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The *Robinia pseudoacacia L.* seed germination and plantlets growth in septic or aseptic conditions under led light of different wavelenghts

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SUMMARY. The lighting of plant vitrocultures using white LEDs expecially the ones of ultrabright type is recomended because – as resulted from the experiments performed in this study, under septic or vitroculture conditions, with *Robinia pseudoacacia L.* seeds and with plants resulted from these – it was shown that compared with red, yellow, green and especially blue light, under white light the organ growth and assimilating pigments synthesis was stimulated. Furthermore the endocellular substances metabolisation was also accelerated. Among other types of light emited by LEDs, only the yellow light has stimulated the increase in the values of the parameters that were investigated. This has favored, in the 40st day of germination the growth of the stemlet in the septic cultures even as high as 51% compared to the control (100% , plantlets under white light LEDs).

Keywords: germination, growth LEDs lighting, plantlets, *Robinia pseudoacacia.*

Introduction

Plant vitrocultures, mainly the ones from the plant micropropagation industry require to set up growth chambers in which the phyto-inoculi or vitroplantlets regenerated „*in vitro*" or the one transferred „*ex vitro*" (to acclimatize them for the septic environment), need to be exposed to light.

The artificial light for lighting such cultures, is installed in growth chambers in which the environmental conditions are automatically adjusted. Most frequently, the lighting in such chambers is made using white light from fluorescent tubes.

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These types of lamps, however, warm the room and as such, an environmental control system has to be used. The cooling of the growth chambers increases the electricity consumption and the maintenance costs of the chambers.

It is also worth to be mentioned that in modern horticulture the planting material is obtained using micropropagation techniques. The nature of the light wavelenght has different influence on the various physiological processes of the plants, depending on the species and developmental stage and also on the organ studied.

The spectral composition of the light is important for the synthesis of different substances or for the various biological processes that occur in plants (Burzo *et al*., 1999).

Solar light consists of a large number of visible electromagnetic radiation with a wavelenght between 400 and 700 nm (red, orange, yellow, green, blue and violet) and also invisible radiation – ultraviolet (UV) light under 400 nm and infrared radiation - above 700 nm (Tarhon, 1992).

Artificial light sources emit differently the spectral wavelenghts, the light being different from the natural one which is an equal blend of all spectral components.

At this moment, in different areas of activity the LED lighting is widespread. It is used as spots, signaling devices, panels of various sizes etc.

LED lighting fixtures are made of lamps consisting of semiconductor diodes that became luminescent when connected to a power supply. Such lamps are 5 -7 mm in size and can be grouped to form light sources with different intensities. They do not heat up and have very low energy consumption (http://ro.wikipedia.org/). The LEDs can be placed in the immediate vicinity of the flasks with the phyto-innoculi, allowing the increase of density of the shelves in the growth chambers for efficient space usage.

Tennessen *et al*. (1995) have studied the effect of LEDs lighting on the photosynthesis in *Solanum tuberosum* vitrocultures under continuous lighting compared to intermittent lighting. The photosynthetic capacity of the vitroplantlets was similar in both cases.

Regarding the distance at which the LEDs can be placed above the vitrocultures, Jao and Fang (2004) studied the *Solanum tuberosum* explants where the lamps were placed 1 cm above the vitrocultures. The lighting duration was as follows: 8 hrs light/16 hrs dark; 12hrs light/12 hrs dark and 16 hrs light/24 hrs dark. The best results were obtained using the last lighting regime. In relation to the use of LEDs for lighting, compared with fluorescent light – placed 30 cm above the cultures – a cost saving of about 17% was estimated.

A series of studies regarding the effect of LED light on plants were performed with *Marigold* and *Salvia* (Jeong *et al*., 2002), *Fragaria* (Nhut *et al.,* 2003), *Chrisantemum* (Petruş and Cachiţă, 2004), *Zantedeschia jucunda* (Jao *et al.*, 2005), *Pisum sativum* (Topchiy *et al.*, 2005), or at seed germination of *Raphanus* and of *Daucus* (Sommer and Franke, 2006), of *Sequoia sempervirens* (Pop and Cachiţă,

2007; 2009; 2013), of *Solanum* (Pop *et al*., 2011), or of *Pinus nigra*, *Brassica oleacea* and *Beta vulgaris* (Matioc-Precup and Cachiţă, 2013), or of kernels of *Sorgum* (Stana and Cachiţă, 2012) or of *Hordeum vulgare* (Matioc-Precup and Cachiţă, 2013). In the majority of the cases, positive results were obtained regarding the growth parameters.

The purpose of the study presented in this work was to investigate the influence of the lighting with LEDs of different colors (red, yellow, green or blue) on the *Robinia pseudoacacia L.* seed germination and on the plantlets growth under septic or aseptic regime for 40 days and exposed to a mono color light of a certain type, for 16/24 hours.

Material and methods

The *Robinia pseudoacacia L.* is a dicotyledonous (dicot) species. The plantlets have epigeal cotyledons that after raising above the ground, turn green and begin photosynthesis as long as they do not enter into senescence and fall (Fig. 1).

In the present work, the experiments were performed either with plantlets from *Robinia pseudoacacia L.* seeds germinated in colorless, transparent plastic containers (seeds placed on filter paper periodically moistened with tap water) or the germination and plantlet growth was done „*in vitro*" on agarised Murashige-Skoog (1962) growth medium without growth regulators.

Depending on the experiments, thesamples were analyzed either after 10 days from germination and after 14 or 40 days. Before „*in vitro*" inoculation, the seeds were disinfected with a „Domestos" solution for 20 minutes. After that, they were washed repeatedly with sterile water.

The inoculation in the culture containers was made in a hood with laminary sterile air flow. In both versions the lighting of the samples was done either with white LEDs (version V_0) or red (version V_1), yellow (version V_2), green (version V_3) or blue (version V_4). From the germination samples, a batch was exposed to natural light (Version V_0) by putting the containers in a northward facing window. The number and density of the LEDs on the lightins panel was choosen to give a light intensity of 2000 lux. The adopted photoperiod in all experiments was 16 hrs light/24 hrs. The LEDs were of ultrabright type and the wavelenghts were 700 nm for the red light, 550 nm for the yellow light, 500 nm for green and 450 for the blue light (Tab.1).

In the 14^{th} day of germination, cotyledons were taken from the plantlets to perform ultrathin transversal sections which were processed according to the specific procedures for this technique (Cachiţă and Crăciun, 1990). Firstly, the plant tissues were fixed in a 2.5% glutaraldehyde solution in 0.15 M phosphtate buffer followed by post-fixation in a 2% osmic acid solution in 0.1 M phosphate buffer. After that,

the samples were dehydrated in increased acetone concentration solutions and embedded in epoxy resin followed by encapsulation in gelatin capsules. The blocks were shaped under the stereomicroscope and ultrahin sections were obtained using a Leica UC6 ultramicrotome and collected on electrolytic grids and doubly contrasted with uranyl acetate and lead citrate. The examination of the sections was done on a Jeol 1010 transmission electron microscope (TEM) and the most representative electronmicrographs are shown in figures 2 and 3. Further, in the 40st day from germination, biometric measurements on the *Robinia pseudoacacia L.* plantlets size were done by measuring the lenght of the embryonary rootlet and stemlet (of the hypocotyl and epicotyl respectively) and by summing their lenghts, the size of the whole plantlet was obtained (Fig. 4 A and B).

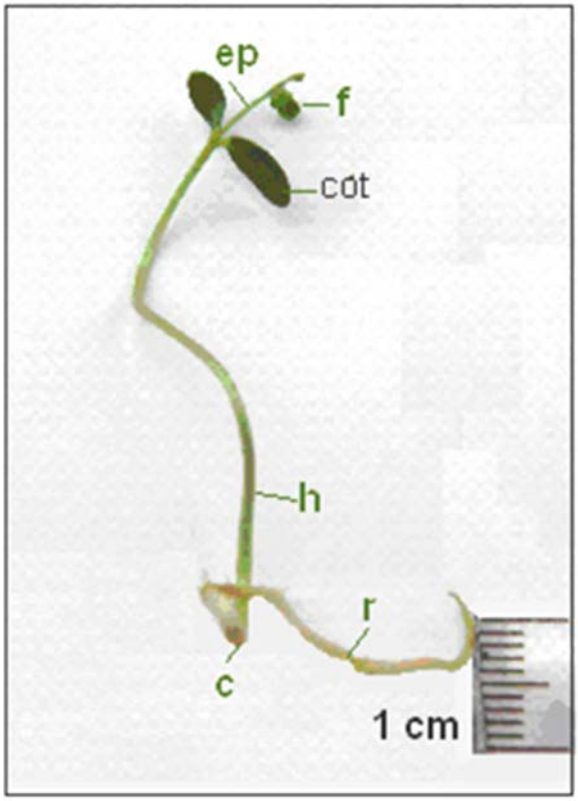

Figure 1. The aspect of a 40 days from germination *Robinia pseudoacacia L.* plantlet (abbreviations: c – root-stem transition zone; cot – cotyledons; ep – epicotyl; f- leaflet foliole; h – hypocotyl; r – embryonary rootlet).

Such measurements were performed both on the plantlets kept in plastic containers and grown on aseptic medium. The biometric measurements were done on 50 plantlets for each experimental version, the average of these values/version were included in the percentage calculations, reporting the values to the reference average evaluated as 100% recorded for the control (V_0) – the sample exposed to white LEDs.

Table 1.

Experimental versions performed to determine the percentage of germinated seeds in the 10th day

Versions	**Percentage of germination**
Natural light (V_{00})	99%
White LED (V_0)	98%
Red LED (V_1) 700 nm	91%
Yellow LED (V_2) 550 nm	96%
Green LED (V_3) 500 nm	99%
Blue LED (V_4) 450 nm	95%

The content in the assimilating pigments, was also determined in the 40st day from germination in the biomass made of a homogenate prepared from the "above the ground" organs of a plantlet – the whole green mass resulted from grounding the hypocotyls, cotyledons, epicotyl and leaflets, where they were developed.

From the fresh plant material, the pigments were extracted with a dimethylformamide solution and the extract measured using a „Spekol 11" spectrophotometer at the following wavelenghts: 664 nm to determine the content in chlorophyll **a**, 647 nm to determine the content of chlorophyll **b**, and 480 nm to determine the content in carotenoid pigments.

From the fresh biomass 50 mg per sample were taken and placed in 5 ml dimethylformamide (DMF) (Moran and Porath, 1980); the mixture was kept in a refrigerator fo 72 hrs at 4ºC. After that, the supernatant was decanted and from this solution the extracted assimilating pigments content was determined (green pigments and carotenoid pigments). By summing the values obtained for each pigment, the total amount of assimilating pigments was obtained.

For each experimental version five measurements were performed.

The data from the spectrophotometer was matematically processed according to a formula proposed by Moran and Porath (1980):

- chlorophyll a (mg/gPM) = 11,65 A664 – 2,69 A647 · v/s;
- chlorophyll b (mg/gPM) = 20,8 A647 – 3,14 A664 · v/sp;
- carotenoids (mg/gPM) = (1000 A480 – 1,28 clorof. a – 56,7 clorof. b) / 245 · v/sp;

where: the numbers near letter ”A” are the wavelenghts in nm;
v – solvent volume in ml;
PM – plant material weight in mg used for extranction/sample.

From the obtained photometric data, average values were calculated and reported to the numbers obtained for the samples under white LEDs (Version V_0) - considered as reference 100%. These percentage values are represented as histrograms in figures 5 A and B.

It is to be emphasised that the experiments from this work consist of a step regarding the examination of the germination percentage evaluated in the 10^{th} day of germination and of studying the ultrastructure aspects in the 14^{th} day of germination under different light in septic regime. The experiments concerned with the determination of the plantlet organs growth and assimilating pigments content were performed on samples exposed to both septic and aseptic vitroculture conditions.

Results and discussions

As it can be seen in Table 1, the data regarding the percentage of germinated seeds– recorded in the 10^{th} day of germination – indicated that under natural light (Version V_{00}) and green light LEDs (V_3), 99% of seed germination was achieved and the LEDs white light induced 98% germination (V_0). The lowest germination percentage – under 91% - was recorded for the seeds under red light (V_1), 95% under blue light (V_4) and 96% under yellow light (V_2). From what is was presented, only the red light produced a 9% inhibition of the *Robinia pseudoacacia L.* seeds germination.

At 14 days from germination, the plantlet cotyledons from each version (V_0 – V_4) were detached to take fragments for fixation for electronmicroscopy studies and to perform transversal sections through this organ. The most representative electronmicrographs are shown in Figures 2 and 3.

From the examination of these images results that at this age, most of the cells had in their vacuolar content various highly electrondense, corpuscular deposits. This suggests the extent to which the endocellular reserve substances are metabolised or not by the growing embryo. It should be mentioned the fact that as the plantlets grow, the storage substances from the cotyledons are exhausted and finally the cotyledons turn yelow and fall.

From previous studies (Cachiţă and Crăciun, 1990) it resulted that in the plant cells vacuolar content there is a tendency to form corpuscules containing anthocyanins (colored in red in acid pH or blue in basic pH) or that different vital dyes administered from the exterior can be included.

Such corpuscles have a liquid crystall type structure (called liposomes or recently nanoparticles). The liquid crystalls can be generated from plant phospholipids, for example soy lecithin (Cachiţă, 1975, 1981 and Cachiţă and Gergely 1990). These formations are of great interest for researchers mainly for their use for medical or cosmetic purposes.

Therefore, in the case of the experiments performed in this work it was considered that in the vacuolar content of the cotyledon cells of *Robinia pseudoacacia L.*, same as in soy seeds, probably there are phospholipids that as the embryo grows, are gradually exhausted and the vacuolar content „clarifies". The cotyledons even if they are green and photosynthesise, their functions gradually stop, they turn yellow and fall.

As it can be seen in Figures 2 and 3, during the first 14 days of germination, in the vacuolar content of the *Robinia pseudoacacia L.* cotyledons cells, transformations of the compounds from the vacuoles occur. Thus, the epidermis of the cotyledons of the germinated plantlets grown under white LED light (V_0) or yellow (V_2) has cells that have in their vacuolar content a very fine suspension (Fig. 2A and 2 E) suggesting that these cotyledons function normally.

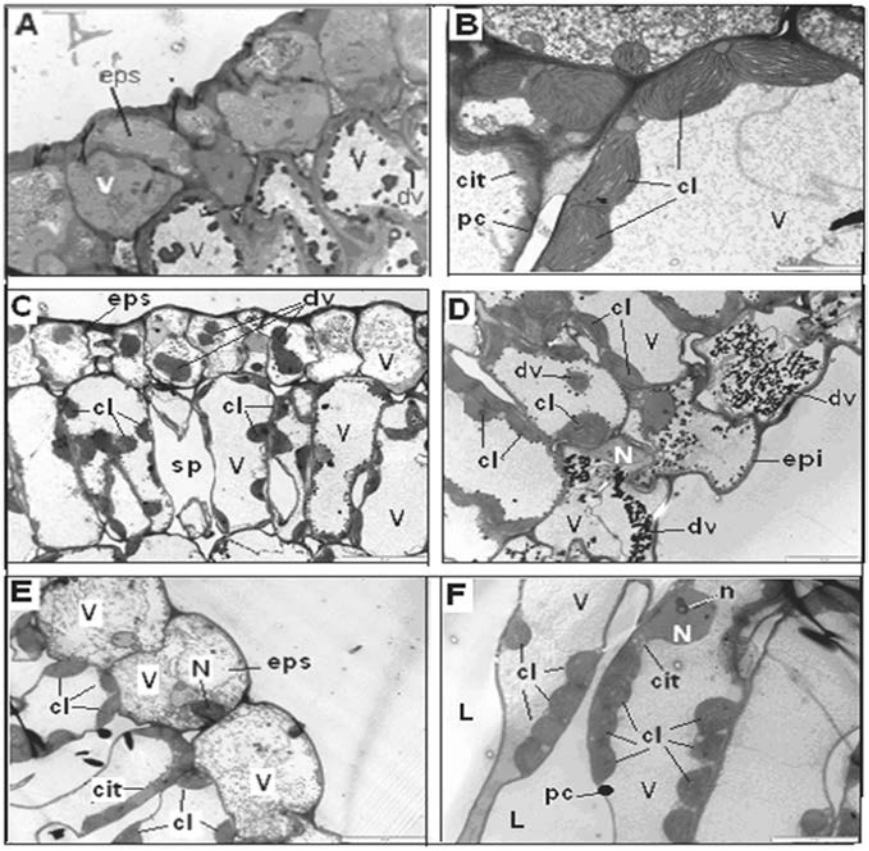

Figure 2. A – F. Transmission electronmicrographs showing the ultrastructure of the cells of *Robinia pseudoacacia L.* cotyledons from the 14 days old plantlets taken from the seeds germinated in plastic containers by placing on filter paper moistered with tap water and exposed to LED light: Fig. 2 A and B – white light – V0; Fig. 2 C and D – red light V1; Fig. 2 E and F – yellow light V2 (abbreviations: cl – chloroplasts; cit – cytoplasm; dv – vacuolar deposits; eps – upper epidermis; epi – lower epidermis; G – air gap; N – nucleus; n – nucleolus; pc – cellular wall; sp – intercellular space; V – vacuole)

The sub-epidermal and deeper cells at the same experimental versions also show the specific structures and the vacuolar content is clear; in the cytoplasm, chloroplasts with a normal aspect can be seen (Fig. 2 B and E – F).

In the case of *Robinia pseudoacacia L.* plantlets exposed to red light (Fig. 2 C and D) or green (Fig. 3 A and B), (versions V_1 and V_3), the epidermal cells of the cotyledons have in the vacuols quite large corpuscular deposits, electrondense, some of them being aggregates, or in section appearing as plates that occupy the whole surface of the vacuole (Fig. 3 B) have normal structures and have well defined chloroplasts.

In the case of the *Robinia pseudoacacia L.* plantlets exposed to blue light (version V_4, Fig. 3 C and D) both, the epidermal and underlying cells have in their vacuolar content very fine particles, in suspension; the chloroplasts are present but are small and look underdeveloped.

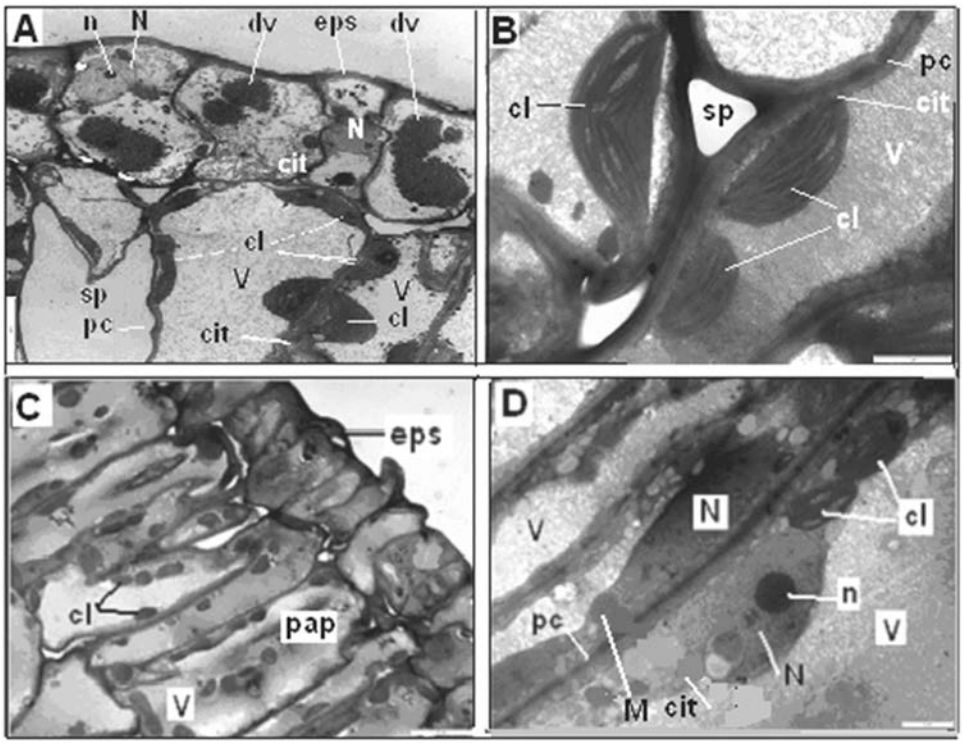

Figure 3. A – D. Transmission electron microscopy aspects identified in the transversal sections through the *Robinia pseudoacacia L.* 14 day old cotyledons from the seeds that have been germinated in plastic containers on filter paper periodically moistened with tap water and exposed 16hrs/day to LED light: version V_3 green LEDs (Fig. 3 A and B) and version V_4 blue LEDs (Fig. 3 C and D) (abbreviations: cl – chloroplasts; cit – cytoplasm; eps – upper epidermis; dv – vacuolar deposit; pap – palisade assimilating parenchyma; M – mitochondrion; mc –cotyledonary mesophyll; N – nucleus; n – nucleolus; pc – cellular wall; sp – intercellular space; V – vacuole).

The presented aspects led to the conclusion that the white and yellow LED lights have a positive influence on the structure, normalising processes and cotyledon functions. This is also reflected in the presented electromicrographs and in the driving of the *Robinia pseudoacacia* L. plantlet growth. This results from the biometric mesaurements done in the 40st day after germination and shown in Figure 4. Thus, the biometric measurements regarding the embryonary rootlet size, the hypocotyl and epicotyl (where was the case – Fig. 4) summed up give the size of the whole plantlet. Such measurements were performed on the plantlets grown both in plastic containers in septic conditions (Fig. 4 A) and „*in vitro*" in aseptic conditions (Fig. 4 B).

To the median value obtained for the plantlets exposed to white light (control 100%), version V_0, all the other averaged values for the different experimental versions (different color LEDs - versions $V_1 – V_4$), were reported.

The differences in growth recorded in the case of the measurements done in the 40st day of germination (Fig. 4 A and B) pointed out the fact that, depending on the size of the plantlets on which biometric measurements were performed, at the samples exposed to white LEDs (version V_0 – control 100%), exposing the plantlets (under septic culture regime) to yellow light (version V_2) has stimulated by 51% the growth in lenght of the stemlet and only by 5% of the rootlet and by 8% the elongation of this organ in the *Robinia pseudoacacia L.* vitroculture maintained in a similar antiseptic regime whereas the growth of the stemlet was only stimulated by 2.5%.

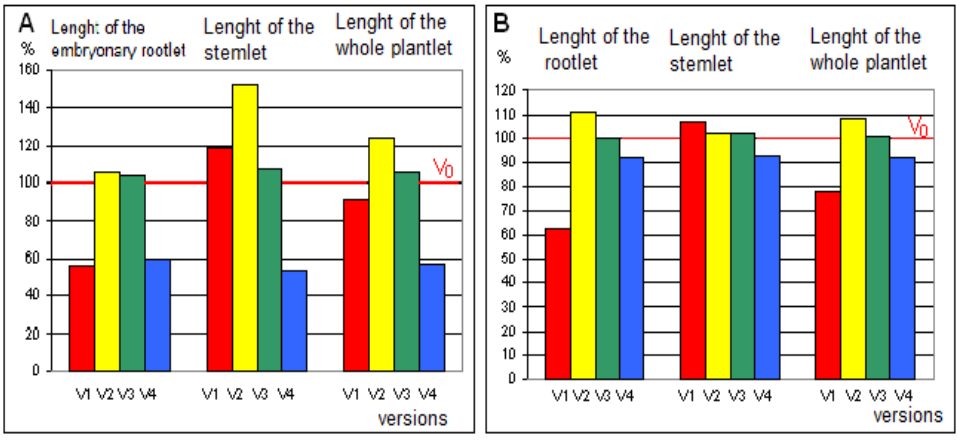

Figure 4. A and **B**. The growth of the *Robinia pseudoacacia L.* plantlets and organs from the germinated seeds after 40 days in plastic containers on filter paper moistered with tap water (4A) or vitro cultivated (4B) and exposed to LED with light of different wavelenghts (versions: V_0 -white light; V_1 - red light; V_2 – yellow light; V_3 – green light; or V_4 – blue light). The histograms show – in percentage values – the data recorded for different versions and organs compared with similarly marked parameters for the control (V_0) considered to be 100%.

In turn, the LEDs red light (version V_1) has inhibited by 45% the growth of the *Robinia pseudoacacia L*. plantlet embryonary rootlet that were maintained in an experimental aseptic regime and by 38% in the experiments done in vitro culture regime (Fig. 4 B). The growth of the stemlet size of the samples from aseptic conditions, under red light was stimulated by 20% whereas in aseptic conditions the results were closed to the control values (Fig. 4 B).

It is also interesting that the LEDs blue light has inhibited by 40% the growth in lenght of the embryonary rootlet (Fig. 4 A) and by 52% the growth in size of the stemlet in the samples from the experiments performed in septic regime. In turn, in the aseptic regime the LEDs blue light has inhibited only by 8% the growth of the *Robinia pseudoacacia L*. organs. This suggests that either a differential „filtration" of the radiation through the glass and culture medium occured or a countering of the positive or negative effects from the containers throught the components present in the culture medium.

In the experiment performed in septic conditions, the size of the whole stemlet was determined mainly by the size of the hypocotyl; the LEDs yellow light (V_2) has stimulated the elongation of this organ whereas the blue light (V_4) has strongly inhibited the growth of all organs and consequently the whole plantlet. The LEDs green light (V_3) has maintained the growth at the same level with the one observed in the plantlets exposed to white light (V_0) (Fig. 4 A).

In the case of a similar experiment performed in vitroculture regime (Fig. 4 B), the differences in growth compared with the control (V_0) were smaller only the inhibition of the rootlet growth in the presence of red light has reduced the size of the whole plantlet by 21% and the blue light by 8%.

Regarding the content in assimilating pigments of the hypocotyl, cotyledons, epicotyl and of the folioles (where they developed) (Fig. 5 A and B), it can be ascertained that in all the types of analysed pigments both green (chlorophyll **a** and **b**) and carotenoids, a strong decrease in their level was recorded – both in septic and aseptic regime – especially under green light. Thus, negative values were recorded down to - 50% for chlorophyll **a**, -72% for chloropyll **b** and -68% for carotenoid pigments, compared with the values obtained for the control (white light, 100%) in both septic (Fig. 5A) and aseptic regime (Fig. 5 B).

In the case of the carotenoid pigments under yellow light (V_2) a 9% increase was recorded. It is interesting that in the samples from the aseptic medium, the LEDs green light has reduced by about 60% the level of all pigments regardless of the type of the pigment, and in the septic regime the recorded values were – 72% for chlorophyll **b** and – 68% for the carotenoids. It was also surprising that the LEDs blue light has inhibited the growth of the *Robinia pseudoacacia L.* organs but in the same time has favored – compared with the red and green light- (and in the case of the samples from vitrocultures even with the values recorded for the yellow light) the synthesis and accumulation of green pigments in the stemlets and leaflets. The red light inhibited the accumulation of assimilating pigments in these organs (Fig. 5 A and B).

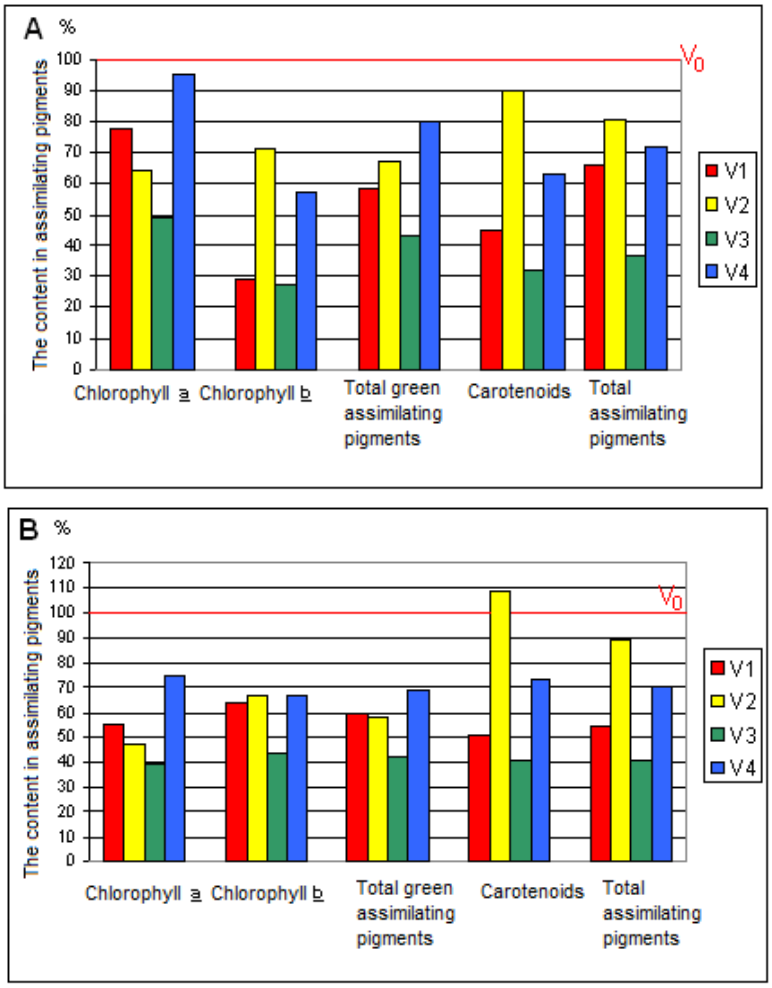

Figure 5. A and B. The content in assimilating pigments in the aerial organs of *Robinia pseudoacacia L.* plantlets (cotyledons, epycotyl and leaflets) from the stemlets of the plantlets from the seeds embryos at 40 days after germination; Fig. A – in plastic container son filter paper moistered with tap water, Fig. B – vitrocultivated and exposed 16 hrs/day to ultrabright LED with light of different wavelenghts. Experimental versions: V_0 – control – plantlets exposed to white light – values considered to be 100%; V_1 – plantlets exposed to red light; V_2 – plantlets exposed to yellow light; V_3 – plantlets exposed to green light and V_4 – plantlets exposed to blue light

Conclusions

Comparing the germination of *Robinia pseudoacacia L.*seeds – placed for germination in plastic containers on filter paper moistened with tap water- and the growth of the plantlets from their embryos for 40 days under 16hrs/day lighting with white, red, yellow, green or blue light LEDs, it was concluded that the white light was the most efficient to obtain the longest plantlets. Only under yellow light, the stemlets were 48% longer than those grown under white light. Same experiment performed *in vitro* culture regime has given far more uniform results regarding the growth of the plantlets under different light color LEDs lighting. This phenomenon is probably due to the filtration of light by the glass covering the vitroculture containers evening out the reaction of the plantlets. The LEDs white and yellow light, in the plantlets germinated in plastic containers, on filter paper moistened with tap water, at the cotyledons level, led to a faster consumption of the reserve substances from the epidermal cells and the cotyledonary storage parenchyma. A slowing down of these processes was observed mainly in the *Robinia pseudoacacia L.* cotyledons under red, green and blue light, in their cells persisting a blockage of the vacuolar content with black colored osmiophile substances that become strongly electrondense and generating corpuscle that are characteristic for the liquid crystall type formations of phospholipidic nature. The white light has also provided the highest level of green and carotenoid pigments in the „above the ground" part of the plantlets. The other wavelenghts have inhibited the formation and accumulation of assimilating pigments in the samples from both septic and aseptic culture regime.

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