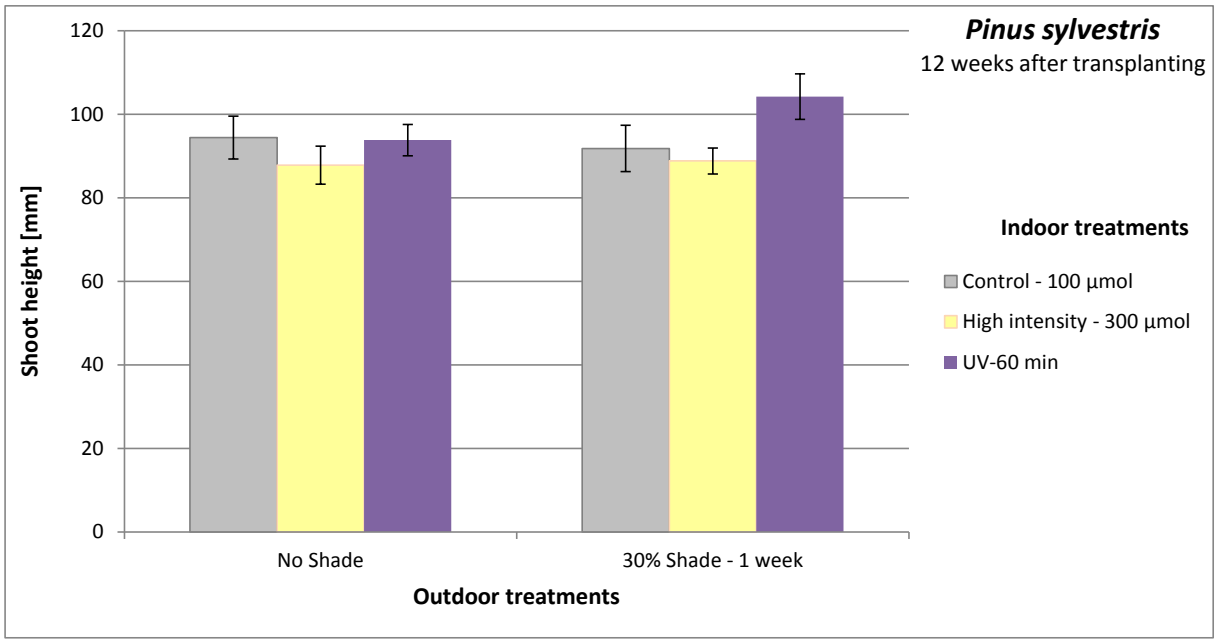


Figure 19: Shoot heights of Pinus sylvestris seedlings comparing some of the indoor and some of the outdoor light shock treatments

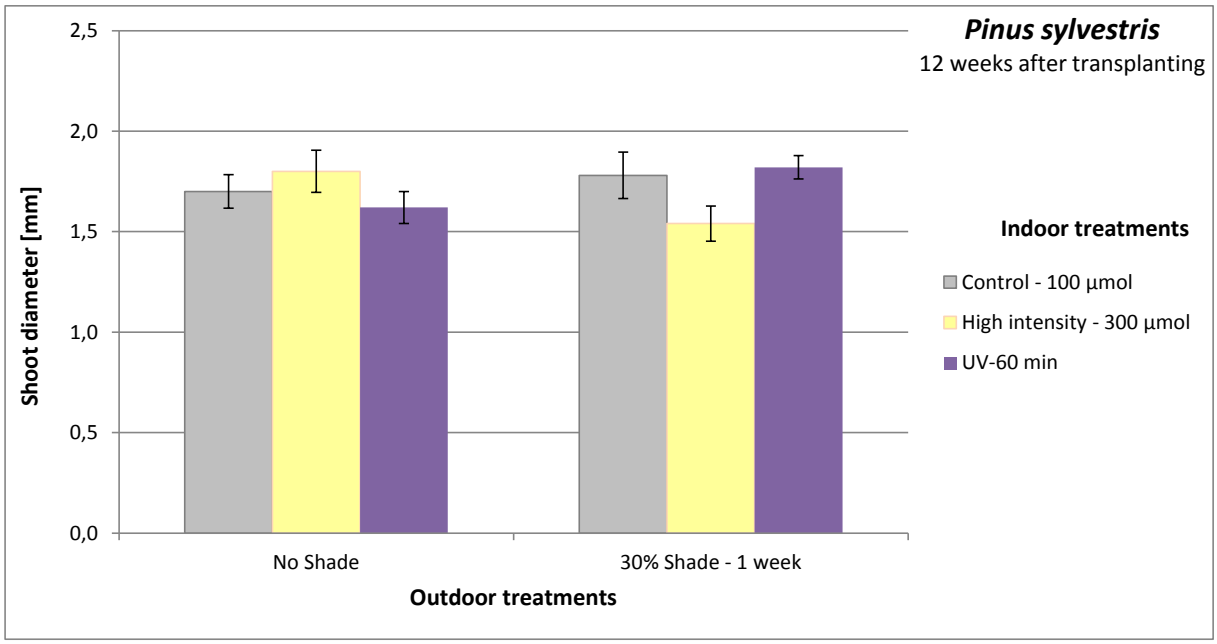


Figure 20: Shoot diameters of Pinus sylvestris seedlings comparing some of the indoor and some of the outdoor light shock treatments

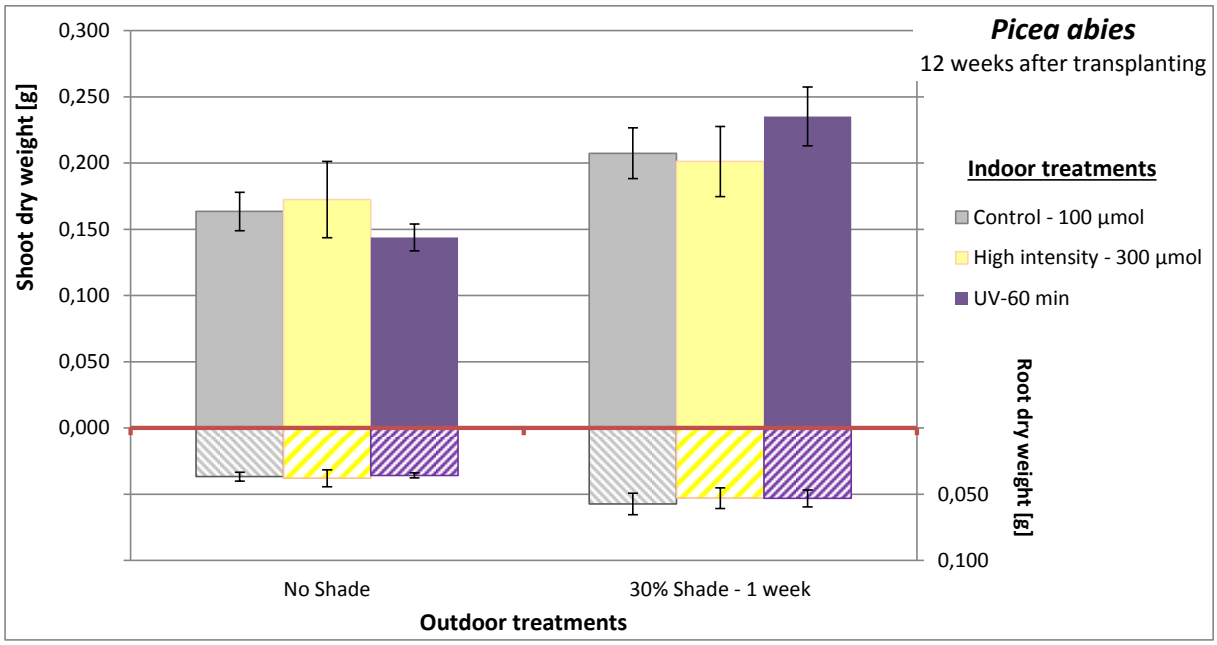


Figure 21: Shoot and root dry weights of Picea abies seedlings comparing some of the indoor and some of the outdoor light shock treatments

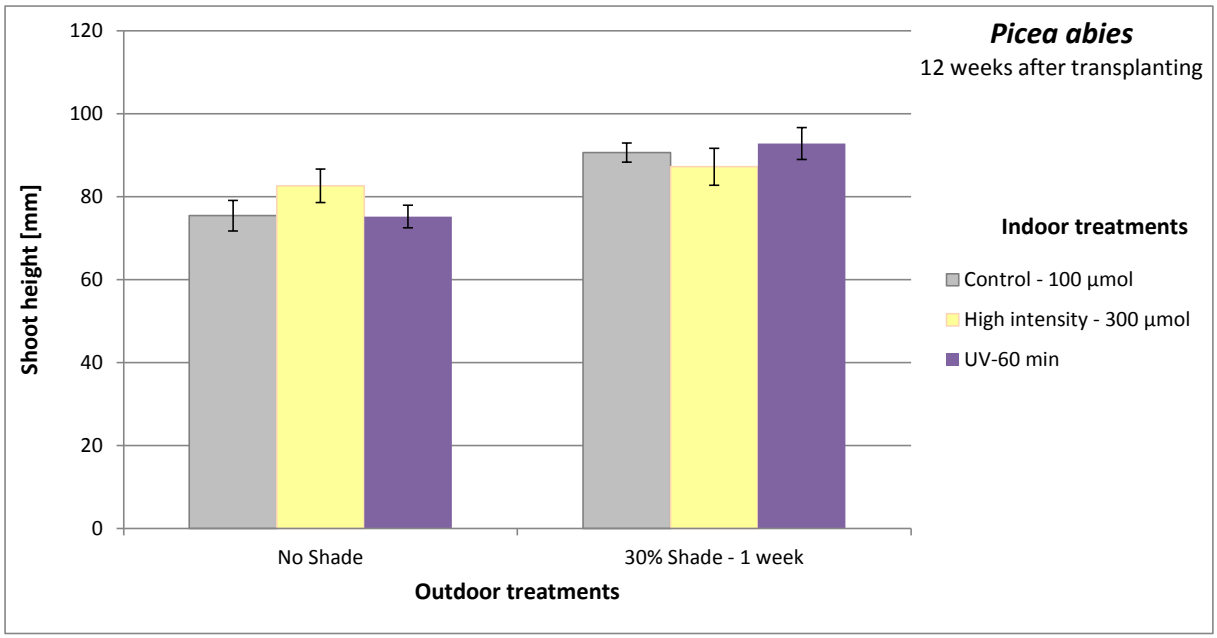


Figure 22: Shoot heights of Picea abies seedlings comparing some of the indoor and some of the outdoor light shock treatments

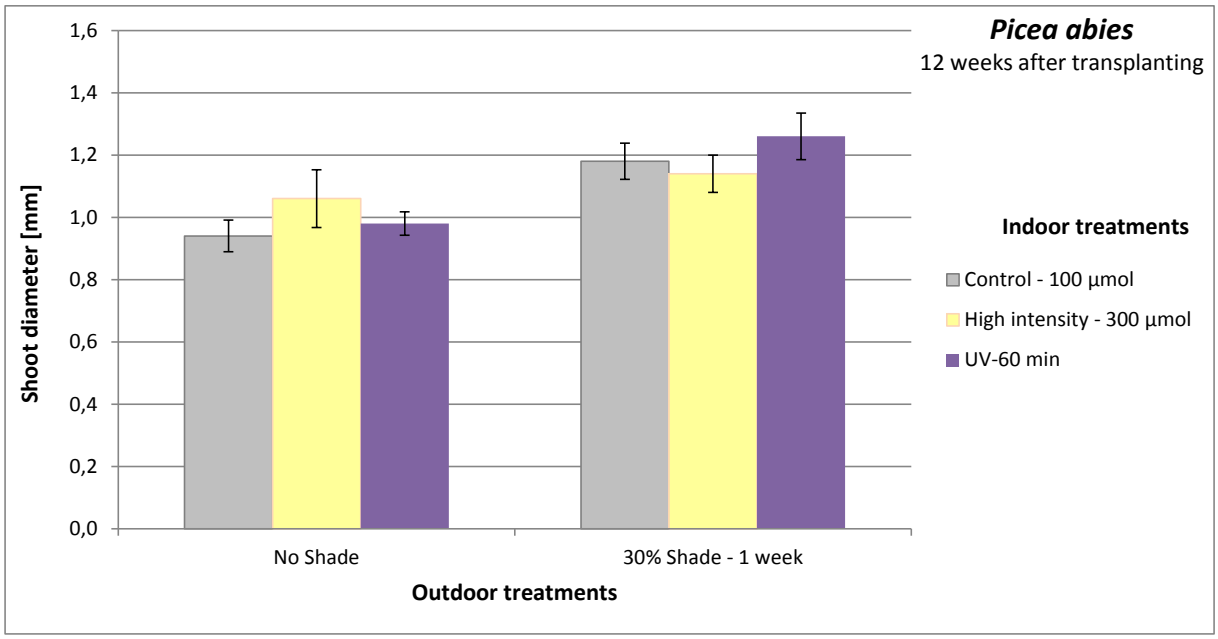


Figure 23: Shoot diameters of Picea abies seedlings comparing some of the indoor and some of the outdoor light shock treatments

4.3.2.4. Gas exchange and Fluorescence measurements

Although the indoor treatments seemed to have little or no influence, the effect of the shading cloths was observable in the Chlorophyll fluorescence since the first day compared to the control seedlings that were exposed to direct sunlight when transplanting (Figure 24 and Figure 25). Control seedlings of *Picea abies* took 3 weeks to get adapted and have similar fluorescence levels to those under the shading clothes. In contrast, seedlings of *Pinus sylvestris* (Figure 26) required only one week to come back to the same levels and started to be negatively affected by too much shading (50%) after 3 weeks.

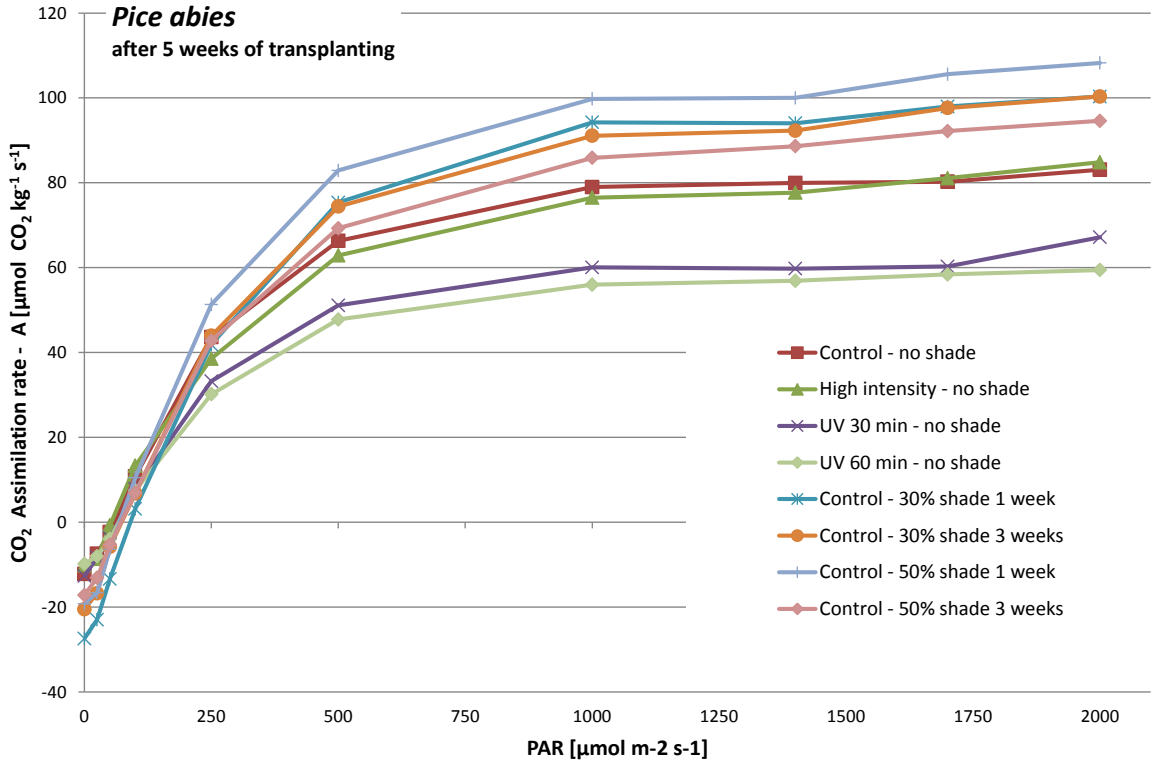


Figure 24: Light response curves of Picea abies seedlings after 5 weeks of transplanting to open land showing the effect of the light shock in the CO2 assimilation

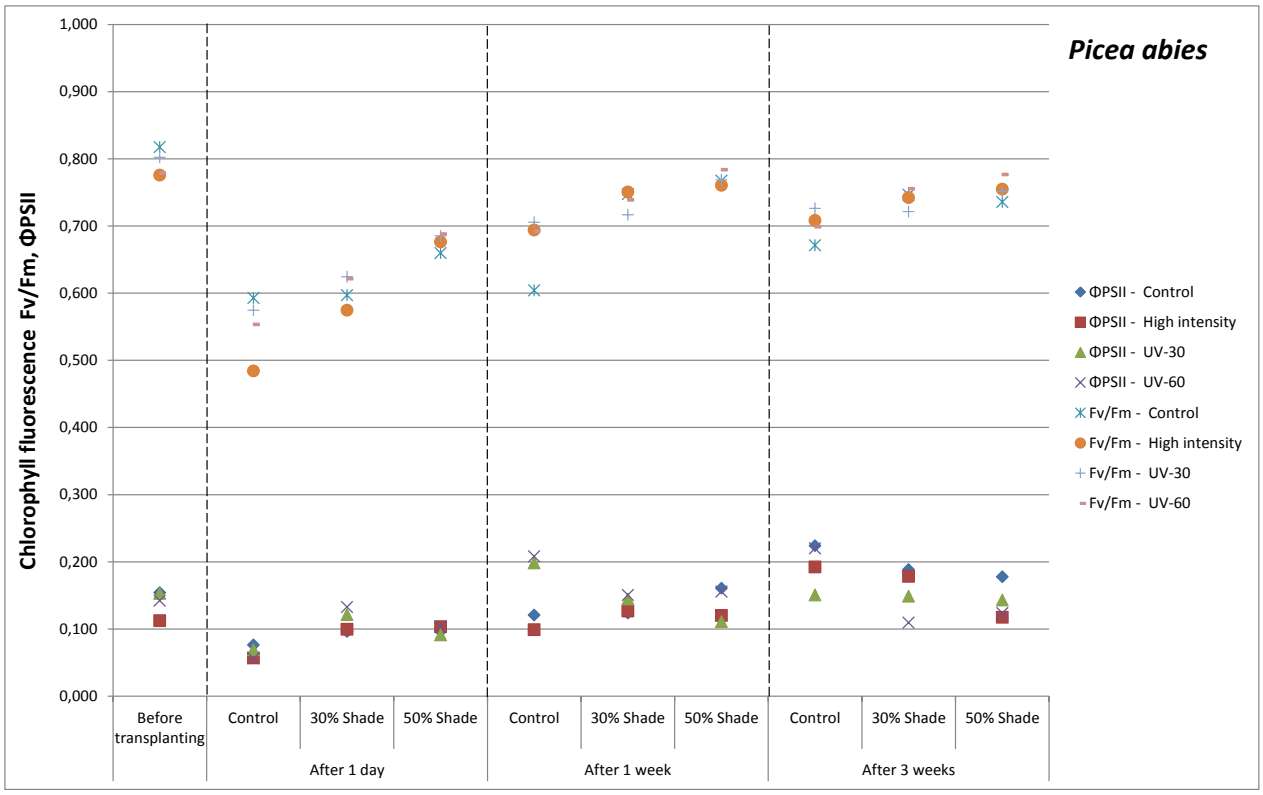


Figure 25: Light shock effect on the Chlorophyll fluorescence of Norway spruce seedlings measured at several points in time after transplanting.

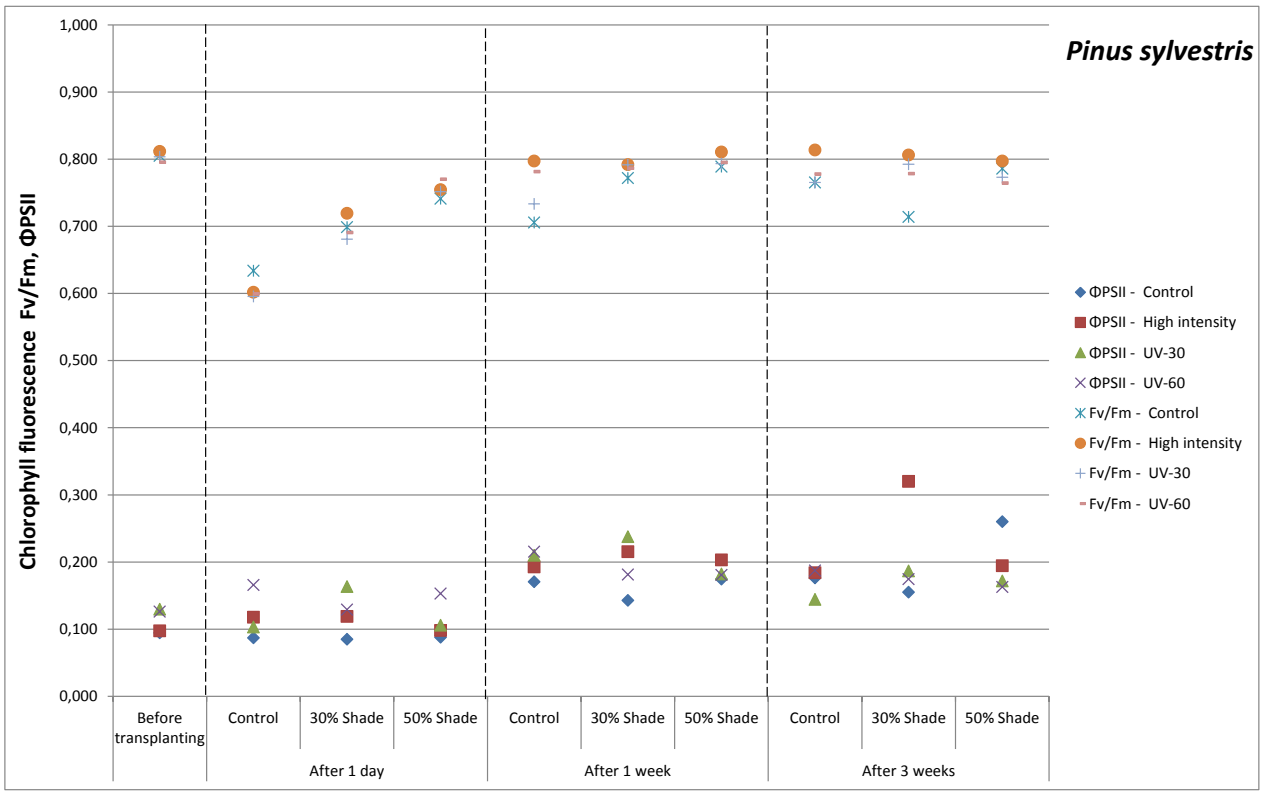


Figure 26: Light shock effect on the Chlorophyll fluorescence of Scots pine seedlings measured at several points in time after transplanting.

4.3.3. Light intensity

After conducting the three sowings, both species showed similar general responses to the different light intensities. In both cases with increasing light intensity the biomass increased (Figure 28 for Norway spruce and Figure 29 for Scots pine); while the shoot height decreased (Figure 30 for Norway spruce and Figure 31 for Scots pine).

Figure 27 shows an example of how the seedlings of both species looked like when grown at the different intensities. One can notice how the height is notably decreasing as the intensity increased but also how the seedling became denser and compact. The needles of the 400µmol treatment were pointing upwards and looked as if they were “closed”. At the same time, the needles of the 50µmol seedlings where much more spread to the sides to be able to catch more light.

It is also possible to observe a color difference in the stem and needles, especially of the *Pinus sylvestris* seedlings. At 50µmol the stem and needles were of the same dark green tone while at 400µmol the needles were yellowish and the stem had some tones of red. In future trials it would be convenient to also perform a chemical analysis of the seedlings’ photosynthetic pigments. Analyzing the chlorophyll and carotenoids content could complement and help to better understand to the gas exchange measurements.

Figure 27: Differences in seedlings of Picea abies (a, c) and Pinus sylvestris (b, d) grown at various intensities.

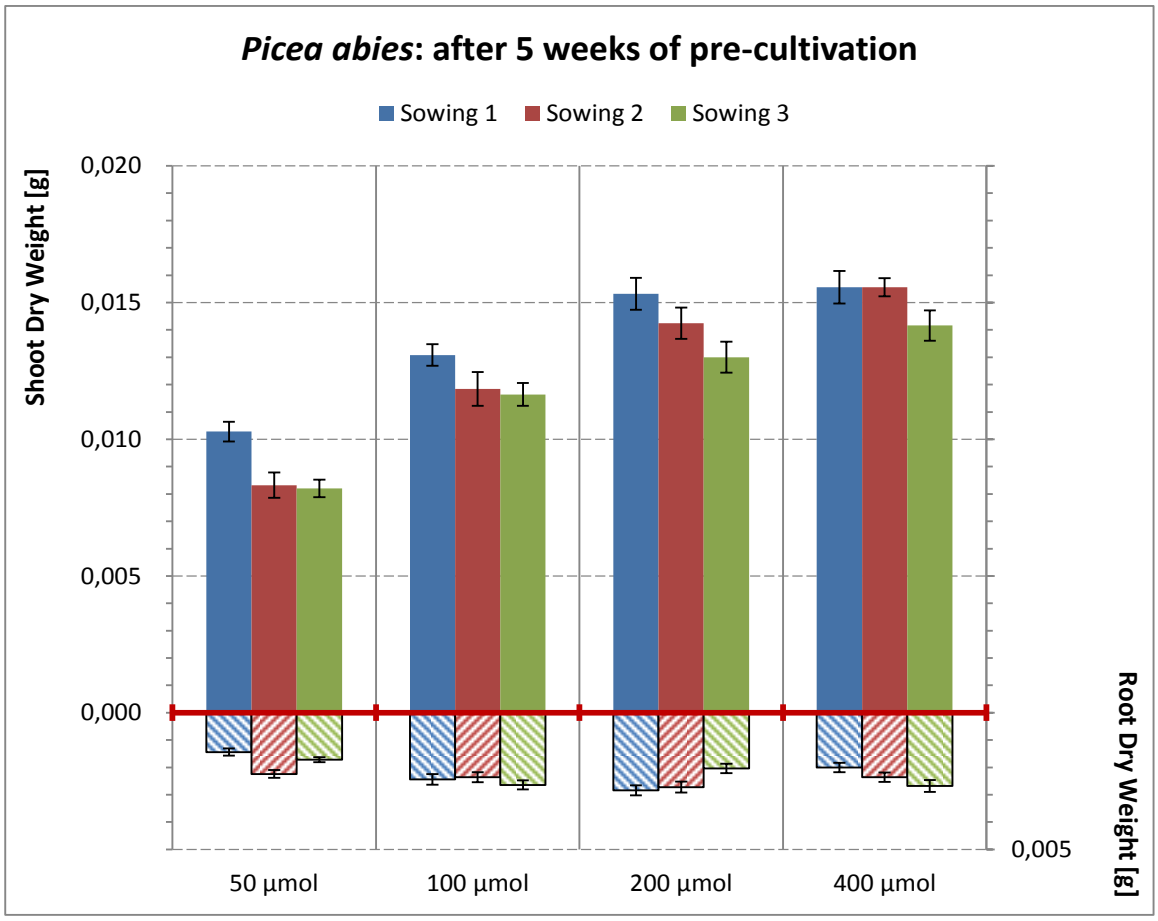


Figure 28: Shoot and root dry weights of Picea abies seedlings

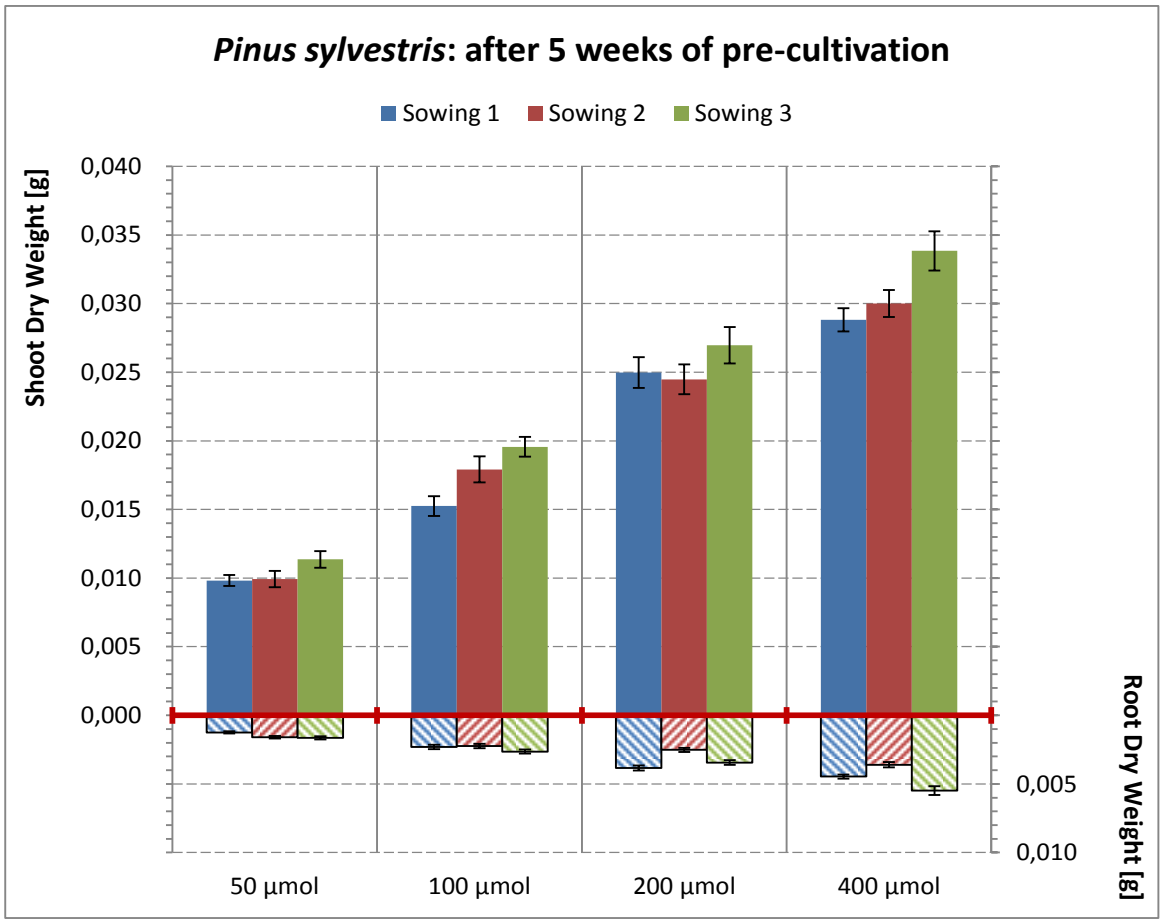


Figure 29: Shoot and root dry weights of Pinus sylvestris seedlings

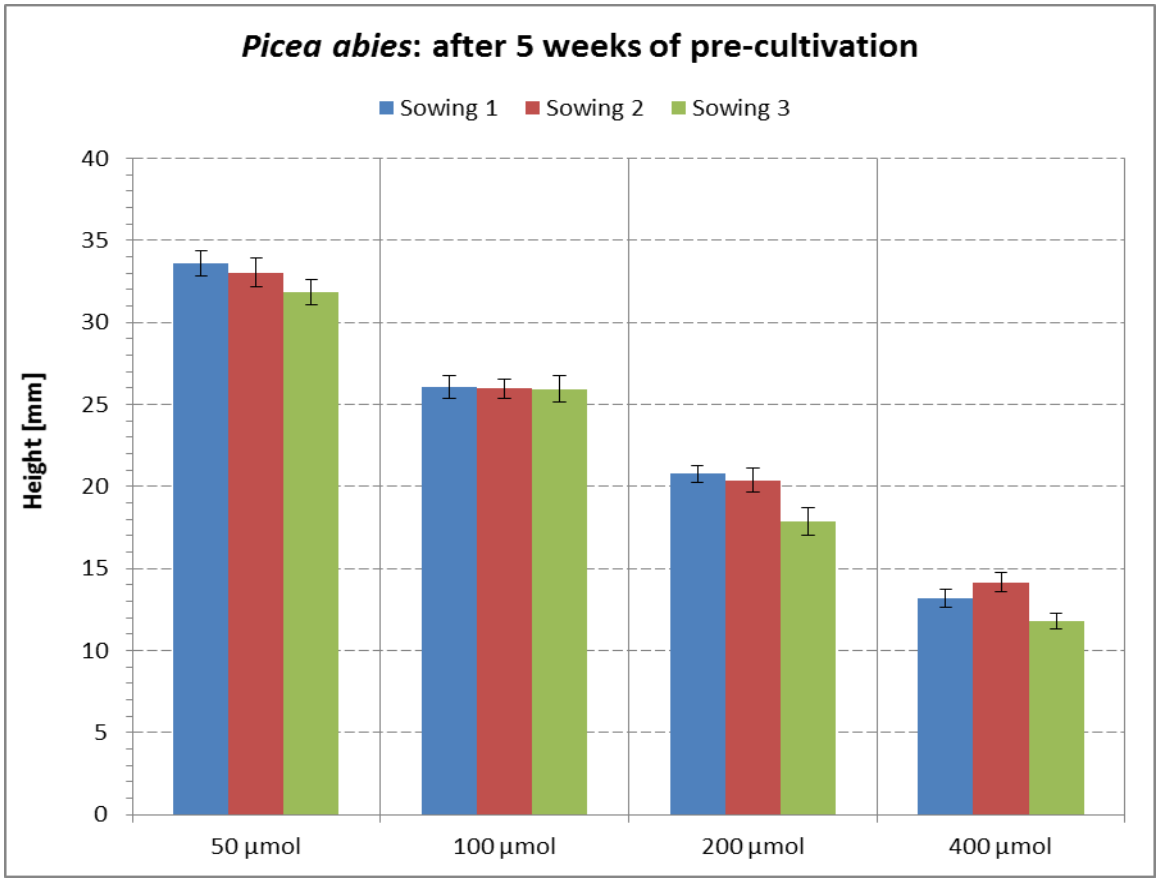


Figure 30: Shoot heights of Picea abies seedlings

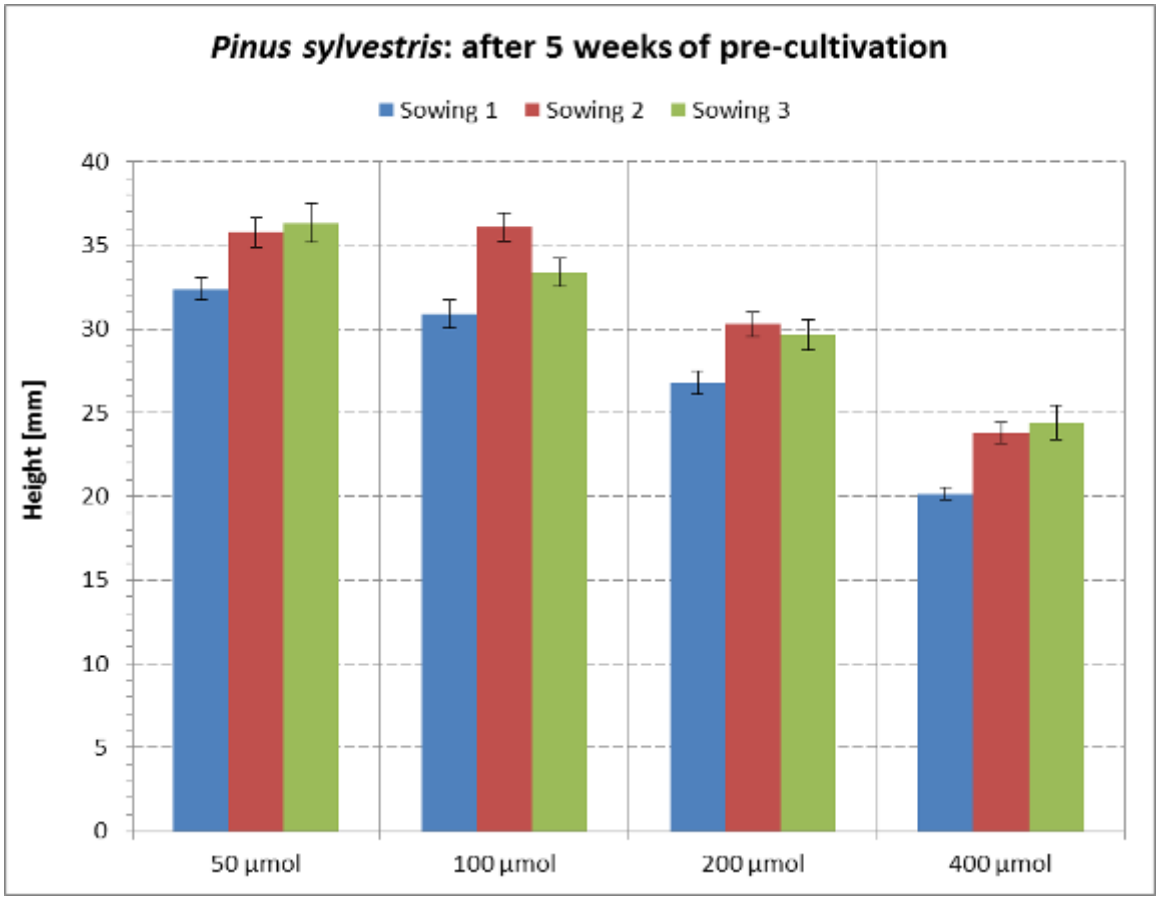


Figure 31: Shoot heights of Pinus sylvestris seedlings

When measuring their CO_2 assimilation rates, seedlings grown under lower intensities were more efficient using the light. This was probably due to the fact that they had developed under limiting conditions and their light receptors had adapted to catch as much light available as possible.

Picea abies is a shade tolerant plant, thus it was to seedlings seemed to reach their light-saturated rate of photosynthesis at 200 µmol/m^{2}s. Meanwhile *Pinus Sylvestris,* being a sun plant, reached its peak at around 400 µmol/m^{2}s.

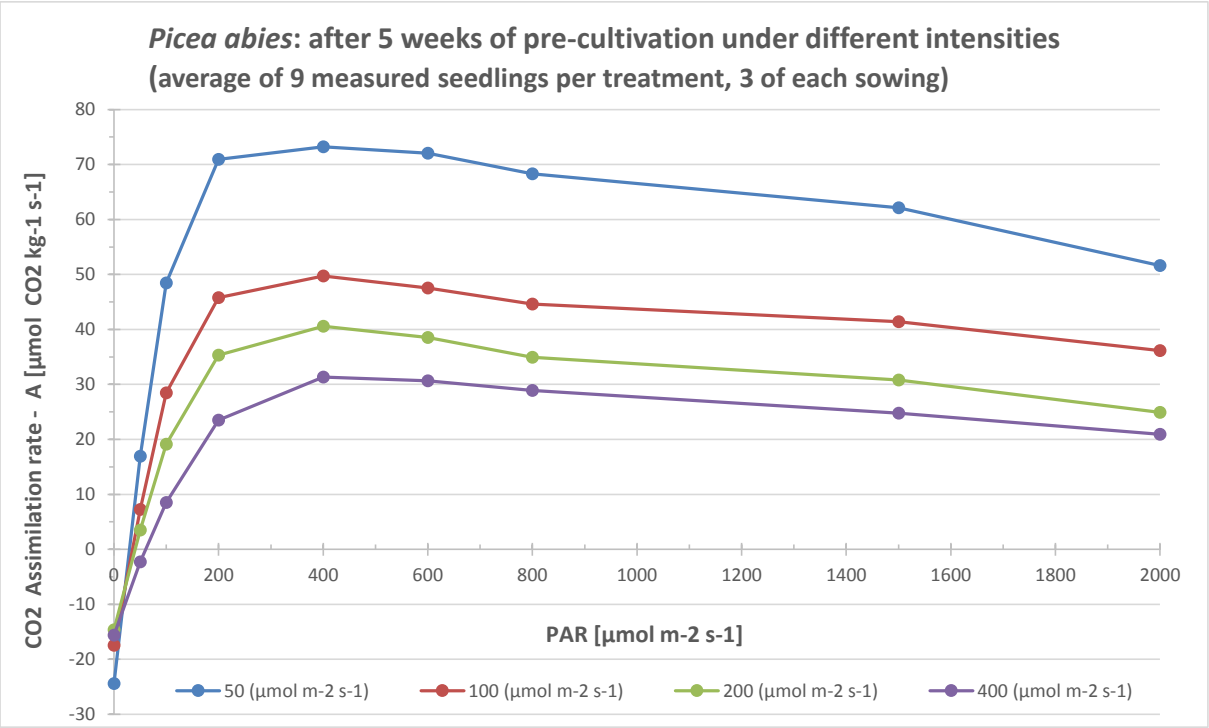


Figure 32: CO_2 Assimilation light curves for Picea abies seedlings that were pre-cultivated at different intensities.

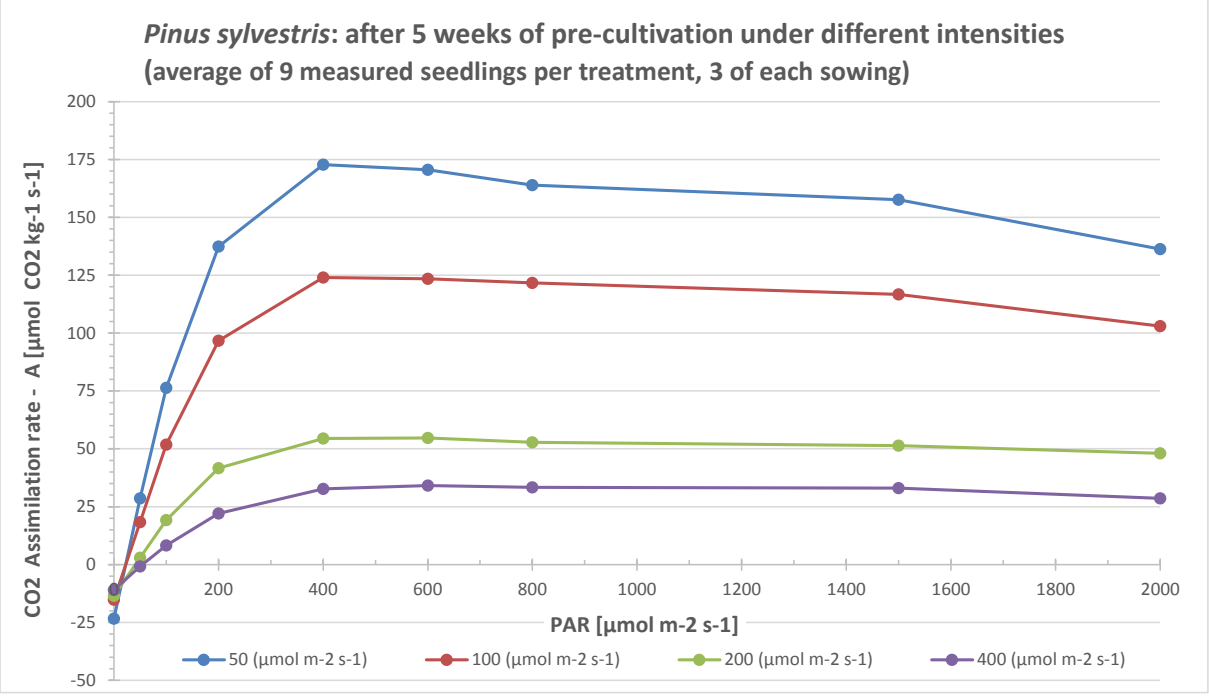


Figure 33: CO_2 Assimilation light curves for Pinus sylvestris seedlings that were pre-cultivated at different intensities.

4.3.4. Forest field trial

After one vegetation period on open land and two vegetation periods in the forest trial, the general tendencies maintain for both *Picea abies* and *Pinus sylvestris* seedlings. The seedlings that had been pre-cultivated under LED lamps were in average at least as tall as those pre-cultivated under fluorescent lamps. Especially the seedlings from the AP67-tube and NS1 had a notable increase in height and stem diameter. In general, the seedlings seem to be performing well and have adapted to the natural conditions despite the challenge of the strong competing vegetation.

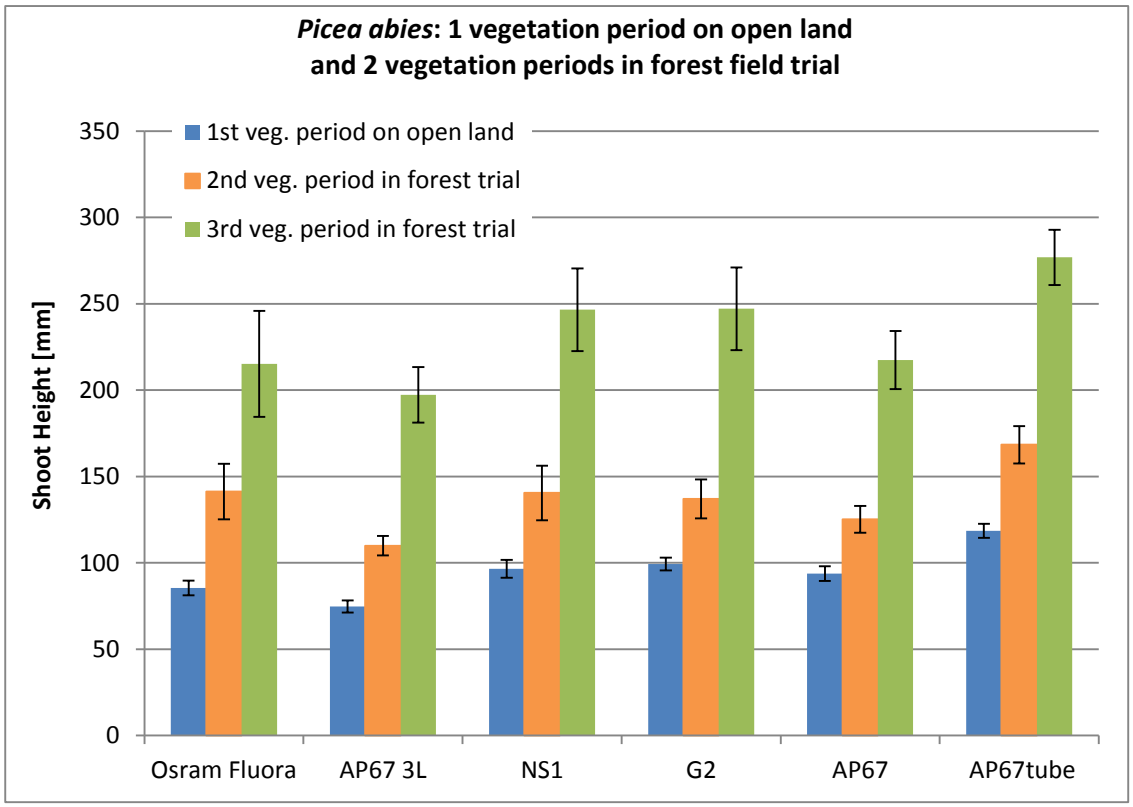


Figure 34: Shoot height comparison of Picea abies seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land and two vegetation periods in field trial in forest.

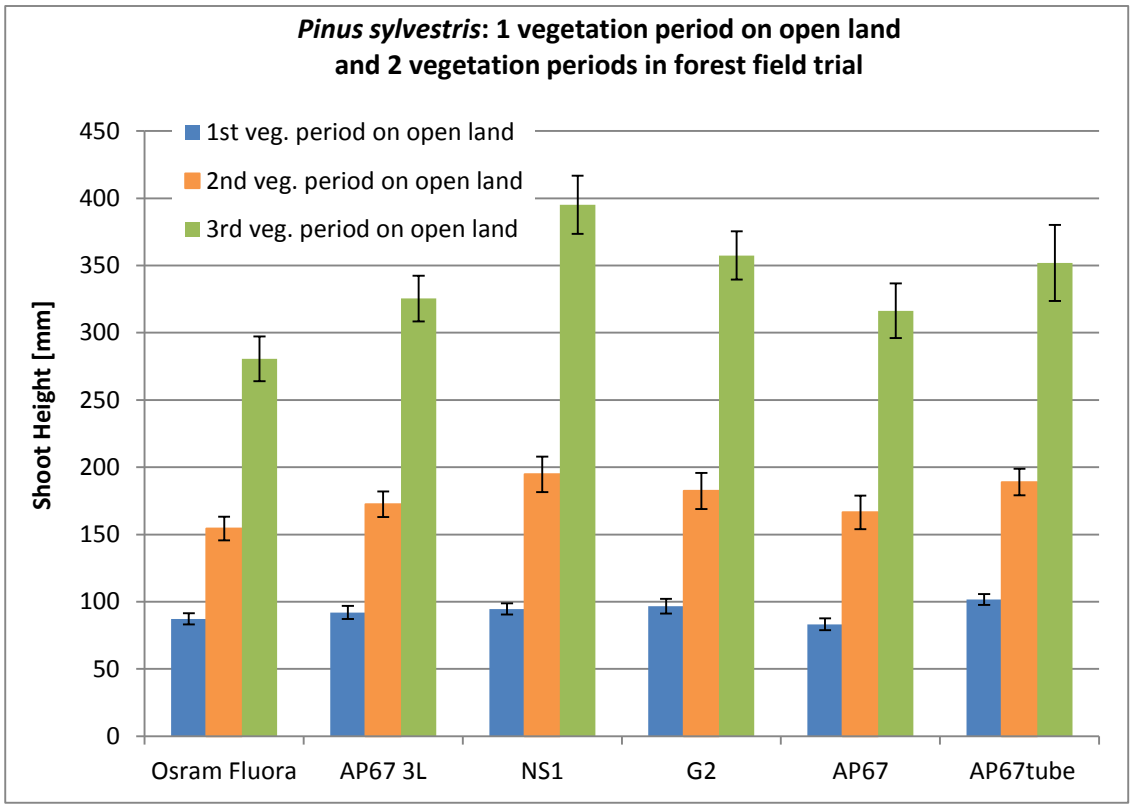


Figure 35: Shoot height comparison of Pinus sylvestris seedlings directly transplanted after pre-cultivation and grown for 1 vegetation period in open land and two vegetation periods in field trial in forest.

4.4. Conclusions

4.4.1. Long night treatments

4.4.1.1. *Pinus sylvestris*

It was found that the temperature during the treatment played a key role of the hardiness process being 5ºC better for *Pinus sylvestris*. The seedlings that were treated at lower temperatures stopped their growth earlier and were more cold harden when taken into the cool storage. Afterwards, when taken out of the storage after several month, those seedlings showed a higher vitality.

Complete dark or very short photoperiods during the LN treatment (0 or 2 hours of light per day) showed low levels of seedlings survival. The cold hardiness process requires a great energy input from the plants which were not receiving enough light to cope with these needs.

From the different photoperiods tested, 5 hours of light during 5 weeks was enough for inducing cold tolerance if treated at 5ºC air temperature. Applying this same treatment during 7 weeks didn't bring any improvement.

4.4.1.2. *Picea abies*

As already reported in D3.2, Norway spruce seedlings are normally LN treated during 5 weeks with a daily photoperiod of 8 hours and a night period of 16 hours at an ambient temperature of 20°C. To test if this treatment also could be applicable for young pre-cultivated Norway seedlings the same procedure was applied.

As the LED light source AP67 in the tube version had proven to be favorable this light source was also chosen during the 5 weeks of pre-cultivation of Norway spruce seedlings. After pre-cultivation LN treatment was conducted during 5 weeks under the same conditions as described for conventionally grown Norway spruce seedlings. The treatment showed to be very effective and after the gene test the result showed that the seedlings were cold tolerant and ready for cold storage.

4.4.2. Light shock

4.4.2.1. *Pinus sylvestris*

As shown from the experiments in D3.2 and from the results parts in this report, *Pinus sylvestris* adapts very quickly to the outdoor conditions and suffers little from light shock. At the end of the vegetation period there were no significant differences between the treated and the control seedlings. In fact, some of the results suggested that leaving the seedlings too long under the shading cloth could affect them negatively.

4.4.2.2. *Picea abies*

Being a shade tolerant species, Norway spruce seedlings are more affected by the change in conditions and suffer from a light shock when being transplanted after the five weeks of pre-cultivation. In order to minimize the risks for damages caused by the excess of sunlight, it is recommended to use a shading cloth to protect the seedling and give them time to acclimate.

From the results of the experiment, reducing the sunlight intensity by 30% using a shading cloth gave positive results. Using a 50% shading cloth did not give significantly better results. The shading should be applied for a week since 3 weeks gave no improvement.

Regarding the indoor treatments applied directly inside the growth chamber before transplanting, more tests are necessary to really understand the way high intensity light and UV-light affect the seedlings. For *Picea abies,* the amount of UV light applied seemed to have further stressed the seedlings instead of helping.

4.4.3. Light Intensity

Similar responses were observed for both species after the 5 weeks of pre-cultivation under different light intensities. In both cases the biomass increased with increasing light intensity while the shoot height decreased, producing more compact plants.

When measuring their CO_2 assimilation rates, seedlings grown under lower intensities were more efficient using the light. This was probably due to the fact that they had developed under limiting light conditions and their receptors where bigger and more open in order to catch as much light available as possible. These doesn't mean a necessary advantage since these seedling would probably present the higher risks of light shock later when being transplanted.

The *Picea abies* seedlings seemed to reach their light-saturated rate of photosynthesis at 200 $\mu mol/m^{2}\cdot s$ while the *Pinus Sylvestris,* being a sun plant, reached at around 400 $\mu mol/m^{2}\cdot s$.

When comparing the seedlings quality to the energy level used, an optimal level seem to be between 100 and 200 $\mu mol/m^{2}\cdot s$. Within this range the seedlings of both species grow with a good quality and using efficiently the light and without needing too much electricity.

4.4.4. Forest field trial

After two years on the field, one can conclude that the seedlings grown under LED lights perform at least as good as those grown under fluorescent lamps. Despite all the external factors that can affect such a field study, it was still possible to observe differences among the treatments. In fact, most of these differences where already existing after the pre-

cultivation phase before taking the seedlings to the field. These shows the importance of the pre-cultivation phase to have a proper start.

4.5. Growth protocols for northern species

The new recommended growth protocols for both Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) are based on the results of the experiments that were conducted during the project. They combine the processes of seedling pre-cultivation and transplanting in the Zephyr concept for a year-round production in northern climates (Figure 36).

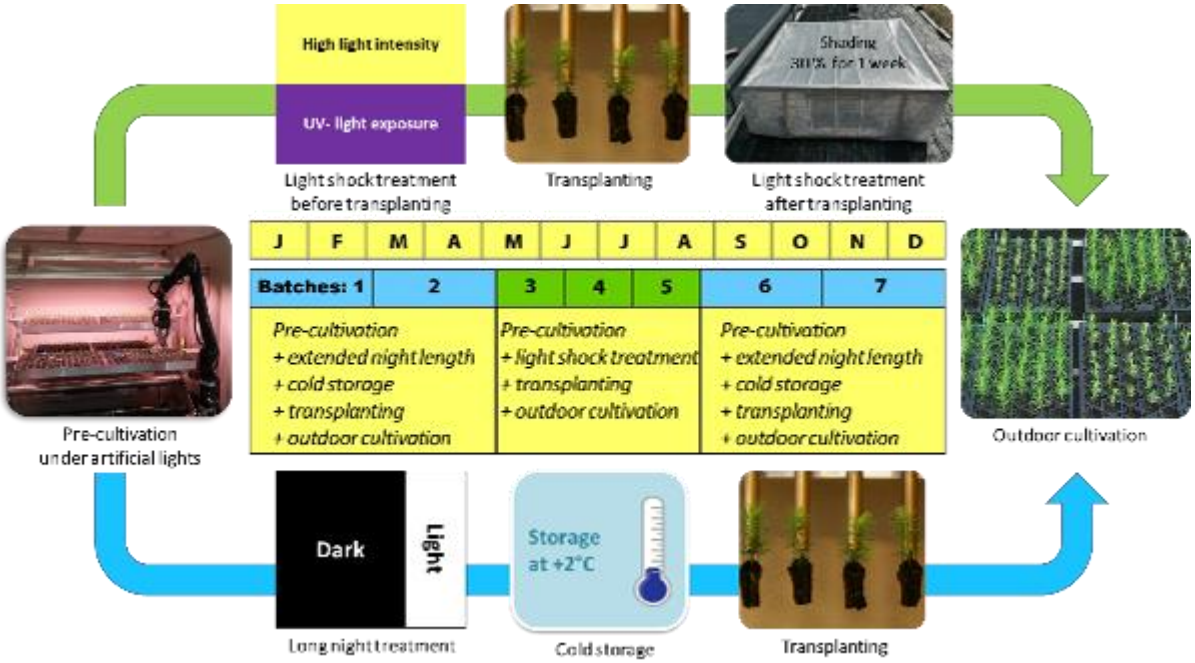


Figure 36: Zephyr concept in a year-round production in northern climates

- **Period of seed harvesting:** *(unchanged from D3.1)*
  - Norway spruce: July to August (southern range), August to September (northern range)
  - Scots pine: August to October (southern range), October to December (northern range)

- **Seed storage and storage period:** *(unchanged from D3.1)*
  At a temperature of -15º C in sealed glass or plastic bottles up to 10 years for both species

- **Pretreatments:** *(unchanged from D3.1)*

No pretreatment necessary

- **Container and substrate:** *(unchanged from D3.1)*
  Quickpot QPD 104 W (Hercuplast) filled with Preforma substrate (Jiffy International)
- **Temperature:** *(unchanged from D3.1)*
  20°C during germination and pre-cultivation
- **Watering:** *(unchanged from D3.1)*
  For both species the ebb and flood method will be used three times a week until the substrate saturation has reached field capacity.
- **Relative humidity:**
  - During germination: 80%
  - During pre-cultivation: 60%
- **Light Spectra and intensity:**
  Valoya AP67 at 100 µmol/m2 s
- **Photoperiod:**
  16 hours light / 8 hours dark during germination and pre-cultivation
- **Duration:**
  - Germination phase: 1 week
  - Pre-cultivation: 4 weeks
- **Treatments before transplanting:** (Depending on the time of the year, see Figure 36)

	Pinus sylvestris	*Picea abies*
Direct transplanting (batches 3,4,5)		
• Indoor light shock treatment before transplanting	* *Further studies should be conducted to explore the effects and possible benefits of exposure to high light intensity* and UV-light *during the pre-cultivation of mini seedlings of both species.*	
• Outdoors acclimation after transplanting	No need for shading after transplanting	The use of a 30% shading cloth for the first week after transplanting is recommended.
Long night treatment and cold storage (batches 1,2,6,7)		
• Temperature during LN	5°C	20°C
• Light Spectra and intensity	Valoya AP67 at 100 µmol/m^2 s	Valoya AP67 at 100 µmol/m^2 s
• Photoperiod during LN	5 hours of light / 19 hours of darkness	8 hours of light / 16 hours of darkness
• Duration of LN	5 weeks	5 weeks
• Temperature during cold storage	+2°C	
• Photoperiod during cold storage	No light, 24 hours of darkness	
• Duration of cold storage	Up to 8 months	

5. INPUT OF UNINSUBRIA

5.1. Material and Methods

5.1.1. Growth protocols of three Azorean plant species (*Frangula azorica*, *Morella faya* and *Prunus azorica*)

From month 18th to month 35th of the project, three Azorean plant species *Frangula azorica* (Tutin), *Morella faya* (Ait.) Wilbur, and *Prunus azorica* Rivas Mart., Lousa Fer. Prieto, E.Dias, J.C. Costa, C. Aguiar *azorica* (Hort. ex Mouil.) were tested. The Azorean species reproduction and conservation is very important for several reasons. These species have an important role as key components of the Azorean Laurel-Forest. This broad-leaved evergreen forest is largely dominated by *Laurus azorica* (a Macaronesian endemic) and *Morella faya* and is considered to be the natural climax vegetation up to an altitude of about 600m above the sea level. Furthermore, seed germination for these species is extremely difficult representing a problem for genetic conservation and thus a challenge for an optimal new germination protocols development. Main threats faced by these species are: habitat degradation mainly due to invasive alien flora species, agriculture expansion and development of infrastructures. Main factors that inhibit the recovery of these species are the dramatic low density of the natural population and its extreme fragmentation resulting in a poor genetic diversity. Seed germination and growth protocols of the Azorean endemic species are almost unknown. In the present project, existing Azorean species protocols were modified. In the case of *Morella faya* protocols were developed according to protocols developed for the same *genus* and similar ecological species.

Frangula azorica protocol based on the protocol indications of *Frangula alnus* (see the References!) and ongoing experiments.

Prunus azorica protocol based on the existent protocol indications (see References) and ongoing experiments.

Myrica faya protocol based on the protocol indications of *Myrica rubra* (see References) and ongoing experiments.

5.1.1.1. Seeds pre-treatment, removal of dormancy

Frangula azorica (Tutin)

Frangula azorica seeds were mixed in a moist draining substrate with 50/50 mixture of compost and sharp sand with enough volume of material to keep the seeds separated. After that the seeds mixture were placed in a clear plastic bag (freezer bags) closed but with a little gap left to provide air exchange. Later, seeds were kept in refrigerator at 4°C for two months. Following seeds sowed in mini-plug plastic trays with a growth substrate made of sphagnum and sand and transferred in the environmentally controlled growth chamber and under different LED light type.

Morella faya (Ait.) Wilbur

Morella faya (firetree) seeds were provided by Azorina Sociedade Gestão Ambiental e Conservação da Natureza, of the year 2014. Seeds are orthodox and for breaking the dormancy were pre-treated in warm (moist sphagnum) stratification for 8 weeks. Seeds of *M. faya* were mixed with moist sphagnum (water content of the sphagnum was about 400% of dry mass). Sphagnum was boiled per half an hour to sterilize it, and left become cold before to mix seeds. Then seeds were sealed inside polyethylene bags (0.04 mm in thickness) and incubated at a day/night temperature of 30/20°C with a 12-h photoperiod supplied by fluorescent light (100-120 µmol). After the two months of stratification seeds were treated with 5.2 mM GA3 solution at room temperature before germination. Seeds washed before in a strainer to remove the stratification substrate. A solution with 5.2 mM GA3, with 0.54 gr of GA3 (potassium salt) and 300 ml of distilled water (ddH_2O), was prepared. Washed seeds kept in the GA3 solution per 20 h at room temperature. Following seeds directly sowed in mini-plug plastic trays with a growth substrate made of sphagnum and sand and transferred in the environmentally controlled growth chamber at the same photoperiod and temperature conditions of the warm stratification waiting the germination.

Prunus azorica (Hort. Ex Mouil.) Rivas Mart., Lousa Fer. Prieto, E.Dias, J.C. Costa, C. Aguiar

Prunus azorica is an Azorean endemic tree considered as one of the top 100 priority taxa for conservation in Macaronesia (Martins et al., 2008), it is one of the constituents of the middle altitude laurel forest species, a type of forest that was largely replaced by other land uses. (Moreover, in Sa˜o Miguel Island, it is also one of the main food sources for the endangered bird *Pyrrhula murina*). After endocarp removal, *Prunus azorica* seeds were transferred into an incubator at temperature of 10°C with a photoperiod of 12 hours provided by a fluorescent lamps (19 - 22 µmol $m^{-2}{\cdot}s^{-1}$) for 3 month. After that no one radicle appeared

so no one seed germinated but one seed was transferred into each cell of the mini-plug trays and transferred into the growth chamber in environmental controlled conditions and under different LED light to try to stimulate the germination.

5.1.1.2. Seed germination and growth conditions

Germination and rate conditions for Azorean species seeds are schematically reported in the following sections.

Prunus azorica seeds dormancy was not removed, despite the pre-treatment applied because after 3 months none seedling appeared, and the trays were full covered by algae and fungus (mildew).

Germination conditions:

Frangula azorica and *Morella faya* seeds were sowed in the mini-plug trays and left to germinate and grow for a period of 43 days and 66 days from the sowing day respectively. A Environmental settings follow reported

- Substrate: sphagnum and sand (VigorPlant) pH = 6,4
- Light: 5 different LED lights (spectra) (Valoya) and Fluorescent light (or tube spectrum) (Osram) as a control

  LED: - AP67 (bar, 2 lamps)

  - AP67 V1 model L20 (tube, 6 lamps)

  - AP67-3L (bar 1 lamp)

  - G2 (bar, 2 lamps)

  - NS1 bar (bar 1 lamp)

  Control: OSRAM L36W/77 FLUORA (Fluorescent)

  PAR: 90-100 µmol m^{-2} s^{-1} (over the substrate surface)
- Photoperiod: 12/12 hours (light/darkness)
- Temperature: 30/20°C day/night
- Relative humidity: For both species: 70 +/- 10% during growth
- Watering: For both species the flood method was used with tap water until substrate saturation (from 10 to 15 minutes) per two times a week.

5.1.2. Plant phenotyping by Optical sensors

5.1.2.1. Image capture system and analysis – shoot height and greenness

Optical sensing system is based on image acquisition and data processing using in-house developed algorithms using hue-saturation-value analysis of the image data. Shoot height sensing is based on analyses of reflected light by using a stereoscopic imaging system (Figure 1). Total leaf area sensing or green biomass is based on analyses of reflected light by using the rate of green ground coverage by the foliage when observed from above. The optical system contains two identical colour cameras from Edmund Optics; 1/1.8" CMOS, 1280 x 1024 pixels, sensor area 6.79 x 5.43 mm, 5 mm fixed focal length lens, field-of-view of 65.5 degree. Rugged USB cable is used for both data transmission and supplying the current to the camera electronics. The same hardware is used for the extraction of plant greenness as for the stereoscopic analysis. The depth of focus of the image is a combination of the size of the sensor, the focal length of the lens, lens aperture and the distance between camera and object. This system can measure for various leave colours (e.g. green, red-brown) and different seedlings height (e.g. 4-5cm, 15-20cm). The control of the cameras is carried out using a vendor-supplied software library, uEye (from IDS GmbH). This library is linked to a graphical user interface in-house developed in Microsoft Visual C++, uEyeDualCam GUI. A separate set of processing tools (uEyeDualCam HeightMap) is also home-developed for the purpose of height-mapping of each stereoscopic pair. The same GUI can also extract the "green-only" information for each picture taken. Additionally, the GUI provides the percentage of green pixels for the currently processed image. The green-pixel selection is sensitive to the light source; the proper configuration is also controlled by the .ini file for the respective camera. A long enough sequence of these images can be used to provide a time-series of plant growth – either averaged over the entire scene, or for individual plants. The achieved resolution of the height map is about 1mm that is adequate to follow the plant development. We have used the hue-saturation-value analysis of the image data to extract the green colours related to plants in a digital photo. The repeatability of lighting conditions is an important to be taken in consideration.

Shoot stereoscopic images were taken at the same time of the destructive sampling. The trays were manually moved into the image capture cabinets where one stereoscopic image – top-view – of each experimental half tray was taken. After image capture, all images were analysed using uEyeDualcam and HeightMap (Acreo Swedish ICT). Plant greenness (%) were estimated by uEyeDualcam software and then HeightMAP software recalculate greenness using uEyeDualCam settings output and create a plant height map (cm) of the tray conferring a value to the pixel of the selected images.

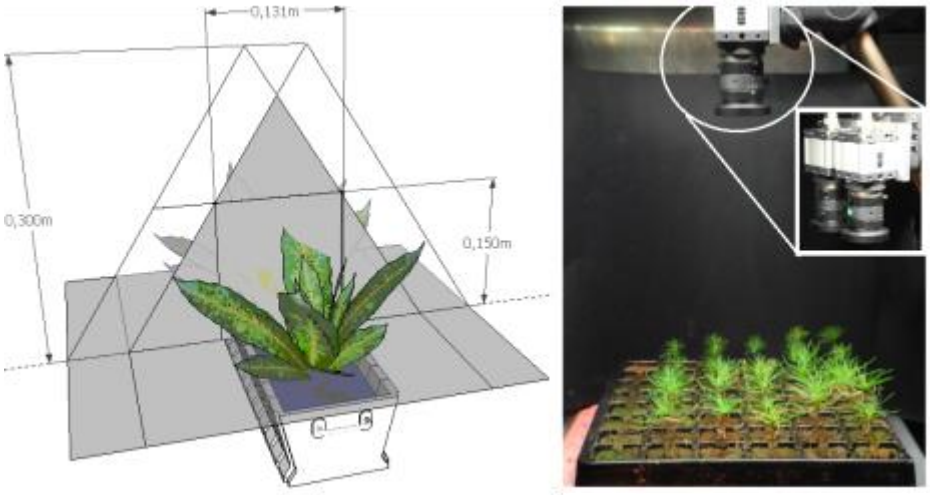


Figure 1

5.2. Results

5.2.1. Seeds germination rate and growth kinetics of Azorean species (*Frangula azorica*, *Morella faya* and *Prunus azorica*)

5.2.1.1. Seeds germination rate

Unfortunately none of the pre-tread seeds of *Prunus azorica* germinated after the treatment under different LED light type. This might be due to the incubation period and would need more time to repeat the protocol. The other two species (*Frangula azorica* and *Morella faya*) showed a highly heterogeneous seed germination in time. In fact, *M. faya* showed a total of 80 seeds germinated in almost 70 days of treatment (Figure 2a) corresponding to a 6% circa of germination rate (Figure 2b). Finally, seed germination showed the highest value of germination (%; Figure 2c) with AP67 LED light type. Seeds under AP67-3L did not germinated at all underling the strong influence of light type on the whole life cycle of plant.

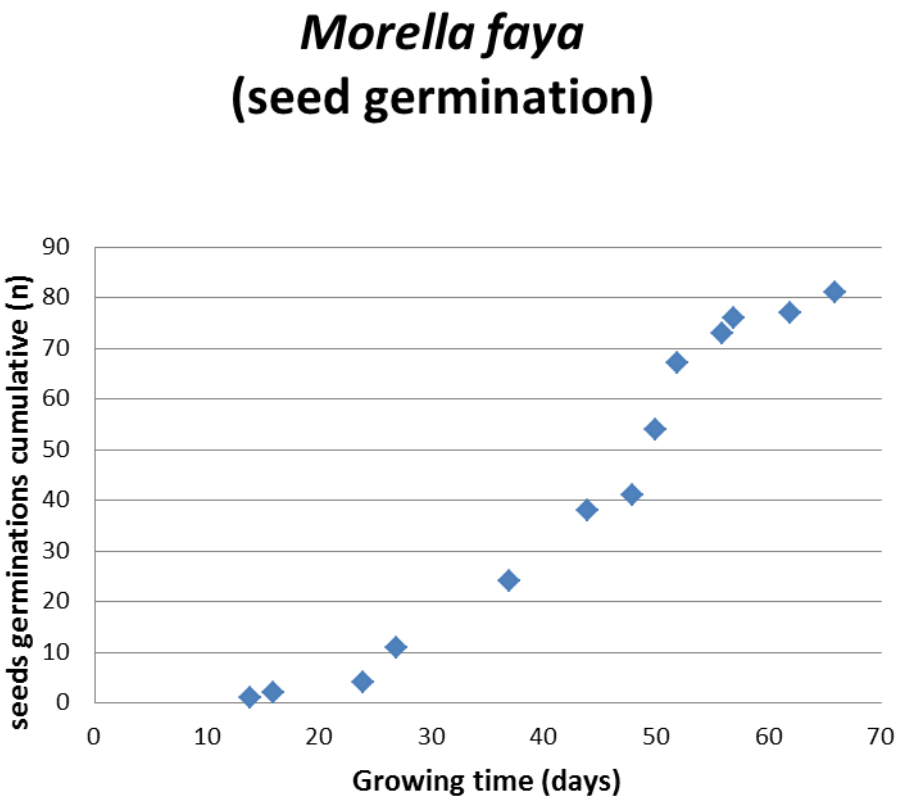


Figure 2a

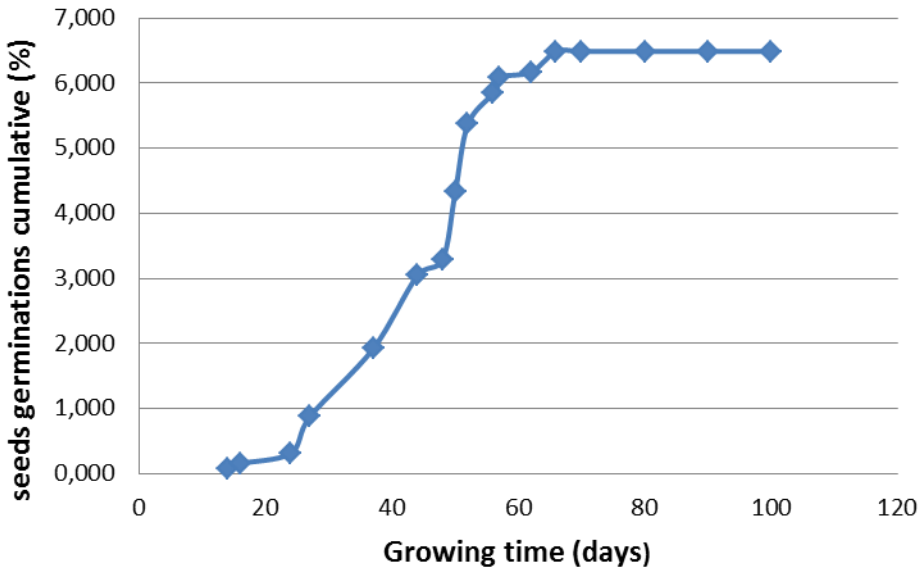


Figure 2b

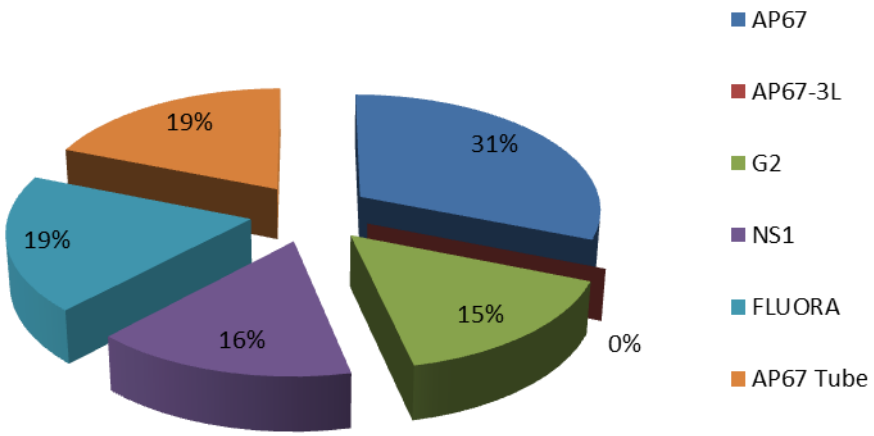


Figure 2c

In the case of *F. azorica*, number of germinated seed was even lower than *M. faya* with a number of almost 60 seed germinated in 70 days of treatment (Figure 3a) corresponding to less than 4% of germination rate (Figure 3b). Finally, also in this case, seed germination showed the highest value of germination (%; Figure 3c) with AP67 LED light type. For this species NS1 and G2 light type as well as AP67-3L did not showed any seed germination.

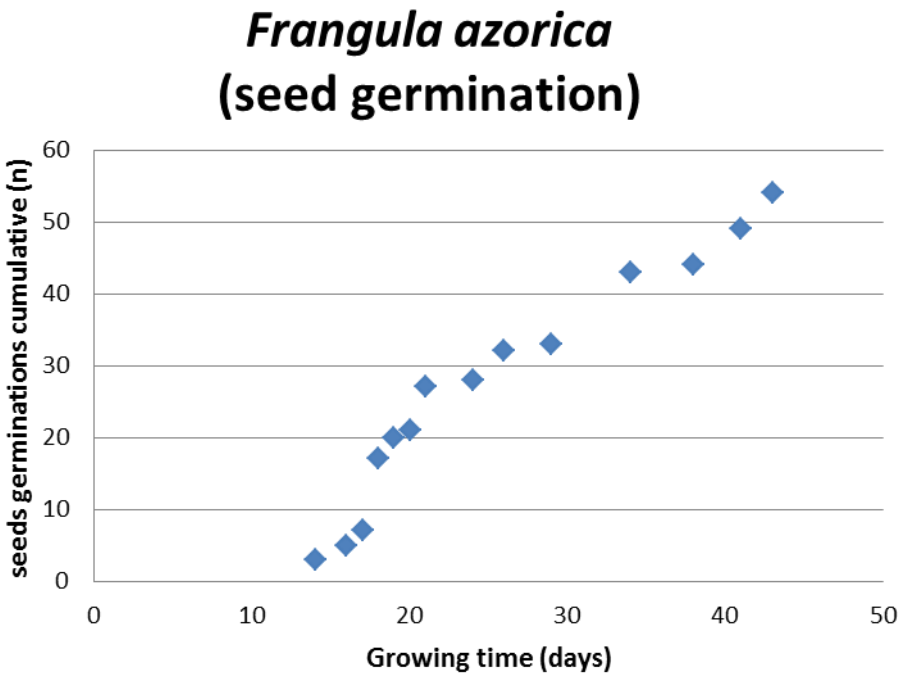


Figure 3a

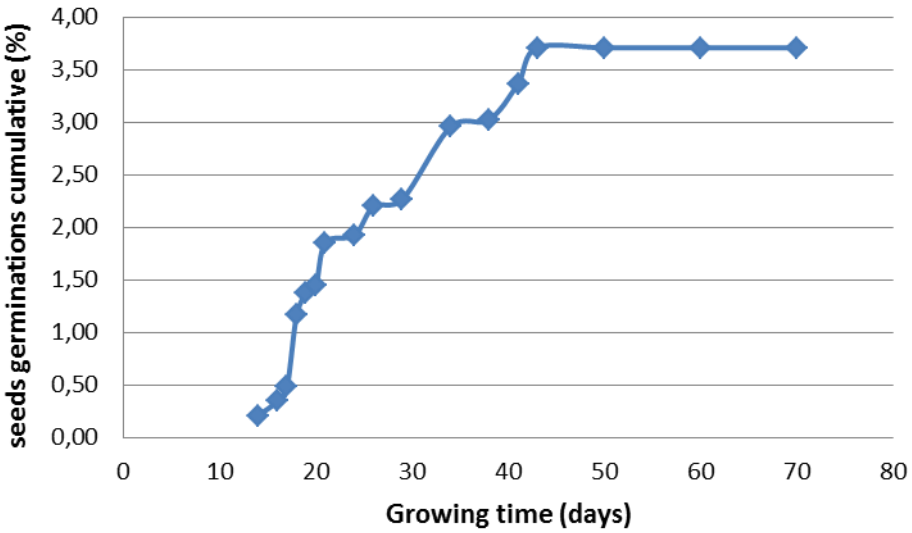


Figure 3b

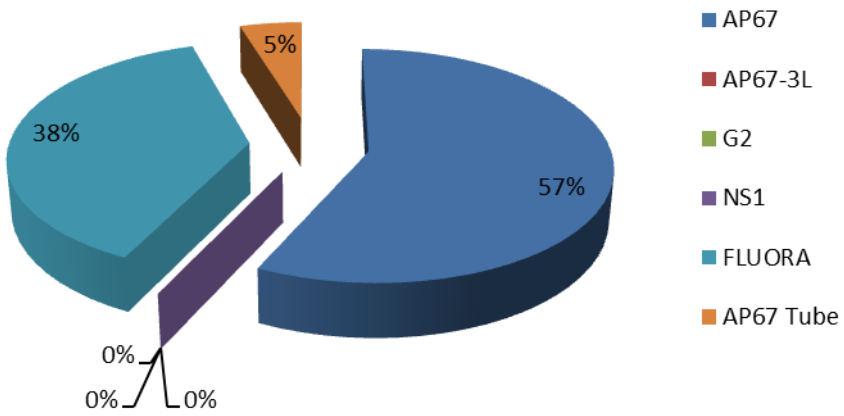


Figure 3c

5.2.1.2. Morphological parameters

Direct measurements of total biomass, plant height, leaves area and root length, were carried by destructive sampling. Due to the heterogeneous seedlings growth, all morphological parameters were considered as increment of each parameter and were calculated as ratio between each parameter and growing time (day).

In the case of *Morella faya* all considered morphological parameter (Figure 4a, b, c and d) showed the highest value for plant grown under AP67 LED type. G2 and NS1 LED light types showed values similar or higher of the control lights (FLUORA). The lowest value was found for AP67 tubes LED type.

Morella faya
(morphological parameters)

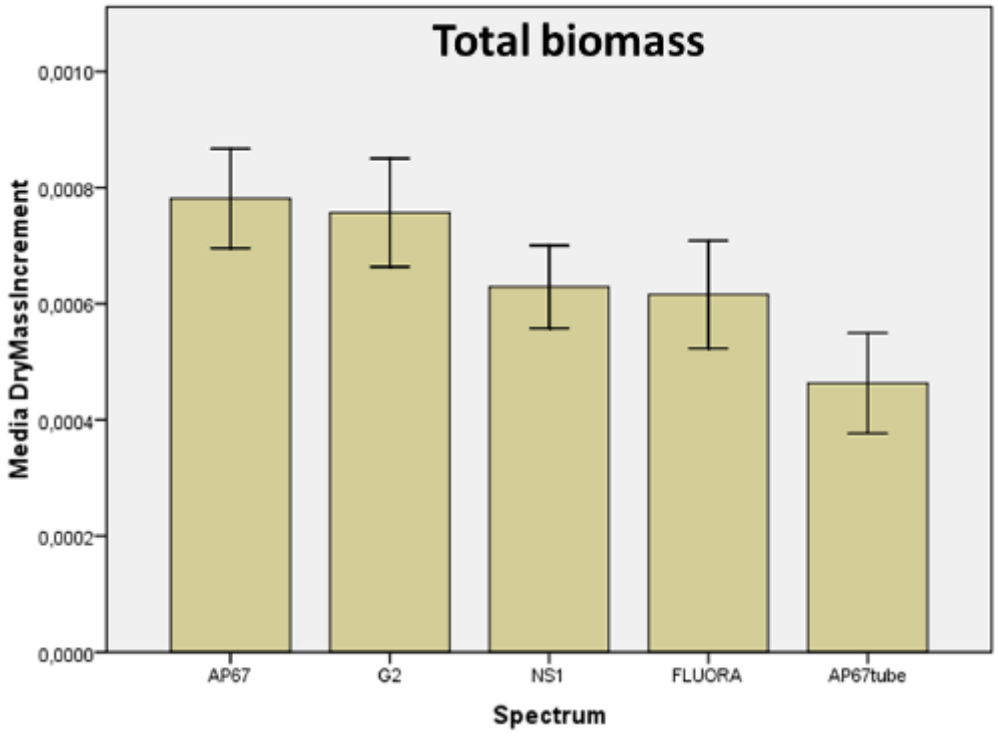


Figure 4a

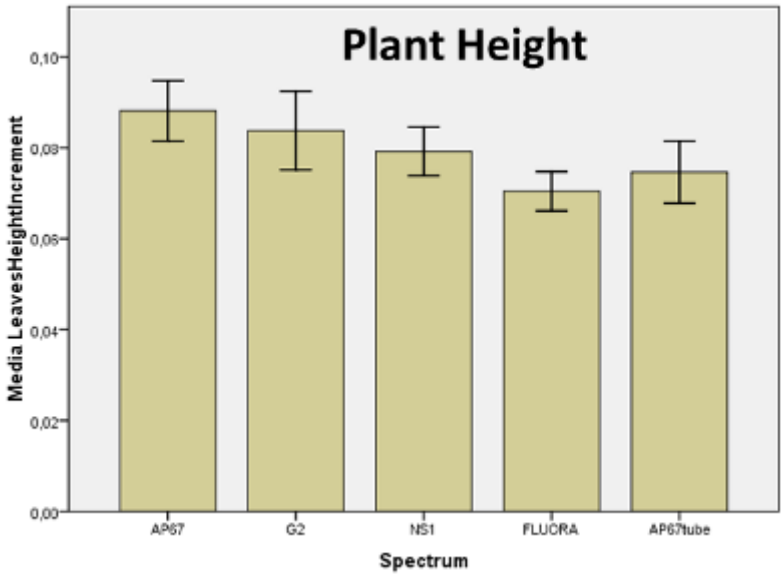


Figure 4b

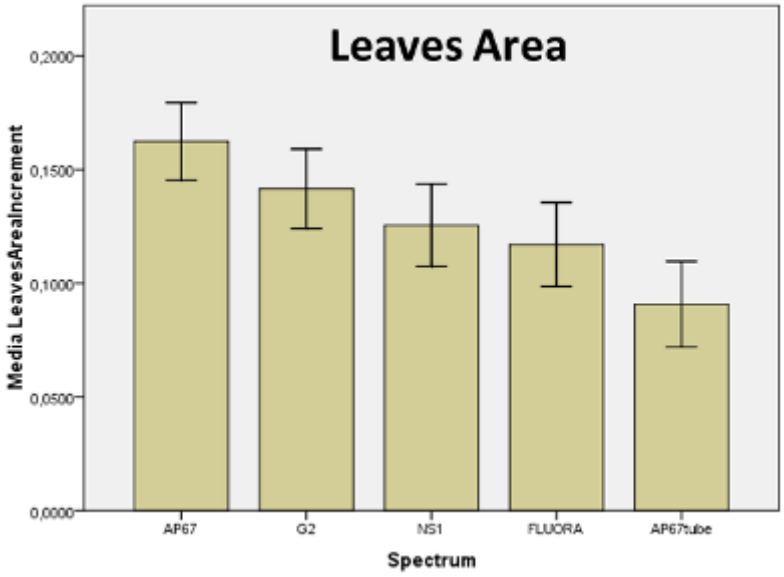


Figure 4c

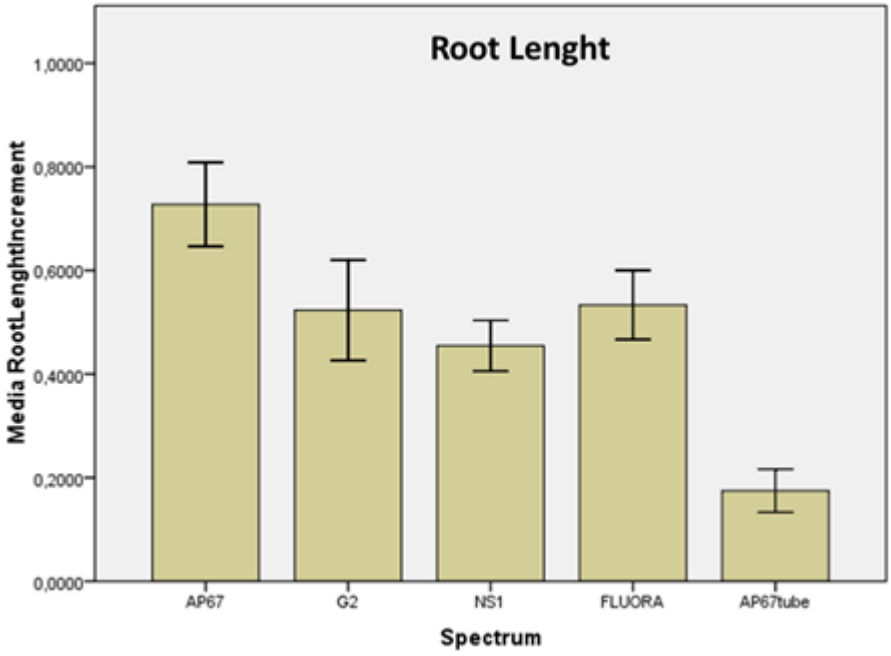

Root Lenght
Media RootLenghtIncrement
1,0000
0,8000
0,6000
0,4000
0,2000
0,0000
AP67
G2
NS1
FLUORA
AP67tube
Spectrum

Figure 4d

In the case of *Frangula azorica* all considered morphological parameter (Figure 5a, b, c, and d) showed that seedlings growth under AP67 LED light type had similar or higher values than seedlings growth under control light (FLUORA). Seedlings growth under NS1 LED light type showed the lowest values for all considered parameters with the only exception of Plant height. Standard errors bars were very high for both Total biomass and Root length parameters highlighting, as in the case of seed germination rate, the high variability of these seedlings of Azorean species.

Frangula azorica (morphological parameters)

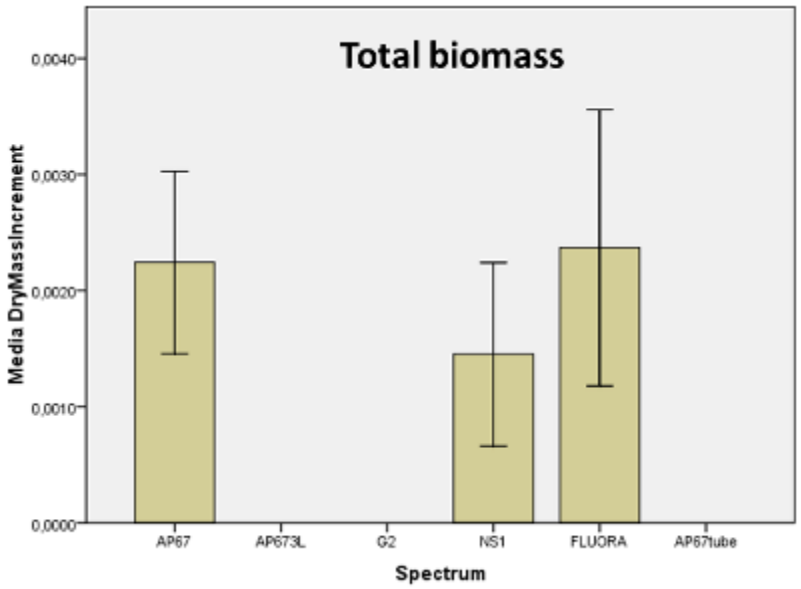


Figure 5a

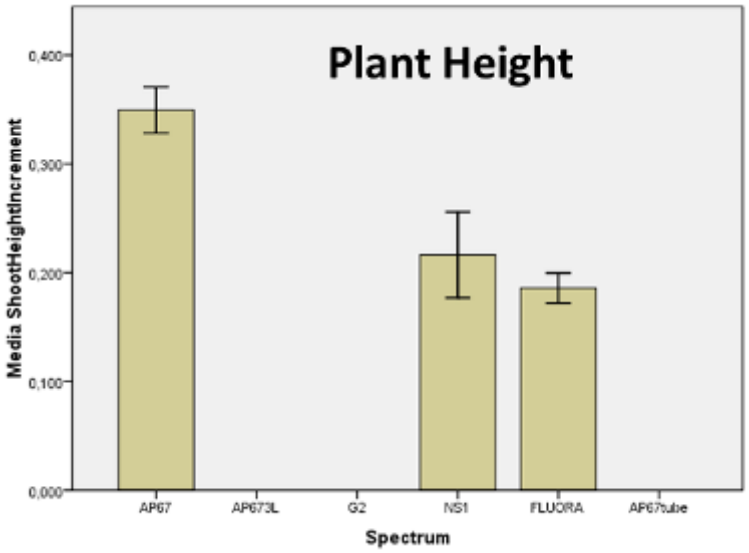


Figure 5b

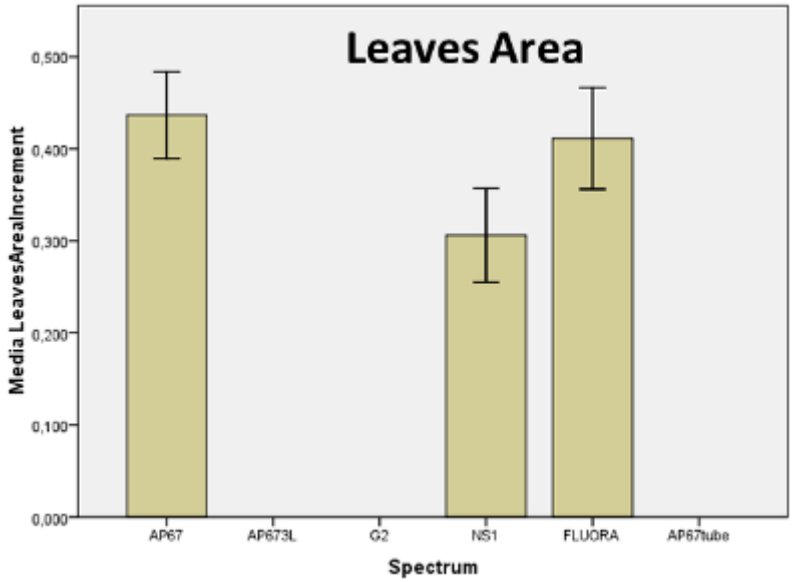


Figure 5c

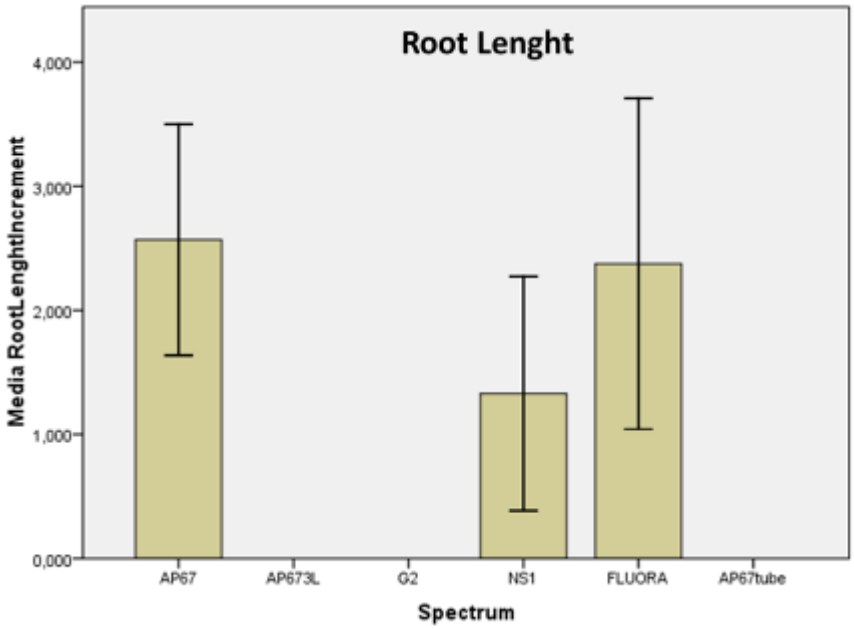


Figure 5d

5.2.2. Seedlings growth performance under different LED type illumination (*Fagus sylvatica*, *Quercus ilex*, *Punica granatum*, *Arbutus unedo*, *Populus nigra*, *Pinus sylvestris* and *Picea abies*)

5.2.2.1. Plant height development

Here are reported data of plant height development (at leaves level) during the experimental time for *Quercus ilex* L. (Figure 6a), *Fagus sylvatica* L. (Figure 6b), *Punica granatum* L. (Figure 6c), *Arbutus unedo* L. (Figure 6d) Data showed, for all species and all different light types, a linear increment of plant height in time. For all four species final plant height (day 57) showed values higher than control light (FLUORA) . In the case of *Q. ilex*, *F. sylvatica* and *P. granatum* the highest values were obtained for seedlings growth under AP76, AP67-3L and G2 LED light types. In the case of *A. unedo* all LED light types showed values similar to control light with the only exception of G2 LED light type that showed the highest values.

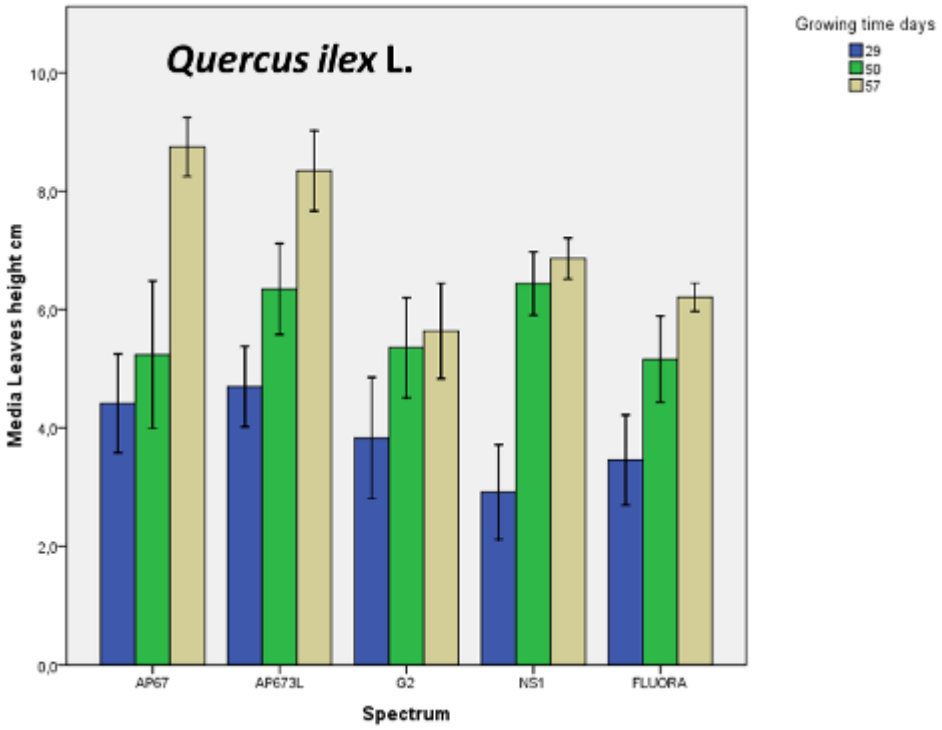


Figure 6a

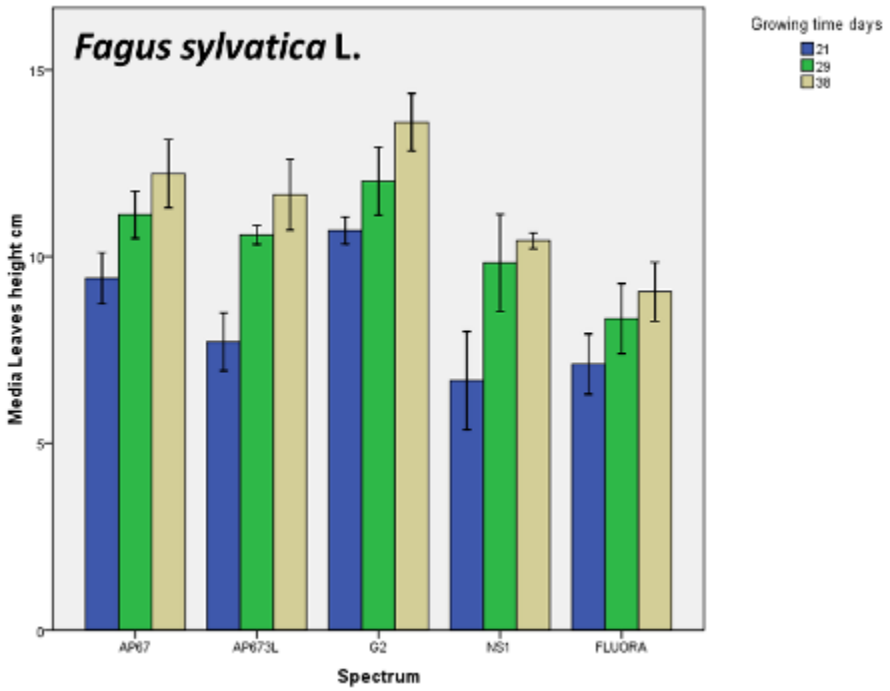


Figure 6b

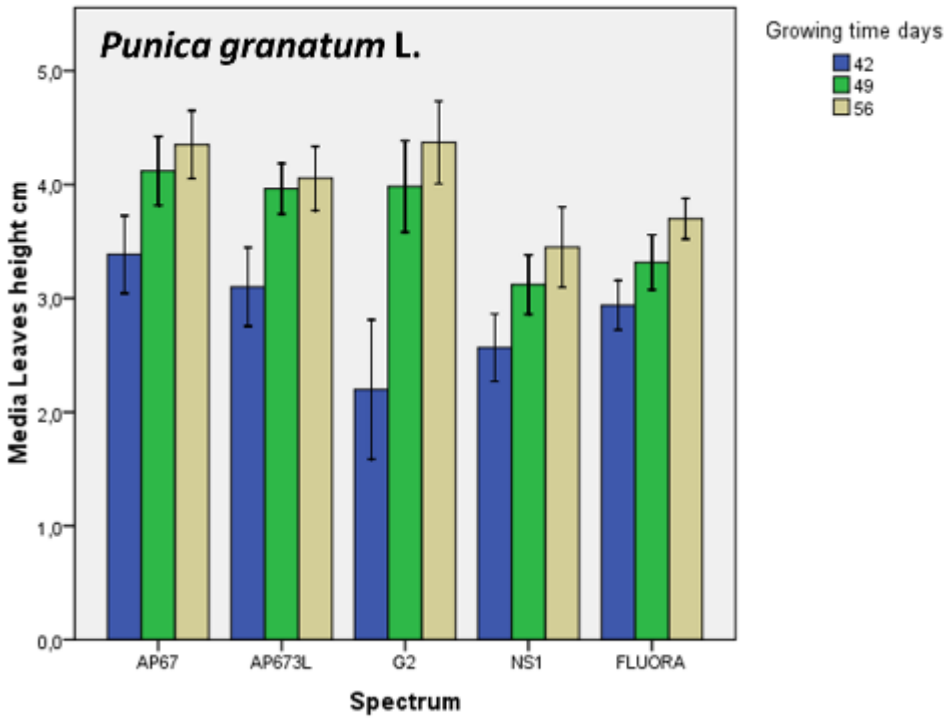


Figure6c

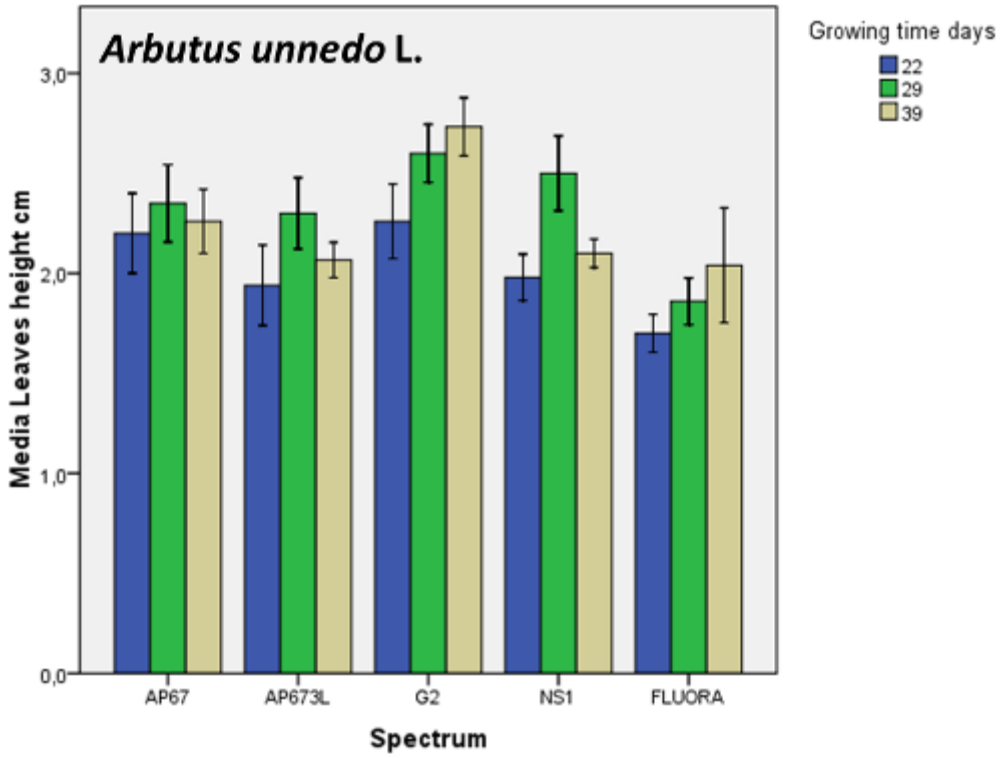


Figure 6d

5.2.2.2. Plant biomass development

Results on plant biomass development during the experiment showed species-specific differences for total biomass and biomass of each pant component.

Fagus sylvatica showed the highest value for the leaves biomass while shoot and root showed similar values each other almost 4 fold lower (Figure 7a).

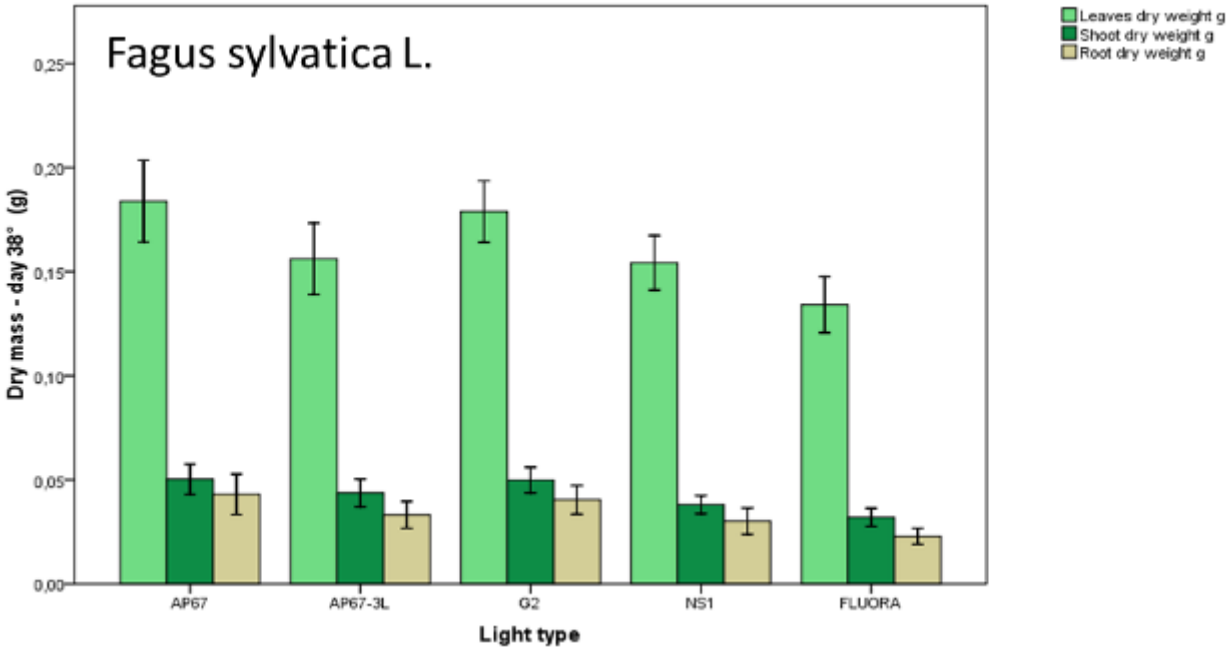


Figure 7a

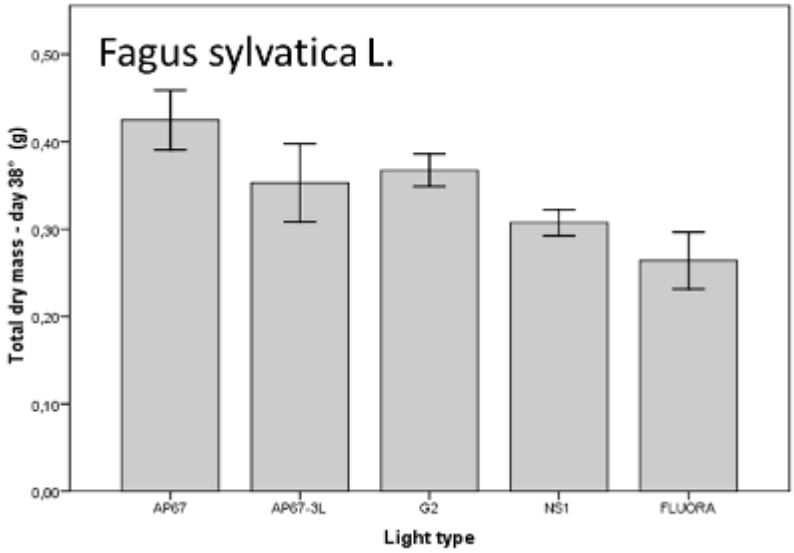


Figure 7b

Total dry mass for *F. sylvatica* seedlings (Figure 7b) showed the highest values for plants growth under AP67 LED light type. Seedlings growth under all the other LED light type showed values similar or higher than control light (FLUORA).

Quercus ilex showed the highest value for leaves biomass while the shoot dry mass was the lowest. Root dry mass showed intermediate values (Figure 8a). Seedling growth under different LED light type showed differences in biomass component. In particular seedling growth under AP67-3L showed the highest values of leaves and root biomass compared to the other LED light type. Moreover, root biomass was almost similar to leaves biomass

showing an higher development with this spectra. Total dry mass for *Q. ilex* seedlings (Figure 8b) showed the highest values for plants growth under AP67-3L LED light type. Seedlings growth under all the other LED light type showed values similar or higher than control light (FLUORA) with the only exception of AP67-3L with values similar to AP67.

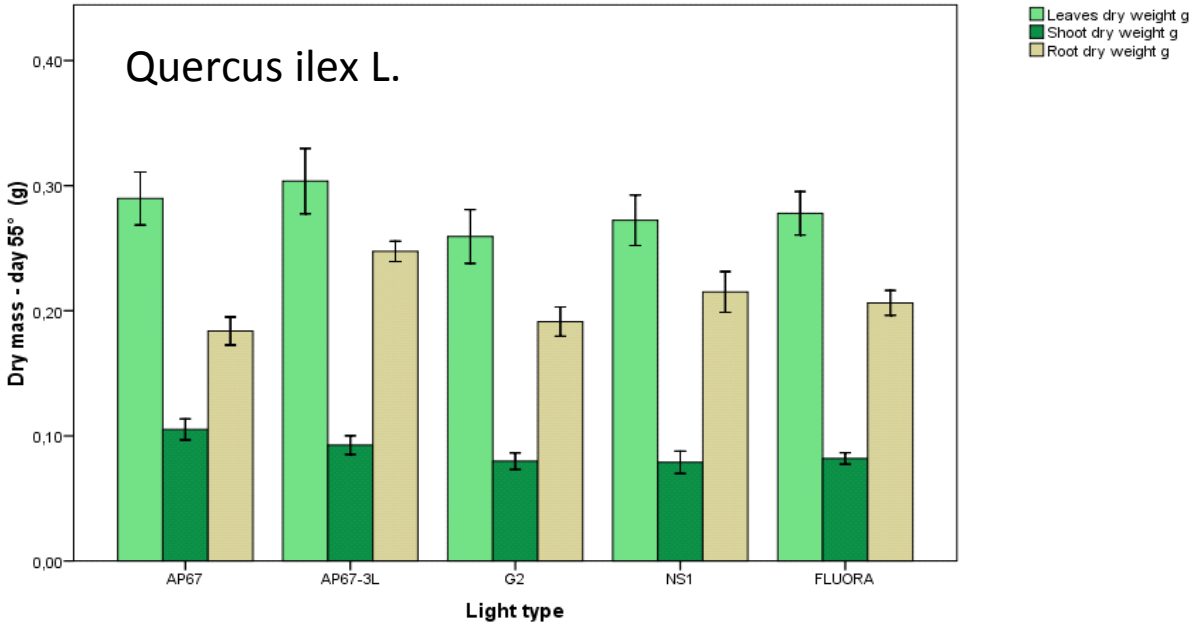


Figure 8a

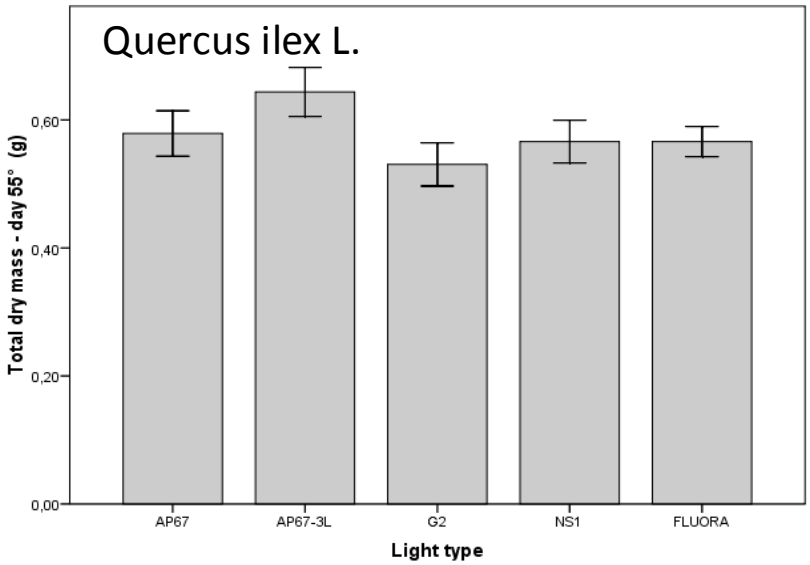


Figure 8b

Punica granatum, again showed highest biomass values for seedlings growth under AP67 LED light type. In particular, leaves biomass resulted the highest of the three considered component (Figure 9a). Root and shoot biomass were similar each other and did not show differences between different LED light type and with control light (FLUORA). Seedlings growth under FLUORA light type showed the highest biomass of all the LED light type (Figure 9b).

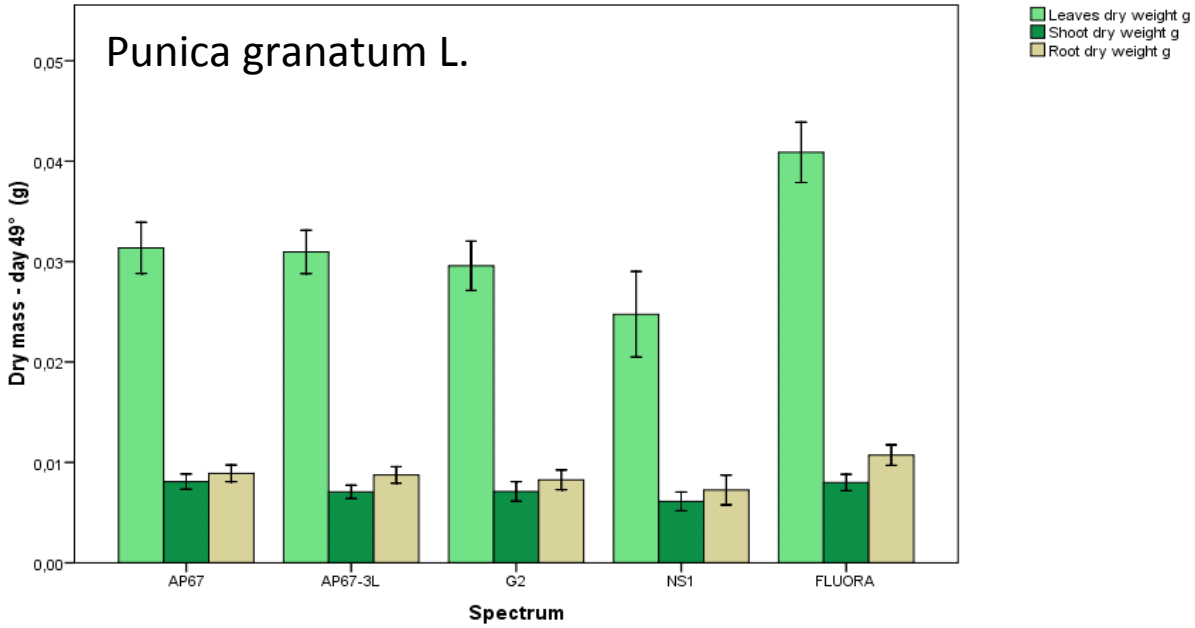


Figure 9a

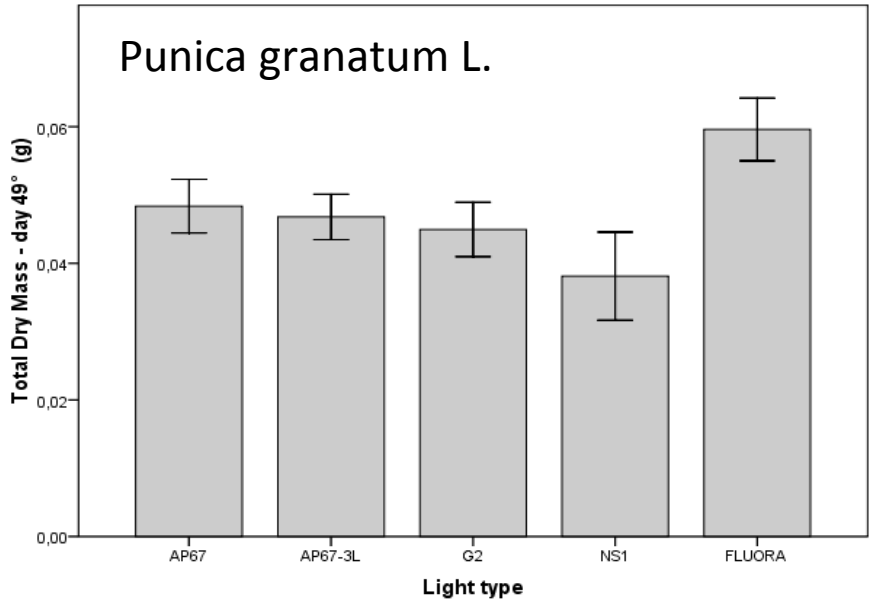


Figure 9b

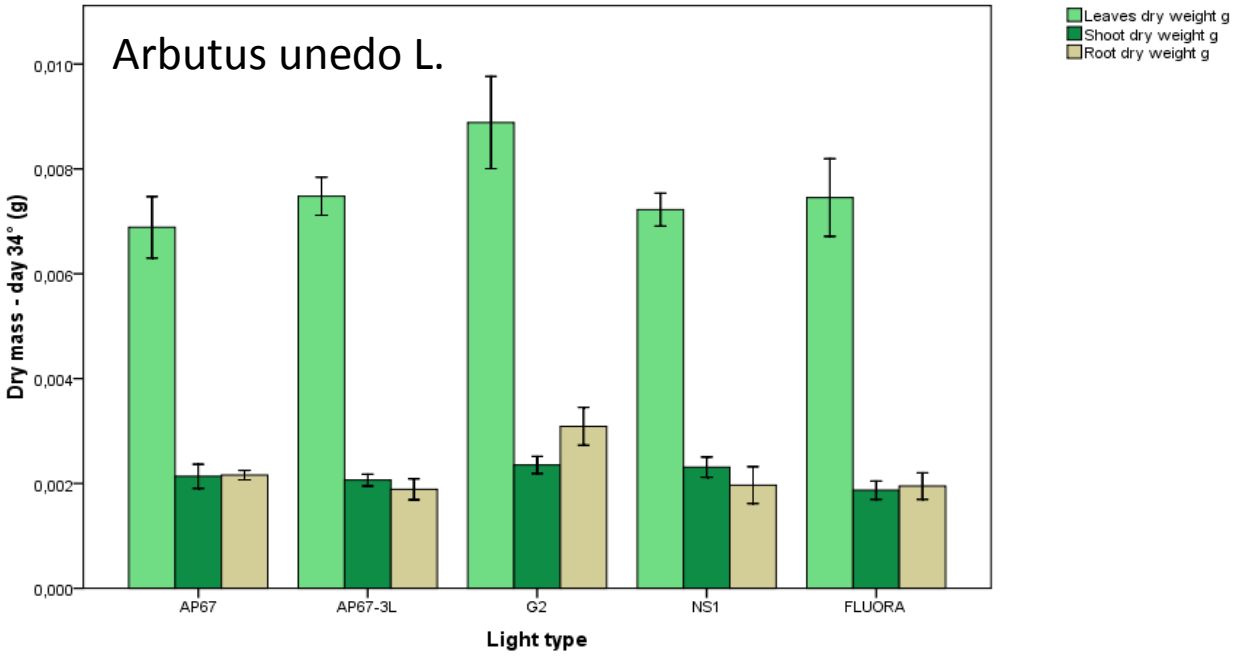


Figure 10a

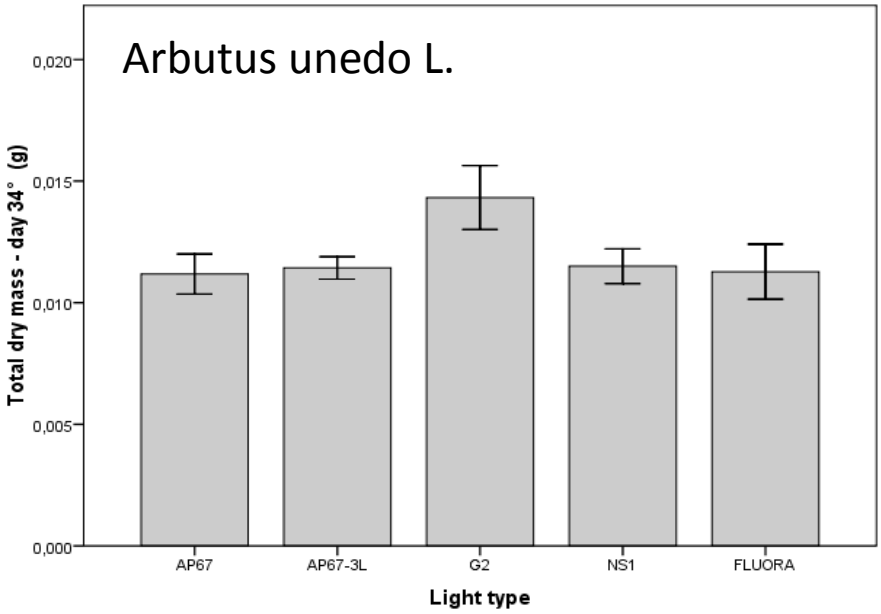


Figure 10b

Arbutus unedo, showed highest biomass values for seedlings growth under G2 LED light type. In particular, leaves biomass resulted the highest of the three considered component (Figure 10a). Root and shoot biomass were similar each other with the only exception of G2 LED light type where root biomass was slightly higher than shoot biomass. Seedlings growth under all the other LED light type showed similar values of seedlings growth under control lights (FLUORA; Figure 10b).

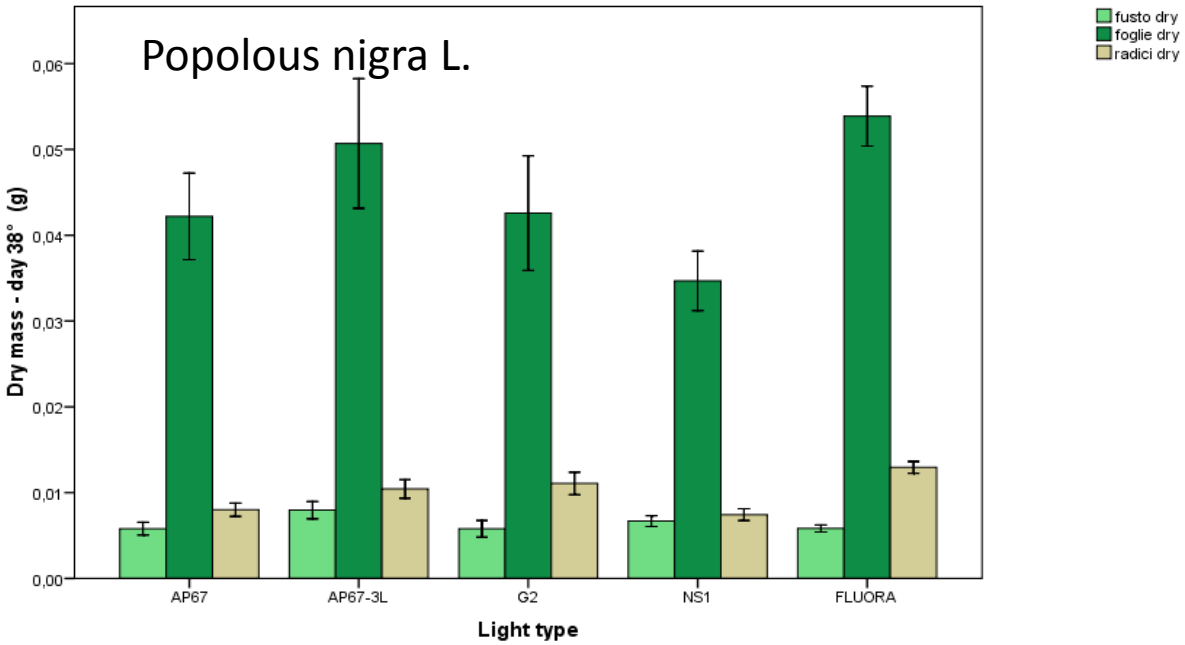


Figure 11a

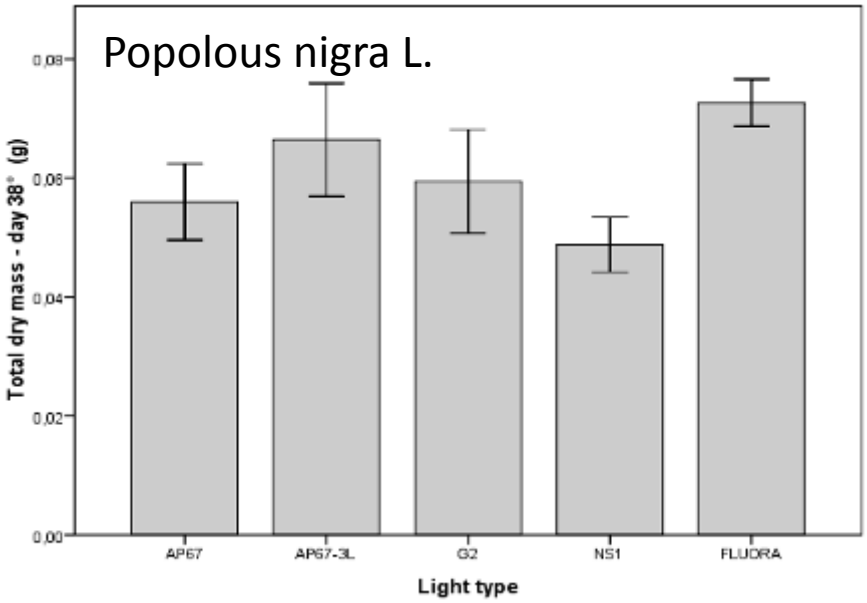


Figure 11b

Populus nigra, showed highest biomass values for seedlings growth under AP67-3L LED light type. In particular, leaves biomass resulted the highest of the three considered component (Figure 11a). Root biomass was higher than shoot biomass for seedlings growth under all LED light type with the only exception of NS1 were shoot and root resulted similar

each other. Seedlings growth under control light (FLUORA) showed the highest values of total biomass ad seedlings growth under NS1 LED light type the lowest (Figure 11b).

Picea abies showed the highest values of biomass for seedlings growth under control light (FLUORA). Seedlings growth under AP67 LED light type showed the highest values between all the other LED light type. Shoot biomass was 4 fold higher than root biomass. Total biomass showed that, with the only exception of NS1, all LED light type had similar values (Figure 12 a, b).

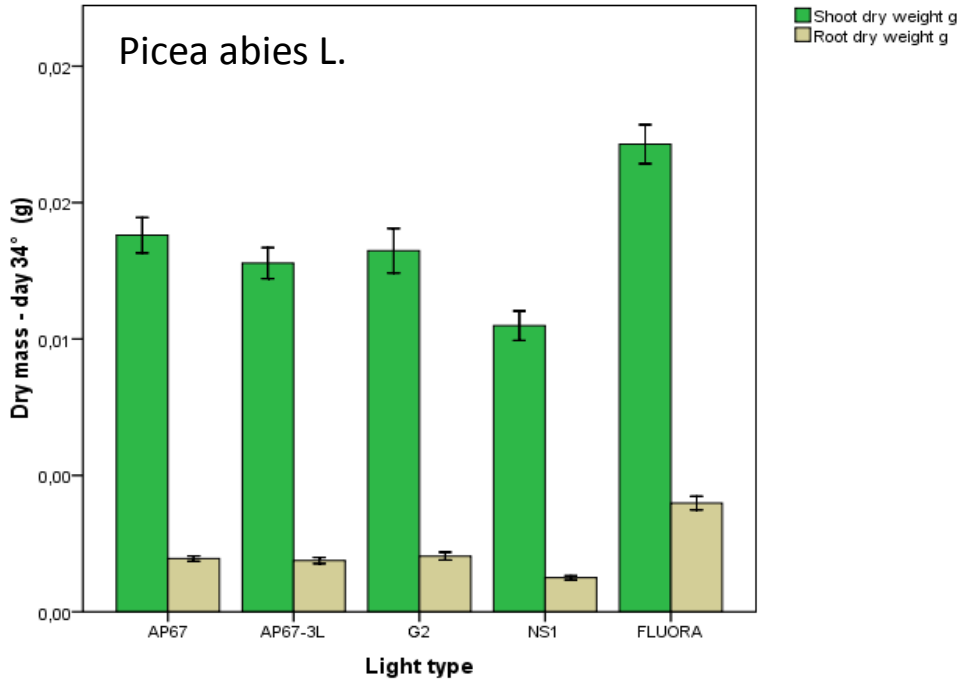


Figure 12a

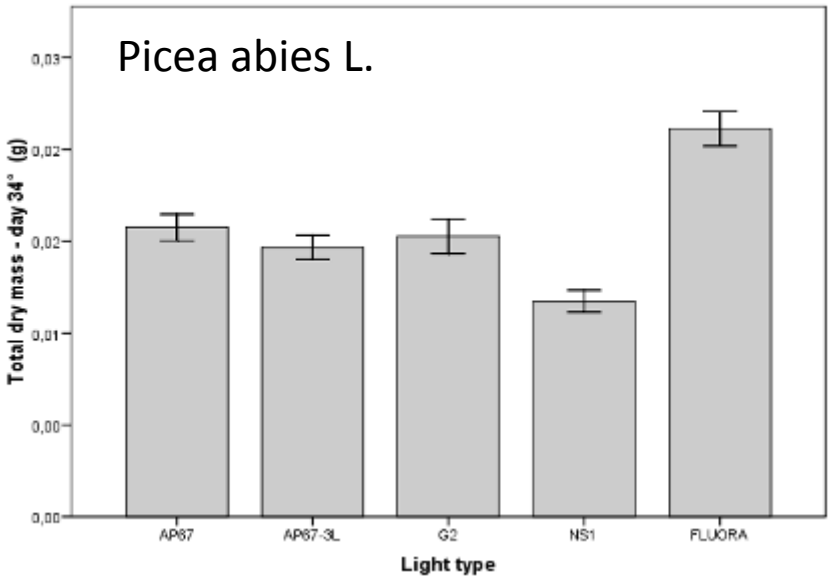


Figure 12b

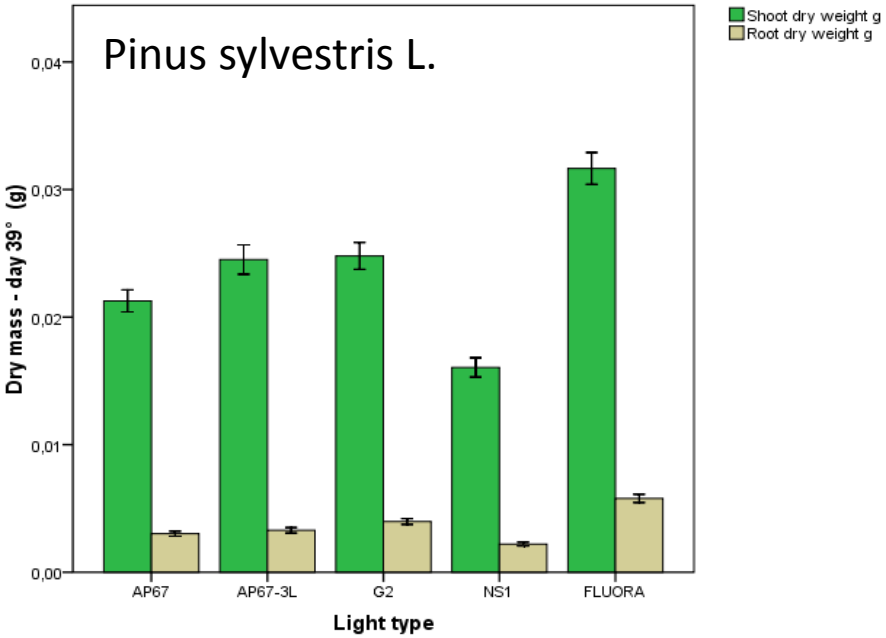


Figure 13a

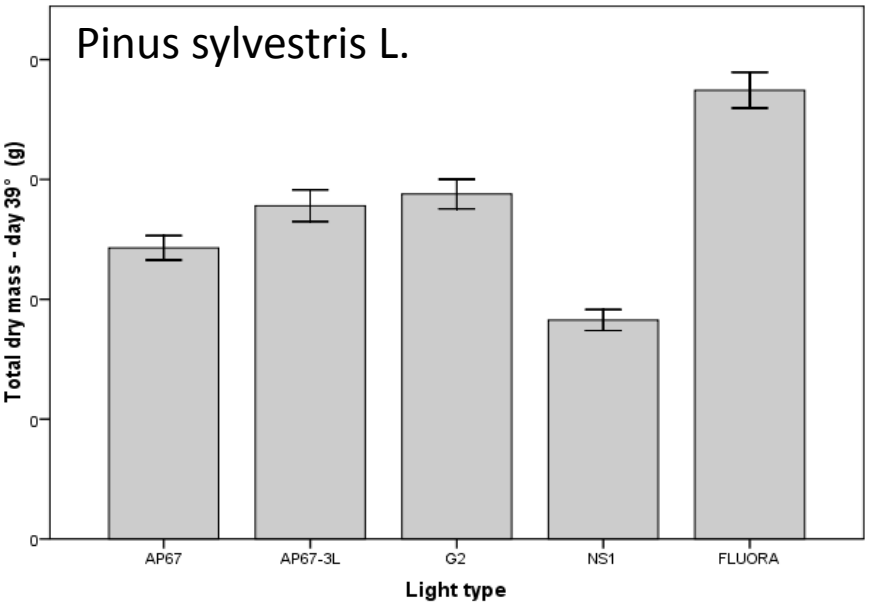


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Figure 13b

Pinus sylvestris showed the highest values of biomass for seedlings growth under control light (FLUORA). Seedlings growth under both AP67-3L and G2 LED light types showed the highest values between all the other LED light type. Shoot biomass was 4 fold higher than root biomass (Figure 13a, b).

5.2.3. Seedlings phenotyping measurements with optical sensors in growth chamber

5.2.3.1. Shoot height and plant biomass

Shoot height throughout the experiment showed different pattern for needle- and broad-leaved species (Figure 14). In the case of both needle-leaved species, after the emergence of cotyledons, a significant increment of the plant height was not detected. This happened because internodes elongation did not occur during the consecutive emissions of new leaves at this early developmental stage. In particular, plant height for *P. abies* reached the maximum value of 3 cm at the first sampling point (day 14th a.g.), without further increment during the duration of the experiment. Seedlings of *P. sylvestris* as *P. abies* reached almost

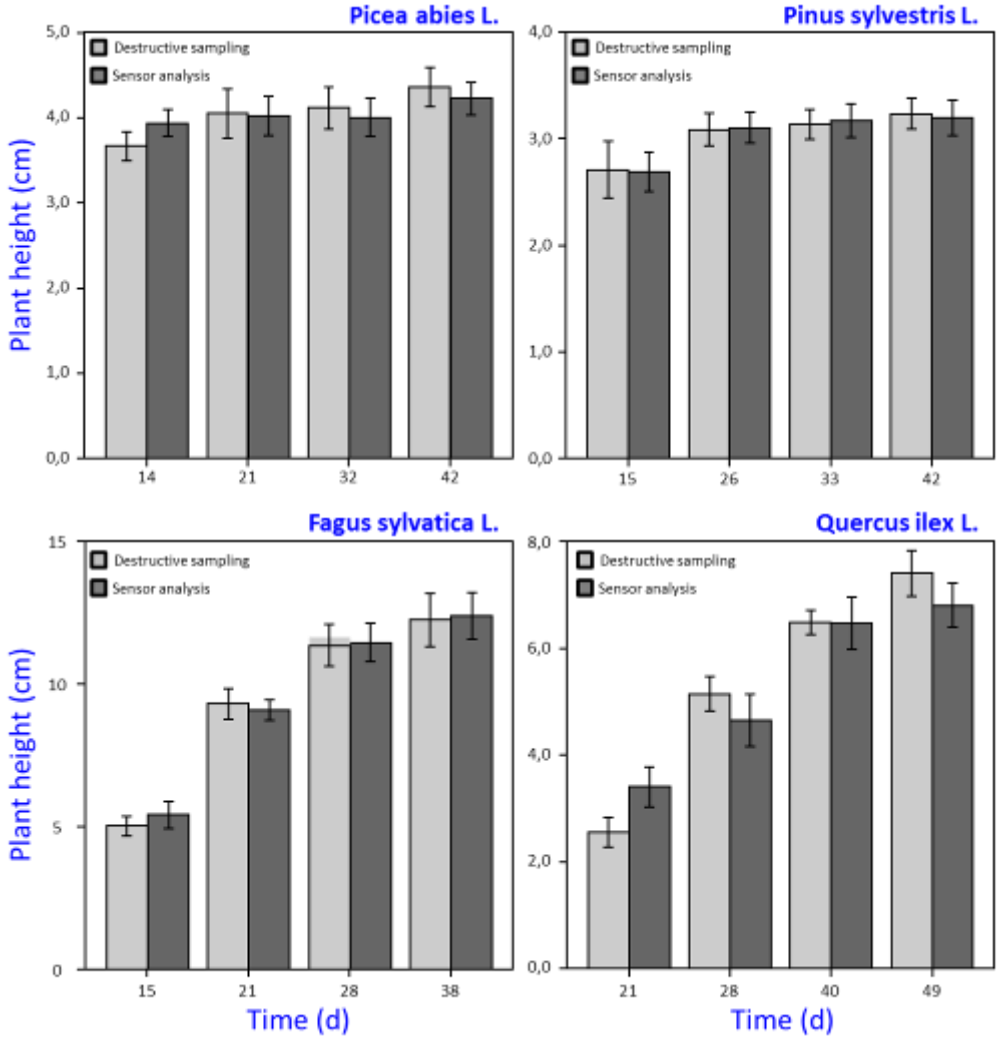

Figure 14

the maximum height at the first sampling point (day 15th a.g.) with a slight increment detectable at the last sampling point (day 42nd after germination). In the case of both broad-leaved species, plant height showed a continuous increment throughout the experiment that reached the maximum value of 13 cm and 8 cm, for *F. sylvatica* and *Q. ilex* respectively, at the third sampling point (day 28th and 40th a.g.), without further increment until the end of the experiment. Results on plant height did not show significant difference between manual and software measurements for all four species and sampling points (Figure 14). Concerning the plant biomass development, all four species showed a linear increase throughout the experiment (Figure 15). Moreover, the two broad-leaved species were characterized by a total biomass 10-fold higher than needle-leaved species.

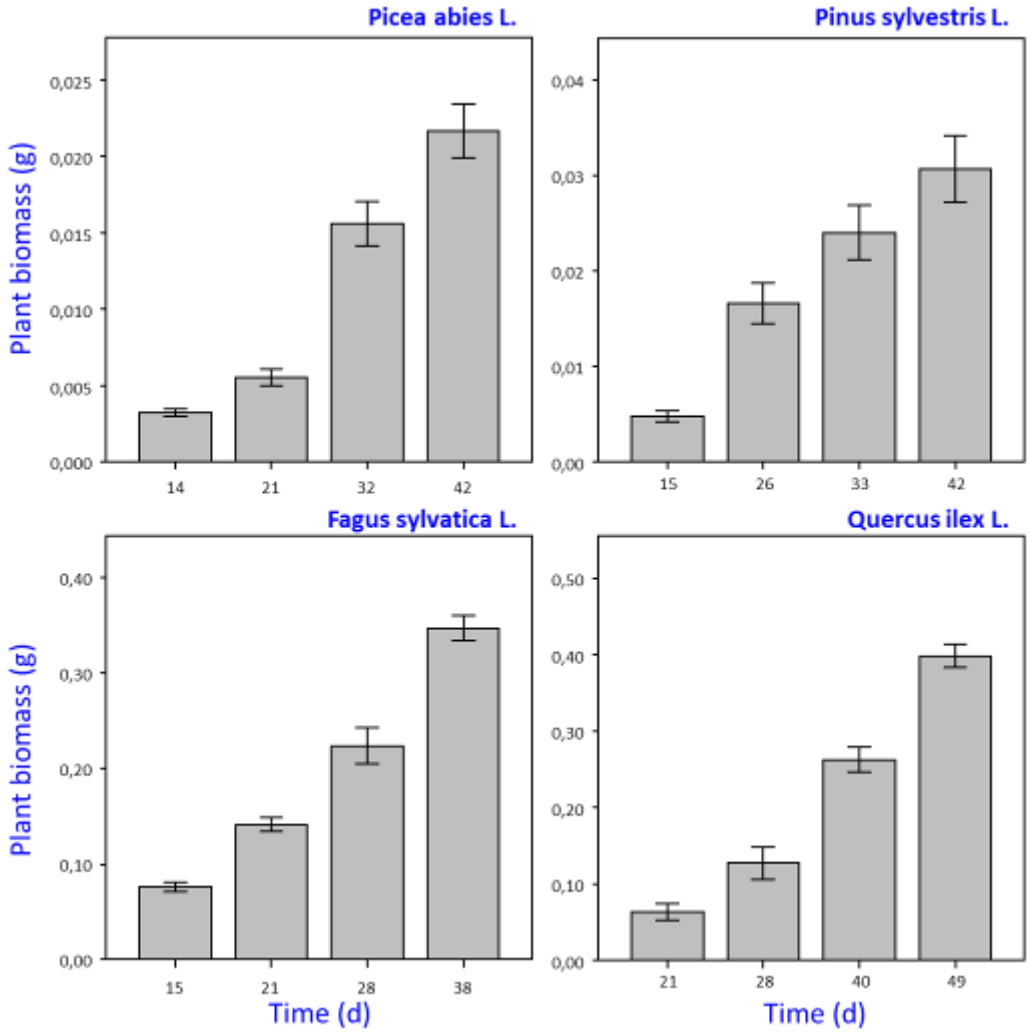

Figure 15

5.2.3.2. Shoot greenness

Seedlings greenness (Figure 16) showed a significant variation throughout the experiment with different patterns for each of the considered species. In the case of *P. abies* maximum value was reached at the third sampling point (day 32 a.g.) remaining stable later until the end of the experiment. *F. sylvatica* seedlings showed a similar pattern of *P. abies* reaching its maximum greenness value at the third sampling point (day 28 a.g.). Both *P. sylvestris* and *Q. ilex* showed a continuous increment throughout the experiment reaching the maximum value at the last sampling point (day 42 and 49 a.g. respectively). In general, broad-leaved species showed values of greenness 10-20 time fold higher than needle-leaved species. Seedling leaves of *F. sylvatica* covered almost all trays at day 20 a.g. while *Q. ilex* covered the 80% of trays at day 49 a.g. On the opposite, *P. abies* and *P. sylvestris* covered less than 7% of the total trays area in 42 days.

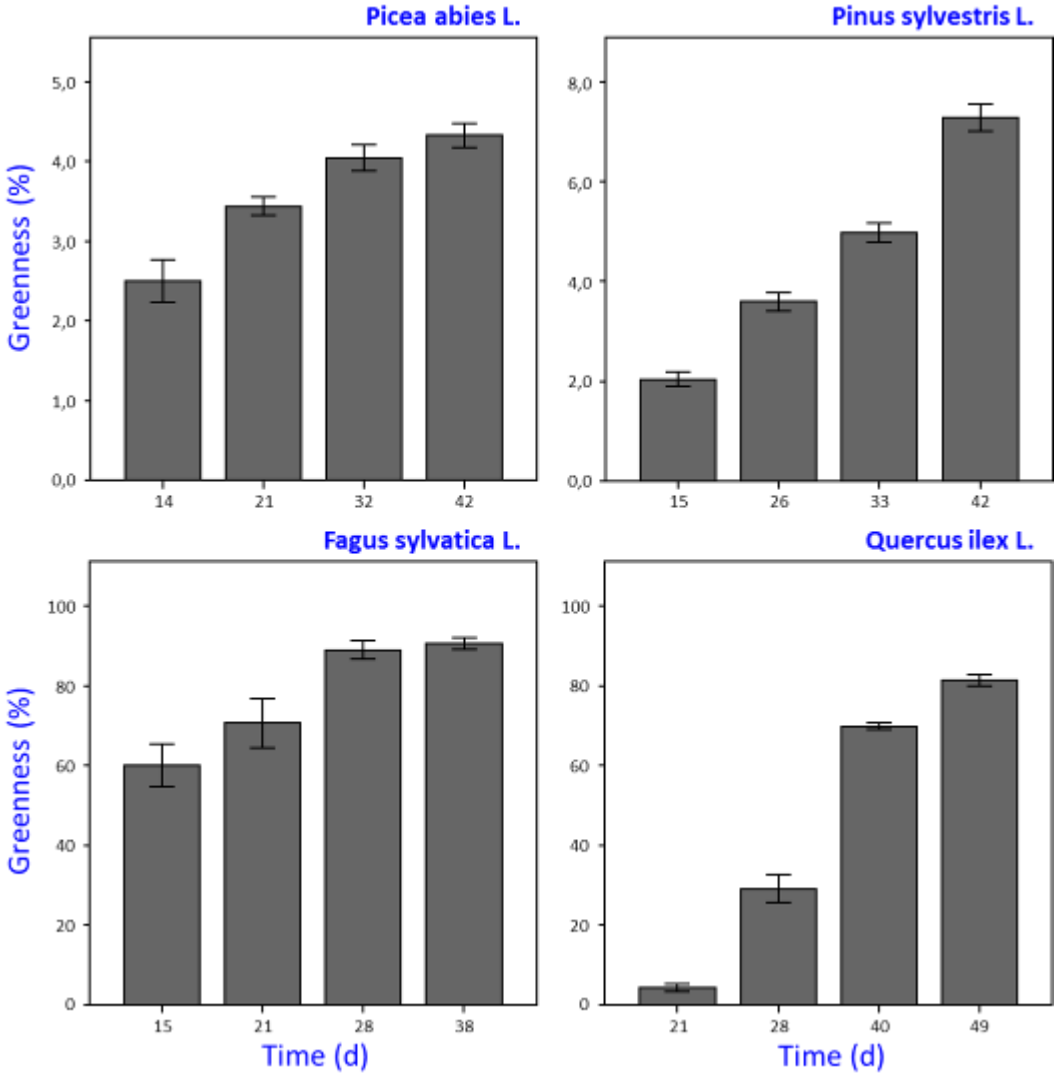

Figure 16

5.2.3.3. Regression model

In order to test the non-destructive measurements as tool for monitoring forest seedling growth, patterns of both seedling tray greenness and height obtained by Software analysis were related to seedling biomass obtained by classical destructive analysis method. The relationship between tray greenness and seedling biomass showed good correlation for all species until the tray got almost fully covered. This was the case of *F. sylvatica* that, as previously stated, covered almost the whole tray in less than one month but its biomass still continue to increase after the full coverage.

As result, the relation between seedling height and biomass (Figure 17a) showed good results with the two broad-leaved species but no relation was found for the two needle-leaved species. Indeed, the constant height of *P. abies* L. and *P. sylvestris* L. didn't relate to the continuous increment of seedling biomass. On the opposite, relationship between seedlings greenness and biomass showed a good results for needle-leaved species (Figure 17b)

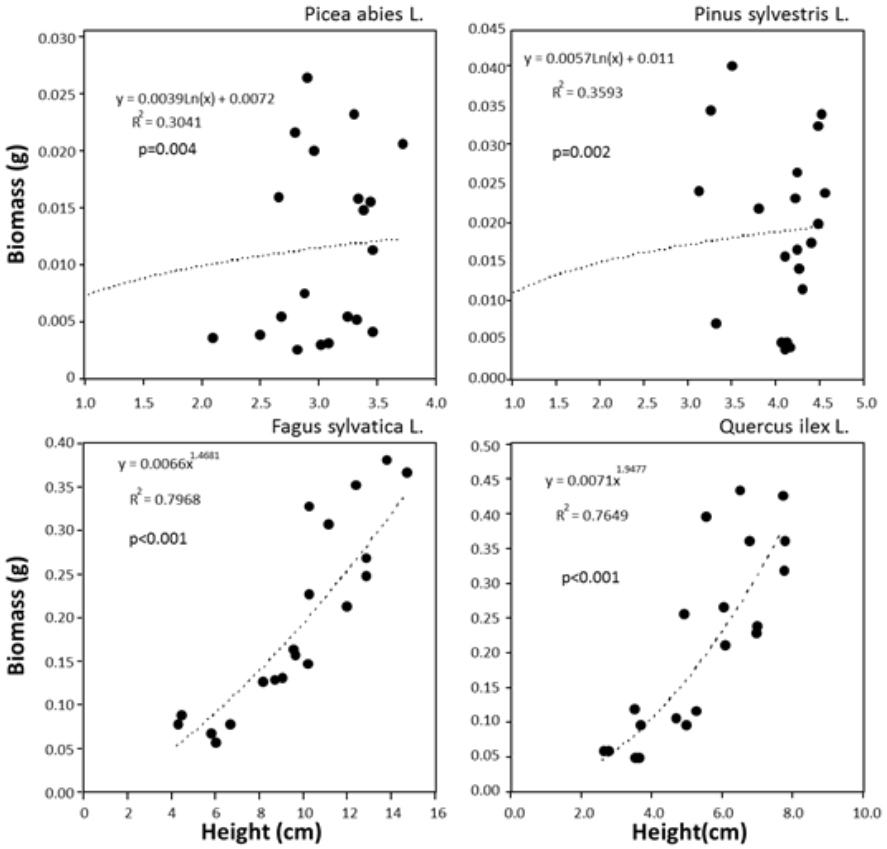

Figure 17a

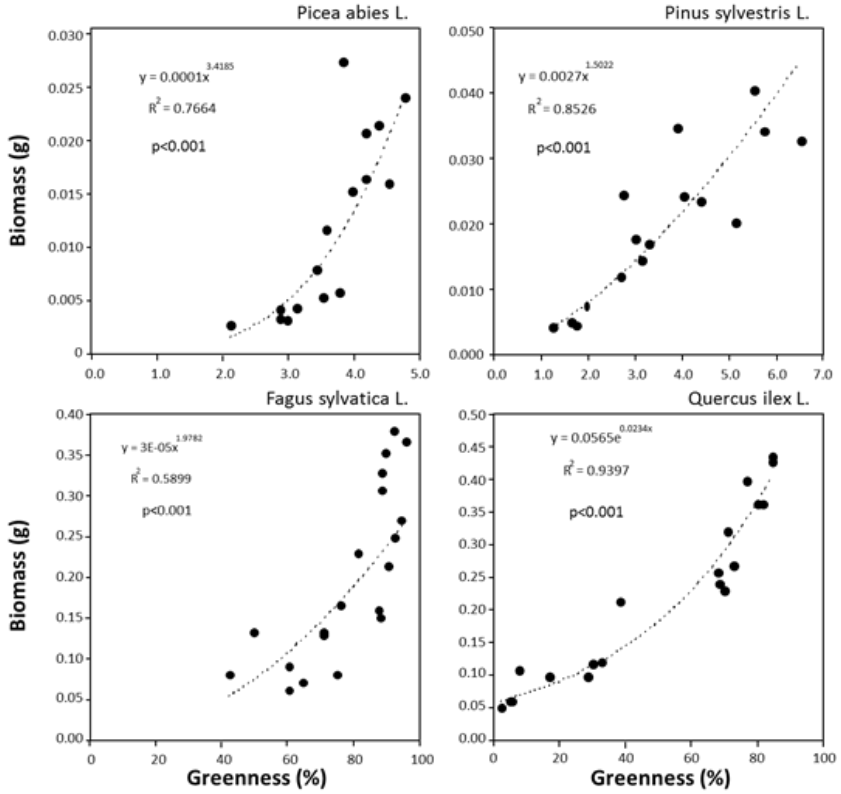

Figure 17b

Thus, the best regression model to explain the relationship between direct biomass data and indirect measurements was based on parameters such as plant height for needle-leaved species and plant greenness for broad-leaved species (Figure 18). Finally, image analysis revealed information on the early seedlings developmental stage.

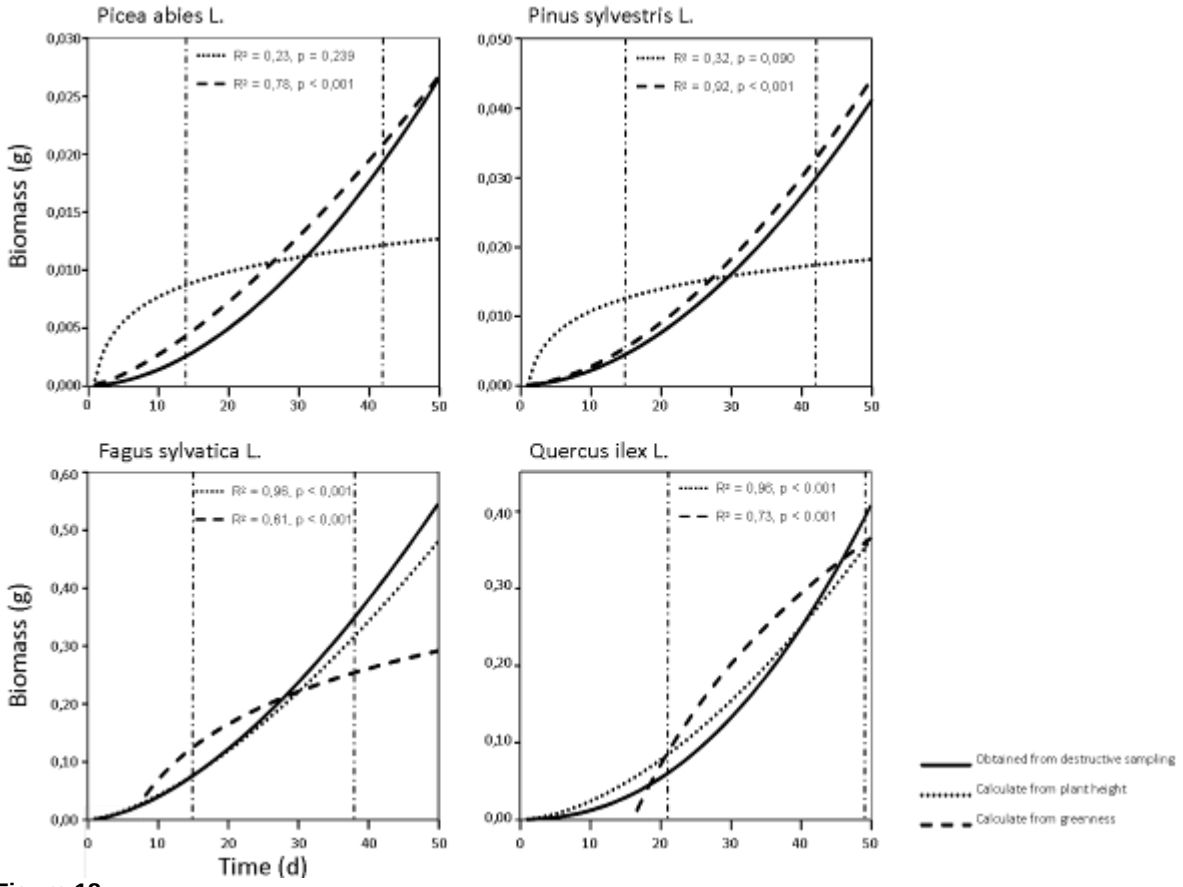

Figure 18

5.2.4. Preliminary results of *Picea abies* and *Pinus sylvestris* seedlings growth in the ZEPHYR prototype: direct and indirect measurements: destructive and optical system

Species	Tray	Image greenness (%)	Background greenness (%)	Plant greenness (%)	Biomass (g) from destructive sampling			Biomass calculated from greenness (g)
					Mean	E.S.		
Picea abies L.	1	3,99	0,70	3,29	0,0052	0,0008		0,0059
	2	4,11	0,80	3,31	0,0058	0,0008		0,0060
	3	3,96	0,70	3,26	0,0052	0,0018		0,0057
Pinus sylvestris L.	1	2,88	0,70	2,18	0,0088	0,0019		0,0087
	2	3,41	0,60	2,81	0,0090	0,0025		0,0127
	3	3,55	0,55	3,00	0,0088	0,0016		0,0141
Data from the first growing session inside the prototype at day 45								

The 21st of September 2015, 20 trays were placed inside the Zephyr prototype. 10 trays were planted with *Picea abies* L. and 10 trays with *Pinus sylvestris* L. Analysis of the greenness was performed before seedlings germination in order to collect data about background noise (background greenness). After 10 and 12 days seedlings of *Picea abies* L. and *Pinus sylvestris* L. started to emerge from soil, respectively.

Trays were left in the prototype for 45 days. A number of 3 trays of each species were sampled (5 seedling for each tray) for destructive analysis. Before sampling, the greenness analysis was performed with the stereo sensor system. Plant greenness was obtained by subtract the background greenness noise to the image greenness.

In order to calculate the biomass, plant greenness allometric equation previously developed in growth chamber (see section 5.2.3) was applied. Biomass data obtained by image analysis were compared with the actual biomass obtained by destructive sampling.

ALLOMETRIC EQUATIONS

Picea abies L.: $BIOMASS = 0.0001 \times GREENNESS^{3,4185}$

Pinus sylvestris L.: $BIOMASS = 0,0027 \times GREENNESS^{1,5022}$

Species	Biomass of seedlings grown in the Zephyr prototype at day 45 from sowing	Biomass of seedlings grown in the growth chamber under the same light type at day 42 from sowing	Biomass of seedlings grown in the growth chamber under the same lights at day 23 from sowing
Picea abies L.	0.0054 ± 0.0011	0.0217 ± 0.0011	0.0055 ± 0.0003
Pinus sylvestris L.	0.0089 ± 0.0020	0.0307 ± 0.0017	0.0166 ± 0.0009
Comparison between data obtain in the Zephyr prototype and in a classic growing chamber. Values of biomass are in grams (g)			

Biomass data obtained from seedlings grown inside the Zephyr prototype showed lower values compared to seedlings of similar age grown inside the growth chamber. Seedlings were grown during the whole system integration operations time. Therefore, environmental conditions were not homogeneous during the whole growth period. Temperature, watering and irradiations were subject to variations due to the interventions. This has provided a stressful environment for plant growth resulting in the relative minor seedlings development. Another main reason for that result could be the different PAR (Photosyntetic Active Radiation) inside the Zephyr prototype and in the growing chamber where allometric equation were developed. The mean, minimum and maximum PAR were measured with continuous measurements using a Photo-radiometer HD2302.0. In the Zephyr prototype the

probe was placed in the middle of the tray and left for 4 hours with rotation at maximum speed (6 minutes for a complete round of the shelve). Data obtained were the following:
Mean PAR : 96.56 µmol / m^2 s
Minimum PAR : 4.09 µmol / m^2 s
Maximum PAR: 261.8 µmol / m^2 s
The mean PAR in the growth chamber was 110 µmol / m^2 s, therefore 12% higher than in the Zephyr prototype. Consequently seedlings grown inside the Zephyr prototype had less energy to invest in their development. Another reason of the lower growth of seedling inside the Zephyr prototype is that the prototype was shot-down due to technical tests or transportation at the final conference in Milan, so overall it was not operative for 9 days.

5.3. Conclusions

Seed germination of Azorean species showed good results for only two of the three considered species. In particular *Prunus azorica* seeds did not show any germination. Unfortunately data on germination of these endemic plant species are very poor. Therefore the effort made during this project to develop a specific protocol for seed germination and optimal plant growth throw some light on this topic. Of course further efforts are needed. Furthermore, seed germination and seedlings growth showed good performances under AP67 LED light type. In particular, other LED light type showed very low or none germination at all. This lowered the total value of germination rate but strongly indicate that the choice of specific LED light type (AP67) is a powerful tool for improving species reproduction and conservation

Seedling growth performance, measured by morphological parameters showed in general a good response of forest tree seedlings to LED light type. Seedling morphology showed a specie-specific response to different LED light type. In particular, best LED light type were AP67, AP67-3L and G2 depending on the plant species. Furthermore, slightly different growth response in different seedling compartments such leaf, shoot and root was detected. This topic need to be further investigated. In the view of a static system where one LED light type must be selected, we can assert that AP67 cover the wider need for all species considered.

The Optical sensor system for containerised seedlings phenotyping, developed during the Zephyr project, is accurate enough to automatically monitoring seedlings growth. As reported in the present document, the best regression model to explain the relationship between direct

biomass data and indirect measurements was based on parameters such as plant height for needle-leaved species and plant greenness for broad-leaved species. Thus, the system here developed, characterised by affordable price, simple hardware and software, provides an efficient and powerful tool for automatic plant analysis.

Preliminary results into the ZEPHYR prototype showed a good plant growth and therefore a good system functioning. Although seedling grown in a non homogeneous environment, due to the technical operation of integration, they showed a good comparison with seedlings growth measured into the growth chamber. Furthermore, Optical sensor system provided good correlation with direct measurements of seedlings in terms of pant greenness and plant biomass.

REFERENCES

Alokam, S.; Chinnappa, C.C.; Reid, D.M., 2002. Red/far-red light mediated stem elongation and anthocyanin accumulation in Stellaria longipes: differential response of alpine and prairie ecotypes. Can. J. Bot. 80: 72–81.

Andreu, L.; Gutierrez, E.; Macias, M.; Ribas, M.; Bosch, O.; Camarero, J.J., 2007. Climate increases regional tree-growth variability in Iberian pine forests. Glob. Chang. Biol. 13: 1–12.

Archibold, O.W., 1995. Ecology of World Vegetation. Chapman and Hall, London.

Aronne, De Micco, 2004. Hypocotyl features of Myrtus communis (Myrtaceae): a many-sided strategy for possible enhancement of seedling establishment in the Mediterranean environment. Botanical Journal of Linnean Society, 145: 195-202.

Arvouet-Grand. A.; Vennat, B.; Pourrat. A.; Legret. P., 1994. Standardization of a propolis extract and identification of the main constituents. Journal de pharmacie de Belgique 49: 462–468.

Athanasiadis, N., 1986b. Forest Botany (Trees and Bushes of the Greek forests), Part II, Thessaloniki. Publ. Giahoudis-Giapoulis, 309 p. (In Greek).

Barbéro, M.; Losiel, R.; Queéze,l P.; Richardson, D.M.; Romane, F., 1998. Pines of the Mediterranean Basin. In: Richardson D.M. (Ed.), Ecology and biogeography of Pinus, Cambridge University Press, Cambridge.

Barrero, J.M.; Jacobsen, J.V.; Talbot, M.J.; White, R.G.; Swain, S.M;, Garvin, D.F.; Gubler,F., 2012. Grain dormancy and light quality effects on germination in the model grass Brachypodium distachyon. New Phytol. 193: 376–386.

Bayala, J.; Dianda, Z.M.; Wilson, Z.J.; Ouédraogo, S.J.; Sanon, Z.K., 2009. Predicting field performance of five irrigated tree species using seedling quality assessment in Burkina Faso, West Africa. New Forest 38: 309–322.

Björkman, O.; Demmig, B., 1987. Photon yield of O, evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170:489-504.

Bolhår-Nordenkampf, H. R.; Lechner, E. G.. 1988a. Temperature and light dependent modifications of chlorophyll fluorescence kinetics in spruce needles during winter. Photosynth. Res. 18: 287-298.

Brockerhoff, E.G.; Jactel, H.; Parrotta, J.A.; Quine, C.P.; Sayer, J., 2008. Plantation forests and biodiversity: oxymoron or opportunity? Biodivers. Conserv. 17: 925–951.

Burdet, A.N., 1990. Physiological processes in plantation establishment and the development of specifications for forest planting stock. Can J For Res 20:415–427.

Carandang, W.M., 1994. Lateral root development and seedling performance of large leaf mahogany (Swietenia macrophylla King). [Ph.D Thesis] College, Laguna, Philippines: University of the Philippines Los Baños : 223.

Carlisle, A.; Brown, A.H.F., 1968. *Pinus sylvestris*. J. Ecol. 56: 269–307.

Carlson, W.C., 1986. Root system considerations in the quality of loblolly pine seedlings. South J Appl For 10: 87–92.

Cha-um, S.; Ulziibat, B.; Kirdmanee, C., 2010. Effects of temperature and relative humidity during in vitro acclimatization, on physiological changes and growth characters of Phalaenopsis adapted in vivo. Aust J Crop Sci 4: 750–756.

Christensen, K.I., 1997. Fagaceae. In: Strid & K. Tan (ed.), Flora Hellenica, Vol. 1. Königstein, Koeltz Scientific Books: 40-50.

Conedera, M.; Krebs, P.; Tinner, W.; Pradella, M.; Torriani, D., 2004. The cultivation of Castanea sativa (Mill.) in Europe, from its origin to its diff usion on a continental scale. Vegetation History and Archaeobotany 13: 161–179.

Corpuz, O.S., 2012. Root Growth Potential, Variability and Heritability of Yemane (Gmelina arborea Roxb) in the Philippines. Philippine Journal of Crop Science 37: 27-35.

Dafis, S., 1973. Greek Forest Vegetation Classification. Scientific Annals of the Forestry Department, Vol. 15(2), Aristotle University of Thessaloniki: 75- 91. (In Greek).

Davis, P.H., 1982. Flora of Turkey and the East Aegean islands, 1-9. Edinburgh, Edinburgh University Press.

Davis, A.S.; Jacobs, D.F., 2005.Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forests 30(2/3): 295–311.

Dickson, A.; Leaf, A.L.; Hasuer, J.F., 1960. Quality appraisal of white spruce and white pine seedlings stock in nurseries. Forest Chron. 36: 10–13.

Downs, R.J. 1977. Incandescent lamp maintenance in plant growth chambers. HortScience 12: 330–332.

Dupont, S.; Mondi, Z.; Willamson, G.; Price, K., 2000. Effect of variety, processing, and storage on the flavonoid glycoside and composition of lettuce and chicory. J. Agri. Food Chem. 48: 3957-3964.

Elvidge, C.D.; Cinzano, P.; Pettit, D.R.; Arvesen, J.; Sutton, P.; Small, C.; Nemani, R.; Longcore, T.; Rich, C.; Safran, J.; Weeks, J.; Ebener, S., 2007. The Nightsat mission concept. International Journal of Remote Sensing, 28(12): 2645 – 2670.

Elvidge, C.D.; Safran, J.; Tuttle, B.; Sutton, P.; Cinzano, P.; Pettit, D.; Arvesen, J.; Small, C. (2007) Potential for global mapping of development via a Nightsat mission. GeoJournal, 69: 45 – 53.

Fernandez, O.A.; Mujica, B., 1973. Effects of some environmental factors on the differentiation of stomata in spirodela intermedia. Bot. Gaz. 134: 117-121.

Fitter, A.H.; Stickland, T.R.; Harvey, M.L.; Wilson, G.W., 1991. Architectural analysis of plant root systems 1. Architectural correlates of exploitation efficiency. New Phytol 118: 375–382.

Folta, K.M.; Koss, L.L.; McMorrow, R.; Kim, H.H.; Kenitz, J.D.; Wheeler, R.; Sager, J.C., 2005. Design and fabrication of adjustable red–green–blue LED light arrays for plant research. BMC Plant. Biol. 5 (17): 1–11.

Ghosh, P.K.; Ramesh, P.; Bandyopadhyay, K.K.; Tripathi, K.M., 2004. Comparative effectiveness of cattle manure, poultry manure, phosphocompost and fertilizer-NPK on three cropping systems in vertisols of semi-arid tropics. II. Dry matter yield, nodulation, chlorophyll content and enzyme activity. Bioresour. Technol. 95: 85-93.

Giannakopoulou, E., 2002. Q. ithaburensis ssp. macrolepis forests (17th-19th century): Economic factor and competition challenge. In: TEI of Lamia (ed.). Proceedings of the

conference “Q. ithaburensis ssp. macrolepis Forests: Past, Present and Future”, Messologi, 17 May 2002: 67-96. (In Greek).

Giliberto, L.; Perrotta, G.; Pallara, P.; Weller, J.L.; Fraser, P.D.; Bramley, P.M.; Fiore, A.; Tavazza, M.; Giuliano, G., 2005. Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. Plant Physiol. 137: 199–208.

Goins, G.D.; Yorio, N.C.; Sanwo, M.M.; Brown, C.S., 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J.Expt. Bot. 48:1407–1413.

Gould, K.S., 2004. Nature’s Swiss Army knife: The diverse protective roles of anthocyanins in leaves. J. Biomed. Biotechnol. 5: 314–320.

Grossnickle, S.C., 2005.The importance of root growth in overcoming planting stress. New Forests 30(2/3): 273–294.

Gupta, V.; Tripathy, B.C., 2010. Effect of light quality on chlorophyll accumulation and protein expression in wheat (Triticum aestivum L.) seedlings. Int. J. Biotechn. Biochem. 6(4): 521-536.

Hallgren, S.W.; Tauer, C.G., 1989. Root growth potential, first-year survival, and growth of shortleaf pine seedlings show effects of lift date, storage, and family. South J Appl For 13: 163–169.

Hanyu, H.; Shoji, K., 2002. Acceleration of growth in spinach by short-term exposure to red and blue light at the beginning and at the end of the daily dark period. Acta Hort. 580: 145–150.

Hatchell, G.E.; Muse, H.D., 1990 Nursery cultural practices and morphological attributes of longleaf pine bareroot stock as indicators of early field performance. Research Paper SE-277. USDA Forest Service,Asheville, NC: 34.

Heo, J.W.; Lee, Y.B.; Kim, D.E.; Chang, Y.S.; Chun, C., 2010. Effects of supplementary LED lighting on growth and biochemical parameters in Dieffenbachia amoena ‘Camella’ and Ficus elastica ‘Melany’. Korean J Hortic Sci Technol 28: 51–8.

Hogewoning, S.W; Trouwborst, G.; Maljaars, H.; Poorter, H.; Ieperen, W.; Harbinson, J., 2010. Blue light dose–responses of leaf photosynthesis, morphology, and chemical

composition of Cucumis sativus grown under different combinations of red and blue light. Journal of Experimental Botany, 61: 3107–3117.

Horton, P.; Ruban, A.V.; Walters, R.G., 1996. Regulation of light harvesting in green plants. Annual Review of Plant Physiology & Plant Molecular Biology 47: 655–684.

Hunt, G., 1990. Effect of controlled-release fertilizers on formation of mycorrhizae in container-grown Engelmann spruce. Tree Improvement, vol. 3, no. 1. Forest Service, British Columbia: 6–8.

Ioannidis, A., 2002. Q. ithaburensis ssp. macrolepis cups and tannery: present status and perspectives. In: TEI of Lamia (ed.). Proceedings of the conference “Q. ithaburensis ssp. macrolepis Forests: Past, Present and Future”, Messologi, 17 May 2002: 128-135. (In Greek).

Johkan, M.; Shoji, K.; Goto, F.; Hahida, S.; Yoshihara, T., 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in ref leaf lettuce. HortScience 45: 1809-1814.

Jönsson, A.M.; Appelberg, G.; Harding, S.; Bärring, L., 2009. Spatio-temporal impact of climate change on the activity and voltinism of the spruce bark beetle Ips typographus. Glob. Chang. Biol. 15: 486–499.

Kaplan, D.; Gutman, M., 1999. Phenology of Quercus ithaburensis with emphasis on the effect of fire. Forest Ecology and Management 115(1): 61-70.

Karpinski, S.; Reynolds, H.; Karpinska, B.; Wingsle, G;, Creissen, G.; Mullineaux, P.M., 1999. Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. Science 284: 654–657.

Kataoka, I.; Sugiyama, A.; Beppu, K., 2003. Role of ultraviolet radiation in accumulation of anthocyanin in berries of ‘Gros Colman’ grapes (Vitis vinifera L.). J. Japan Soc. Hort. Sci. 72: 1–6.

Kim, H.H.; Goins, G.D.; Wheeler, R.M.; Sager, J.C., 2004. Green-light supplementation for enhanced lettuce growth under red-and blue-light-emitting diodes. HortScience 39: 1617–1622.

Kim, H.H.; Wheeler, R.M.; Sager, J.C.; Yorio, N.C.; Goins, G.D., 2005. Light-emitting diodes as an illumination source for plants: A review of research at Kennedy Space Center. Habitation (Elmsford) 10:71–78.

Kim, K.; Kook, H.S.; Jang, Y.J.; Lee, W.H.; Kamala-Kannan, S.; Chae, J.C.; Lee, K.J., 2013. The Effect of Blue-light-emitting Diodes on Antioxidant Properties and Resistance to Botrytis cinerea in Tomato. J Plant Pathol Microb, 4: 1-5.

Kitajima, M.; Butler, W. L., 1975. Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim. Biophys. Acta 376: 105-115.

Krasowski, M.J., 2003. Root system modifications by nursery culture reflect on post-planting growth and development of coniferous seedlings. For Chron 79(5):882–891.

Ksontini, M.; Louguet, P.; Laffray, D.; Rejeb, M.N., 1998. Comparison of the water stress effects on stomatal conductance, photosynthesis and growth of Mediterranean oak seedlings (Quercus suber L., Q. faginea, Q. coccifera) in Tunisia. Annales des Sciences Forestieres 55: 477–495.

Kubinova, L., 1991. Stomata and mesophyll characteristics of barley leaf as affected by light: stereological analysis. Journal of Experimental Botany 42: 995–1001.

Kuper, J.H., 1994. Sustainable Development of Scots Pine Forests. Wageningen Agricultural University Papers 94.2.

Landis, T.D.; Pinto, J.R.; Dumroese, R.K., 2013. Light Emitting Diodes (LED) – Applications in Forest and Native Plant Nurseries. Forest Nursery Notes 5: 5-13.

Lattif, A.N.; Younis, H., 1984. Comparative anatomy and identification of native oak-woods in northern Iraq. Iraqi Journal of Agricultural Sciences Zanco 2(2): 96-99.

Lee, S.H.; Tewari, R.K.; Hahn, E.J.; Park, K.Y., 2007. Photon flux density and light quality induce changes in growth, stomatal development, photosynthesis and transpiration of Withania somnifera L. Dunal. plantlets. Plant Cell Tissue & Organ Culture 90: 141–151.

Li, G.L.; Lui, Y.; Yang, J.; Sun, H.Y.; Jia, Z.K.; Ma, L.Y., 2011. Influence of initial age and size on the field performance of Larix olgensis seedlings. New For 42: 215–226.

Li, H.; Tang, C.; Xu, Z.; Liu, X.; Han, X., 2012. Effects of different light sources on the growth of non-heading Chinese cabbage (Brassica campestris L.). Journal of Agricultural Science 4: 262-273.

Llorach, R.; Martinez-Sanchez, A.; Francisco, A.; Gil, M.I.; Ferreres, F., 2008. Characterization of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem. 108:1028-1038.

Luis, V.C.; Peter, J; Gonzalez-Rodrıguez, A.M.; Jimeez, M.S.; Morales, D., 2004. Testing nursery plant quality of Canary island pine seedlings under different cultivation methods. Phyton Ann. Rei Botanicae 44: 231–244.

Lynch, J., 1995. Root architecture and plant productivity. Plant Physiol 109: 7–13.

Makri, O.; Kintzios, S., 2007. Ocimum sp. (Basil): Botany, cultivation, Pharmaceutical properties, and biotechnology. Journal of Herbs, Spices & Medicinal Plants 13: 123-150.

Marques, V.B.; De Paiva, H.N.; Gomes, J.M.; Neves, J.C.L., 2006. Effects of nitrogen sources and levels on the growth of sabia (Mimosa caesalpiniaefolia Benth.) seedlings. Scietia Forestalis/For. Sci. 71: 77–85.

Martínez-Vilalta, J.; López, B.C.; Adell, N.; Badiella, L.; Ninyerola, M., 2008. Twentieth century increase of Scots pine radial growth in NE Spain shows strong climate interactions. Glob. Chang. Biol. 14: 1868–2881.

Mason, W.L.; Alia, R., 2000. Current and future status Scots pine (*Pinus sylvestris* L.) forests in Europe. Invest. Agr.: Sist. Recur. For.: Fuera de Serie n 1–2000: 317–333.

McNellis, T.W.; Deng, X.W., 1995. Light control of seedling morphogenetic pattern. J. Plant Cell 7: 1749–1761.

McPhee, K., 2005. Variation for seedling root architecture in the core collection of Pea germplasm. Crop Sci 45:1758–1763.

Mexal, J.G.; Cuevas, Rangel R.A.; Landis, T.D., 2008. Reforestation success in central Mexico: factors determining survival and early growth. Tree Planters' Notes 53:16–22.

Morrissey, R.C.; Jacobs, D.F.; Davis, A.S.; Rathfon, R.A., 2010. Survival and competitiveness of Quercus rubra regeneration associated with planting stocktype and harvest opening intensity. New For 40: 273–287.

Mouterde, P., 1966. Nouvelle flore du Liban et de la Syrie. Tome 1. Beyrouth, Éditions de l'Imprimerie catholique.

Muller, J.; Brandl, R., 2009. Assessing biodiversity by remote sensing and ground survey in montainous terrain: the potential of LiDAR to predict forest beetle assemblages. J. Appl. Ecol. 46: 897–905.

Mullineaux, P.; Karpinski, S., 2002. Signal transduction in response to excess light getting out of the chloroplast. Current Opinion in Plant Biology 5: 43–48.

Murray, J.R.; Hackett, W.P., 1991. Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase in Hedera helix L. Plant Physiology 97: 343–351.

Naidu, R.A.; Krishnan, M.; Nayudu, M.V.; Gnanam, A., 1984. Studies on peanut green mosaic virus infected peanut (Arachis hypogaea L.) leaves. II. Chlorophyll-protein complexes and polypeptide composition of thylakoid membranes. Physiol. Plant Pathol. 25: 191-198.

Nambiar, E.K.S., 1984. Significance of first-order lateral roots on the growth of young radiata pine under environmental stress. Aust For Res 14: 187–199.

Oh, M.M.; Carey, E.E.; Rajashekar, C.B., 2009. Environmental stresses induce health – promoting phytochemicals in lettuce. Plant Physiol. Bioch. 47: 578-583.

Ohasi-Kaneko, K.; Takase, M.; Kon, N.; Fujiwara, K.; Kurata, K., 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environ. Cont. Biol. 45: 189–198.

Oliet, J.A.; Planelles, R.; Artero, F.; Valverde, R.; Jacobs, D.F.; Segura, M., 2009b. Field performance of Pinus halepensis planted in Mediterranean arid conditions: relative influence of seedling morphology and mineral nutrition. New For 37: 313–331.

Öquist, G.; Wass, R., 1988. A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. Physiol. Plant. 73: 211-217.

Ouzounis, T.; Frettéa, X.; Rosenqvistb, E.; Ottosenc, C.O., 2014. Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanulas. Journal of Plant Physiology 171: 1491–1499.

Padilla, F.M.; Pugnaire, F.I., 2007. Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Funct Ecol 21: 489–495.

Pardo, G.P.; Aguilar, C.H.; Martinez, F.R.; Pacheco, A.D.; Martinez, C.L.; Ortiz, E.M., 2013. High intensity LED light in Lettuce seed Physiology. Acta Agrophysica, 20(4): 665-677.

Pignatti, S., 1982. Flora d'Italia, Vol.1. Roma, Edragricole.

Pinho, P.; Lukkala, R.; Särkka, L.; Teti, E.; Tahovnen, R.; Halonen, L., 2007. Evaluation of lettuce growth under multi-spectral-component supplemental solid state lighting in greenhouse environment. Intrnational Review of Electrical Engineering 2: 680-854.

Porra. R.J.; Thompson, W.A.; Kriedemann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta 975: 384-394.

Radoglou, K.M.; Jarvis, P.G., 1990. Effects of CO2 enrichment on four poplar clones. II. Leaf surface properties. Annals of Botany 65: 627–632.

Ramalho, J.C.; Marques, N.C.; Semedo, J.N.; Matos, M.C.; Quartin, V.L., 2002. Photosynthetic performance and pigment composition of leaves from two tropical species is determined by light quality. Plant Biol. 4: 112–120.

Ravazzi, C., 2002. Late quaternary history of spruce in southern Europe. Rev. Paleobot. Palyno. 120: 131–177.

Ritchie, G.A.; Dunlap, J.R., 1980. Root growth potential: its development and expression in forest tree seedlings. N Z J For Res 10: 218–248.

Ritchie, G.A., 1984. Assessing seedling quality. In: Duryea, M.L., Landis, T.D. (Eds.), Forest Nursery Manual:Production of Bareroot Seedlings. Martines Nijhoff/Dr.W.Junk. Publishers, Hague/Boston/Lancaster: 243– 259.

Rodríguez-Calcerrada, J.A.; Pardos, .JA.; Gil, L.; Reich, P.B.; Aranda, I., 2008. Light response in seedlings of a temperate (Quercus petrae) and sub-Mediterranean species (Quercus pyrenaica): contrasting ecological stratgegies as potential keys to regeneration performance in mixed marginal populations. Plant Ecol 195: 273-285.

Roller, K.J., 1976. Field performance of container-grown Norway and black spruce seedlings. Can. For. Serv. Dept. Environ. Inf. Rep.: 64-68.

Ruehle, J.L.; Kormanik, P.P., 1986. Lateral root morphology: a potential indicator of seedling quality in northern red oak. Southeasten Forest Experiment Station: 344-346.

Rushforth, K., 1999. Trees of Britain and Europe. Collins ISBN 0-00-220013-9

Sager, J.C.; Edwards, J.L.; Klein, W.H., 1982. Light energy utilization efficiency for photosynthesis. Trans. ASAE 25:1737–1746.

Salisbury, E.J., 1927. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. Philosophical Transactions of the Royal Society (London) B216: 1-65.

Sarris, D.; Christodoulakis, D.; Körner, C., 2007. Recent decline in precipitation and tree growth in the eastern Mediterranean. Global Change Biol. 13: 1187–1200.

Satyavati, G. V.; Gupta, A. K.; Tandon, N., 1978. Medicinal Plants of India, vol. 2, Indian Council of Medical Research, New Delhi, India.

Schelhaas, M.J.; Nabuurs, G.J.; Schuck, A., 2003. Natural disturbances in the European forests in the 19th and 20th centuries. Glob. Chang. Biol. 9: 1620–1633.

Schoch, P.G.; Jacques, R.; Lecharny, A; Sibi, M., 1984. Dependence of the stomatal index on environmental factors during stomatal differentiation in leaves of Vigna sinensis L. II. Effect of different light quality. J. Exp. Bot. 35: 1405-1409.

Schreiber, U.; Bilger, W. 1993., Progress in chlorophyll fluorescence research: major developments during the past years in retrospect. Progr. Bot. 54: 151-173.

Schultz, R.C.; Thompson, J.R., 1996. Effect of density control and undercutting on root morphology of 1+0 bareroot hardwood seedlings: five-year field performance of root-graded stock in central USA. New For 13: 297–310.

Schultz, R.C.; Thompson, J.R., 1997. Effect of density control and undercutting on root morphology of 1+0 bareroot hardwood seedlings: five-year field performance of root graded stock in the central USA. New For 13: 301–314.

Seelye, J.F.; Mullan, A.C., 2010. Light-emitting diode lights: the future of plant lighting. International Plant Propagators' Society, Combined Proceedings 60 (2010): 171-178.

Serce, S.; Ercisli, S.; Sengul, M.; Gunduz, K.; Orhan, E., 2010. Antioxidant activities and fatty acid composition of wild grown myrtle (Myrtus communis L.) fruits. Pharmacogn. Mag. 6(21): 9-12.

Sestak, Z., 1996. Liminations for finding linear relationship between chlorophyll content and photosynthetic activity. J. Biol. Plant, 8: 336-346.

Siddiqui, S.; Arshad, M., 2014. Osteogenic potential of punica granatum through matrix mineralization, cell cycle progression and runx2 gene expression in primary rat osteoblasts. Research Article. Journal of Pharmaceutical Sciences 22: 72.

Singh, S.; Kumari, R.; Agrawal, M.; Agrawal, S.B., 2011. Growth, yield and tuber qualityof Solanum tuberosum L. under supplemental ultraviolet-B radiation at different NPK levels. Plant Biol. 13: 508–516.

Singleton, V.L.; Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-158.

South, D.B., 2000. Planting morphological improved pine seedlings to increase survival and growth. Report no. 1. Alabama Agricultural Experiment Station (Auburn University), Auburn, Alabama, USA.

South, D.B.; Harris, S.W.; Barnett, J.P.; Hainds, M.J.; Gjerstad, D.H., 2005. Effect of container type and seedling size on survival and early height growth of Pinus palustris seedlings in Alabama, U.S.A. For Ecol Manage 204: 385–398.

Stankovic, M.S.; Topuzovic, M.D., 2012. In vitro antioxidant activity of extracts from leaves and fruits of common dogwood (Cornus sanguinea L.). Acta Botanica Gallica: Botany Letters 159: 79-83.

Steven, H.M.; Carlisle, A., 1959. The Native Pinewoods of Scotland. GC Book Publishers, Edinburgh.

Tanaka, Y.; Walstad, J.D.; Borrecco, J.E., 1976. The effect of wrenching on morphology and field performance of Douglas-fir and loblolly pine seedlings. Can J For Res 6: 453–458.

Talbott, L. D.; Hammad, J. W.; Harn, L.C.; Nguyen, V.H.; Patel, J.; Zeiger, E., 2006. Reversal by green light of blue light-stimulated stomatal opening in intact, attached leaves of Arabidopsis operates only in the potassium dependent, morning phase of movement. Plant Cell. Physiol. 47: 332-339.

Terashima, I.; Fujita, T.; Inoue, T.; Chow, W.S.; Oguchi, R., 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. Plant Cell Physiol. 50: 684–697.

Thomas, W.P.; Woodward, F.I.; Quick, W.P., 2003. Systemic irradiance signaling in tobacco. New Phytologist 161: 193–198.

Travese, 2001. Ecology of Fruit-color Polymorphism in Myrtus communis and Differential Effects of Birds and Mammals on Seed Germination and Seedling Growth. Journal of Ecology., 89: 749-760.

Tsakaldimi, M.; Zagas, T.; Tsitsoni, T.; Ganatsas, P., 2005. Root morphology, stem growth and field performance of seedlings of two Mediterranean evergreen oaks species raised in different container types. Plant Soil 278: 85–93.

Tsormpatsidis, E.; Henbest, R.G.C.; Davis, F.J.; Battey, N.H.; Hadley, P.; Wagstaffe, A., 2008. UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. Environ. Exp. Bot. 63: 232–239.

Vendramin, G.G.; Anzidei, M; Madaghiele, A.; Sperisen, C., Bucci, G., 2000. Chloroplast microsatellite analysis reveals the presence of population subdivision in Norway Spruce (*Picea abies* K.). Genome 43: 68–78.

Vilela, A.E.; Rennella, M.J.; Ravetta, D.A., 2003. Response of tree-type and shrub-type Prosopis (Mimosaceae) taxa to water and nitrogen availabilities. Forest Ecology Management 186: 327–337.

Villaey,s A., 1990. L'Albanie forestière. Rev. For. Fr. XLII(5) : 531-541.

Viuda-Martos, M.; Fernandez-Lopez, J.; Peerez-Alvarez, J. A., 2010. "Pomegranate and its many functional components as related to human health: a review," Comprehensive Reviews in Food Science and Food Safety, 9: 635–654.

Walentowski, H.; Ewald, J.; Fischer, A.; Kölling, C.; Turk, W., 2004. Handbuch der naturlichen Waldgesellschaften in Bayern. Geobotanica, Freising.

Walters, R.G.; Shephard, F.; Rogers, J.J.M.; Rolfe, S.A.; Horton, P., 2003. Identification of mutants of Arabidopsis defective in acclimation of photosynthesis to the light environment. Plant Physiology 131: 472–481.

Warrington, I.J.;. Mitchell, K.J; Halligan, G. 1976. Comparisons of plant growth under four different lamp combinations and various temperature and irradiance levels. Agr. Meteorol. 16: 231–245.

Wermelinger, B., 2004. Ecology and management of the spruce bark beetle Ips typographus—a review of recent research. For. Ecol. Manage. 202: 67–82.

Willmer, C.; Fricker, M., 1996. Stomata. Chapman & Hall Publisher, London.

Wilson, E.R.; Vitols, K.C.; Park, A., 2007. Root characteristics and growth potential of container and bare-root seedlings of red oak (Quercus rubra L.) in Ontario, Canada. New For 34: 163–176.

Yanagi, T.; Okamoto, K.; Takita, S., 1996. Effects of blue, red and blue/red lights of two different PPF levels on growth and morphogenesis of lettuce plants. Acta Horticulturae 440: 117-122.

Yanovsky, M.J.; Alconada-Magliano, T.M.; Mazzella, M.A.; Gatz, C.; Thomas, B.; Casal, J.J., 1998. Phytochrome, affects stem growth, anthocyanin synthesis, sucrosephosphate-synthase activity and neighbour detection in sunlight-grown potato. Planta 205: 235–241.

Yeh, N.; Chung, J.P., 2009. High-brightness LEDs—Energy efficient lighting sources and their potential in indoor plant cultivation. Renewable and Sustainable Energy Reviews 13: 2175–2180.

Yorio, N.C.; Goins, G.D.; Kagie, H.R.; Wheeler, R.M.; Sager, J.C., 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. HortScience 36: 380–383.

Zhang, T.; Folta, K.M., 2012. Green light signaling and adaptive response. Plant Signaling & Behavior, 7: 1-4.

Zhou, Y.; Singh, B.R., 2002. Red light stimulates flowering and anthocyanin biosynthesis in American cranberry. Plant Growth Regul. 38: 165–171.

Zohary, M., 1973. The geobotanical foundations of the Middle East. Stuttgard, Gustav Fisher.

Internet site: www.giaroots.org. GiA Roots: Software for the High Throughput Analysis of Plant Root System Architecture (manuscript). Taras Galkovskyi, YuriyMileyko, Alexander Bucksch, Brad Moore, Olga Symonova, Charles A Price, Christopher N Topp, Anjali S Iyer-Pascuzzi, Paul Zurek, Suqin Fang, John Harer, Philip N Benfeyand Joshua S Weitz.