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Effect of light quality on *in vitro* germination, seedling growth and photosynthetic pigments production in wheat (*Triticum aestivum* L.)

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Due to its economic importance, wheat (*Triticum aestivum* L.) has been the subject of most breeding studies, most of which having as a starting point the *in vitro* cultures required for initiating different cell and tissues cultures such as protoplasts, ovules and pollen cells. All these procedures are used to obtain new varieties or hybrids regarding the improvement of different qualities such as higher productivity, more resistant towards pests and diseases, and to handle different environmental stress factors. This study presents specific results in attempt to optimize the efficient initiation and growth of wheat plantlets into *in vitro* cultures, by analysing the effects of different light wavelengths for the growing phase. The results showed that the vegetation stage of *in vitro* plantlets is highly influenced by the light wavelength, which may either stimulate a considered normal growth (in white artificial light) or contrary to decrease it and being relevant for maintaining wheat gene banks as *in vitro* culture for a long time (in red artificial light). The natural light may support the initiation of callus generation and protoplast cultures. The efficient use of light may further contribute to the cost efficiency of the process, ensuring the reduction of the carbon footprint of biotechnological protocols.

Key words: *In vitro*, *Triticum aestivum*, light, amelioration, seedlings, germination.

INTRODUCTION

Wheat is one of the main raw materials for the production of a wide range of food products, bioethanol, etc. Grains and products have long shelf life, and can be transported for a long time and distances without affecting their quality. This plant is one of the most important cereal crops and the most cultivated for the entire quantity of

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food required by humans and animals (Oyewole, 2016). Recently, Egyptian researchers have conducted studies to increase wheat productivity in order to minimize the gap between production and consumption by increasing the cultivated area (Rady et al., 2019). According to FAO data, the globally harvested wheat area has decreased by more than 7 million hectares (3.66%) less in the last 10 years, and wheat production during same period increased by more than 53 million tons (7.32%) (FAO, 2020). This increase is primarily due to the progressively productive varieties improved in specialized laboratories, and the reduction of the harvested area is a method to improve agricultural practices and decrease the carbon footprint. A growing interest in obtaining progressive productive varieties and hybrids can be noticed especially due to climate change effects on food security at the global level. This political interest at the global level is substantiated by the continuous growth of the world's population and on the other hand due to the adoption into agricultural practices of environmental protecting rules. Under such circumstances, each country as a Party to the Plant Treaty needs to address national surveys on all plant genetic resources for food and agriculture (PEGRFA) and, prioritizing their efforts in the quest to fight for food security and allow full access to genetic resources (Antofie, 2011). An example include the wheat case in Romania (Antofie and Sava, 2018).

Among the physical factors, the light studies on wheat morphogenesis was considered as extremely important. Thus, the efforts of researchers in the field of photomorphogenesis are aimed at the production of plants under artificial lighting, upon understanding the entire process of development. Under these circumstances, a complex knowledge approach to gain access to agricultural, biology and physiology features of the species development as well as considering the deep molecular mechanisms for responding to different environmental factors, has been applied to improve wheat genetic diversity and particularly to understand the growth development of wheat depending on lighting conditions. It has been proven that the blue light reduces cell expansion and consequently it inhibits leaf growth and stem elongation (Goto, 2003). Scientists have discovered the effects of red and blue light on the acquisition of the leaf shape, plant development and the accumulation of high levels of antioxidants such as phenolic compounds. Thus, red light stimulated the growth of the lettuce plant, and blue light was effective for the accumulation of phenolic compounds (American Society for Horticultural Science, 2013).

Thus, low intensity or lack of light of a certain wavelength negatively influences crops morphogenesis. Red and blue optical spectra with wavelengths between 640 and 660 nm and between 430 and 460 nm, were most effective for plants growth (Tertyshnaya and Levina, 2016). Light emitting diode known today as LED, accelerates growth and physiological processes in plants, in addition to reducing lighting costs. In the propagation phase, the red light stimulated the elongation of the shoots in *Vanilla planifolia* Andrews, as well as the synthesis of chlorophyll. During the rooting phase, the blue light stimulated rooting (that is, the number of developed roots) as well as leaf development (that is, the number of leaves). The treatments with different LEDs quality of light, increased the synthesis of photosynthetic pigments. The integration of these results into *in vitro* protocols may further contribute to the improvement of the micropropagation process of any species (Ramirez-Mosqueda et al., 2017).

Studies on the effect induced by different light intensities on germination and *in vitro* growth in wheat have been successfully conducted (Yao et al., 2017), but information on the effect of light with different wavelengths on this plant species *in vitro*, are very few. The high light intensity determined a high photosynthetic activity, increased biomass production and development efficiency. At lower light intensities and at blue and red light, separately, the results were antagonistic, offering an alternative for maintaining *in vitro* plants for longer period of time. A high level of blue light prolonged the juvenile phase. Both blue and red light altered the starch and protein contents into plantlets. Lights with different wavelengths are effective for experimental wheat cultivation and can be used to optimize growing conditions and manipulate metabolism, productivity and quality (Monostori et al., 2018).

Knowing the importance of *in vitro* cultures as a precursory phase to today plants breeding, as well as the fact that not all types of vegetative tissues are suitable for the initiation of cells and callus cultures, the purpose of our studies was to improve the efficiency of initiating and cultivating wheat into *in vitro* conditions. To this end, the analysis is on the effects induced by the light with different wavelengths, on the germination and growth of *in vitro* cultures of wheat (*Triticum aestivum* L). The objectives of the study was to find out which type of light stimulates germination, seedlings growth and development of wheat plantlets, to achieve as soon as possible, an *in vitro* plantlets collection, to be accessed for further biotechnological procedures (such as callusogenesis, protoplasts cultivation, and micropropagation). Using different lighting techniques proposed in this study, it is possible to shorten the time needed to maintain *in vitro* plantlets, to acquire performance and cost efficiency of the process in terms of energy consumption also. As a consequence, the study also tries to integrate into old biotechnological protocols, new approaches related to energy saving, lowering the carbon foot print and limiting environmental pollution.

MATERIALS AND METHODS

The plant material consisted of caryopses of *T. aestivum* L. varie

'CCB INGENIO C1', provided from commercial market. This variety was chosen after the analysis of germination capacity (GC) parameter (the percentage of seeds that would normally germinate under optimal conditions for the species). It was determined that the value of GC was 96% in 15 days, considering it is suitable for the use of the caryopse lot from which it came, for the initiation of *in vitro* culture.

Sterilization

The wheat caryopses was sterilized with 5% sodium hypochlorite solution of Domestos (commercial bleach).

Culture medium

The culture medium used was the basic Murashige-Skoog liquid (1962) (MS62) (Murashige and Skoog, 1962), modified, hormone free, pH =5.6.

Cultivation technique

Technique applied was "Blidar" type filter paper bridges (Blidar, 2004, 2014), which allows caryopses a better access to nutrients from liquid culture media for 21 days.

Growing conditions

The growth room provided low humidity (under 20%), a temperature of 26°C during the day and 25°C during the night with a photoperiodicity of 16 h at day and 8 h at night, under continuous ventilation.

Experimental procedure

In each culture, a single caryopsis on the 'Blidar' filter bridge was introduced to each vessel; after that, the culture vessels formed four experimental groups, dependent on the light conditions as follows: V_0 – White fluorescent light (λ = 400-700 nm); V_R – Red fluorescent light (λ = 610-700 nm); V_B – Blue fluorescent light (λ = 450-500 nm); V_N – Natural light. Each experimental lot consisted of 150 culture vessels.

Measurements, observations, and data analysis

During the whole experiment (of 21 days), measurements at 7, 14 and 21 days were performed, taking into account several morphological and physiological aspects. The following parameters were investigated during this experiment: root length (mm), number of roots, coleoptile length (mm), true leaf length (mm) and quantity of pigments at leaf level (µg pigment/g of sample sp): chlorophyll *a*, chlorophyll *b* and carotenoids (carotene, xanthophyll). The amount of pigments with photosynthesis role, accumulated at foliar level, was measured using a spectrophotometer (PG Instruments model T80+), according to the Moran (1982), the calculation formulas being those of Wellburn (1994).

Statistical analysis

V_0 was considered as the control group and the recorded values were taken as references for other experimental variants. All statistical analyses were made using Microsoft Excel; values are significantly different at $P<0.05$ according to the Student's t-test. The experiment was repeated three times, in the same conditions, using caryopsis from the same genotype.

RESULTS AND DISCUSSION

At day 7, after starting the experiment, the seed group enlightened with natural light (V_N) showed the highest values of all the following parameters: roots, coleoptiles and leaves being the best developed compared to all other experimental variants and compared to the control group (V_0) (Figure 1A and Table 1). On the contrary, the weakest results were recorded for the plants enlightened with blue light, except for the length of the leaves, the smallest growth increases were marked on red light, the difference being statistically significant. It is more proven that the germination of some seeds is influenced by light quality. The quality of the light influences the wheat growth rate even under continuous light at a constant temperature. In addition, the red light can play an important role in regulating the rate of wheat development, independent of the photoperiod (Kasajima et al., 2007). The blue light at the end of the visible light spectrum is active in promoting germination. Light-sensitive seeds will become ready for germination in any light or dark conditions, other conditions being appropriate, and the function of light seems to be only that of the final stimulus, either activating or inhibiting. The red region (= 650 nm) of the spectrum is most effective in stimulating the germination of *Lactuca* seeds, and the dark red radiation in the 730 nm region inhibits germination (Flint and Mcalister, 1935). The smallest increase was recorded for the leaf length parameter in the variant enlightened with red light (V_R) which showed an unfavourable difference of 76.82%, compared to V_0 (Table 1).

According to specialized studies, it is known that of the entire package of pigments, chlorophylls *a* are the most important pigments due to the fact that without them the photosynthetic process cannot take place (Sumanta et al., 2014). In the event of acclimation of *in vitro* plantlets, their presence in thier highest amount has a beneficial effect on the easier transition, from heterotrophic to autotrophic nutrition. For this reason, the amount of pigments with a role in photosynthesis has been studied and analysed.

According to the results on the first date of observations (7 days), of all the types of lights used, in absolute values, the blue light (V_B) most effectively stimulated the production of pigments; compared to control V_0, the chlorophyll *b* content was higher by 4.01% and the carotenoids by 22.69% (Figure 2A). This process can be explained by the fact that blue light, having a shorter wavelength and a higher frequency, was more easily absorbed by plants, thus intensifying the synthetic process of these pigments, even if the nutrition of *in vitro* plantlets was heterotrophic (Lesar et al., 2012). It should

Figure 1. General image of *in vitro* seedlings of *Triticum aestivum* enlightened under different lighting conditions, where: V_0 - white fluorescent light; V_R - red fluorescent light; V_B - blue fluorescent light and V_N - natural light, at 7 days (A) and 21 days (B) from the moment of initiating the experiments.

be noted that for all 3 variants, V_R, V_B, V_N, chlorophyll *a* recorded a lower value compared to the control group. The lowest values were highlighted in the version enlightened with natural light (V_N), where the pigments had lower increases than the control.

From a morphological point of view, artificial white light (V_0) and natural light (V_N) led to rapid growth of wheat seedlings, in some cases exceeding the available space of the used vials (Figure 1A). The group enlightened with natural light (V_N) due to the lack of differentiation may be optimal for the initiation of callus, cells, or protoplast cultures. Callus is an unorganized tissue or mass of cells, proliferated by undifferentiated cells (Suffness, 1995). Thus, young, and undifferentiated plants can be initiated from callus and/or cell cultures or protoplasts. At the end of 7 days, the highest reactivity was recorded for seedlings enlightened by the natural variant (V_N).

In the case of 14 days of observations, oscillations were noticed. The highest values being recorded both for the control variant (V_0) and the variant enlightened with fluorescent red light (V_R), depending on the analysed biometric parameter. Thus, the best overall results were recorded in the variant enlightened with white, fluorescent light (V_0) because it recorded the highest values of the roots and leave length (Table 1). The weakest results of the analysed parameters were highlighted in blue, fluorescent light, the difference being significant. As it is known, the coleoptiles of monocotyledons are sheath-shaped leaves whose role is to protect the embryonic bud. This leaf is pierced by the embryonic bud, and the moment it ceases to play its role, its growth is either inhibited or stopped, so that in a few days it enters senescence, and then into necrosis (Davet, 2004). The lowest increase was recorded for the length parameter of the leaf enlightened with blue light (V_B) with a very significant statistical value of -69.96%, compared to V_0 (Table 1). For wheat, the optimal conditions can be defined as hydration in the dark, conditions that wheat seeds experience in the field during sowing. The degree of inhibition of germination by blue light was dependent on light intensity (Jacobsen et al., 2013).

At day 14, there is an increase in the amount of analysed pigments, blue light (V_B) being the most effective, the accumulation increases compared to the control being 109.02% for chlorophyll *a*, 107.84% for chlorophyll *b* and 136.8% for carotenoids (Figure 2B).

Table 1. Statistical data on the average number of roots (A), root length (B), coleoptile length (C) and leaf length (D) in *Triticum aestivum* seedlings enlightened with V_0 - white fluorescent light; V_R - red fluorescent light; V_B - blue fluorescent light and V_N - natural light, during the 21 days of vitro culture.

Parameter	Days	Control group	Statistic data	Experimental variants	Statistic data		Statistical significance
			X ± Sx		X ± Sx	p (testul *t*)	
				V_R	4.083 ± 1.78	0.044	**
	7		5.5 ± 1.45	V_B	4 ± 1.41	0.020	**
				V_N	6.73 ± 1.19	0.037	**
				V_R	5.3 ± 1.42	0.438	Ns
A. Roots no./plant	14	V_0	4.8 ± 1.398	V_B	2.875 ± 0.99	0.0036	***
				V_N	6.1 ± 1.29	0.044	**
				V_R	4.5 ± 0.76	0.777	Ns
	21		4.6 ± 0.699	V_B	3.5 ± 0.93	0.0157	**
				V_N	5.8 ± 0.92	0.0044	***
				V_R	13.25 ± 4,14	0.00209	***
	7		20.083 ± 5.32	V_B	8.091 ± 4.61	0.00001	***
				V_N	25.73 ± 6.13	0.029	**
				V_R	21.4 ± 4.74	0.330	Ns
B. Roots length (mm)	14	V_0	23.7 ± 5.498	V_B	10.375 ± 5.18	0.00008	***
				V_N	19.1 ± 3.21	0.0379	**
				V_R	16.25 ± 5.365	0.188	Ns
	21		20.3 ± 7.13	V_B	9.75 ± 5.599	0.0029	***
				V_N	20 ± 4.37	0.911	Ns
				V_R	10.17 ± 3.93	0.00014	***
	7		17.58 ± 3.99	V_B	9.36 ± 3.38	0.00003	***
				V_N	38 ± 5.69	0	***
				V_R	14.4 ± 2.72	0.0058	***
C. Coleoptile length (mm)	14	V_0	18.4 ± 2.99	V_B	8.875 ± 2.47	0	***
				V_N	33.2 ± 7.81	0.00013	***
				V_R	12.375 ± 2.92	0.0029	***
	21		18.7 ± 4.62	V_B	14.75 ± 7.34	0.211	Ns
				V_N	30 ± 3.89	0.00002	***
				V_R	12.42 ± 8.31	0.0001	***
	7		53.58 ± 24.93	V_B	14.18 ± 8.4	0.00016	***
				V_N	72.36 ± 15.77	0.042	**
				V_R	47.3 ± 24.47	0.0919	*
D. Leaf length (mm)	14	V_0	69.5 ± 30.78	V_B	20.87 ± 11.06	0.0006	***
				V_N	58.4 ± 35.32	0.464	Ns
				V_R	43.25 ± 16.11	0.829	Ns
	21		45.3 ± 23.45	V_B	21.75 ± 12.98	0.0168	**
				V_N	71 ± 22.326	0.022	**

X ± Sx [mean ± standard deviation]; depending on the value of *p* (significance of the difference compared to the control group): ns – not-significant difference (p> 0.1); *Weakly significant difference (0.05≤p <0.1); **Significant difference (0.001≤p <0.05); ***Very significant difference (p <0.01).

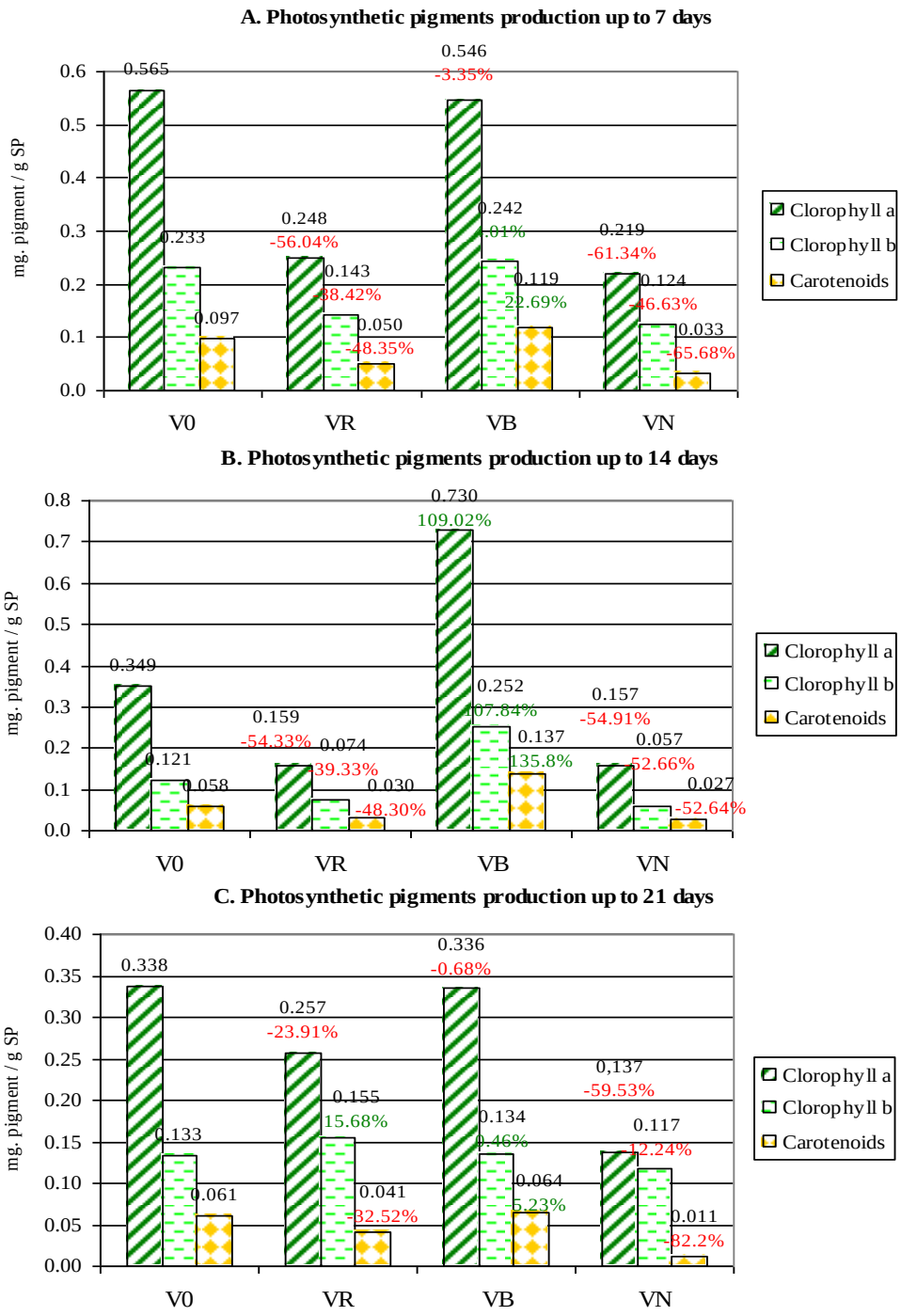

Figure 2. A general comparison of the amounts of chlorophyll pigments in the leaves of *Triticum aestivum* seedlings at 7 (A), 14 (B) and 21 days (C) from the initiation of *in vitro* cultures.

The variants enlightened with natural light (V_N) and red light (V_R) showed negative increases similar to the control variant (V_0), both on the first date of the observations and on the second. In the latter case, the differences compared to the values of the V_B group being highly significant.

On this experimental date, the best overall results were recorded in the variant enlightened with white artificial light (V_0) because it recorded the highest values of root and leaf length, which is effective for possible acclimation procedures. It should be noted that among the experimental variants enlightened with coloured light, red

light induced the largest increase in the range of 7 to 14 days, but in absolute terms, the recorded data remained lower than natural light. Blue light did not play such an important role in growing but is useful for storing *in vitro* plantlets for a long time in the containers.

On the 21st days of *in vitro* culture, the highest values for parameters number of roots, coleoptile length and leaf length were recorded in the variant enlightened with natural light (V_N), where the highest increase was 60.43% for the length of the coleoptile, statistically significant difference compared to the control group (Table 1). Similar to the results obtained at 14 days, the lowest values for almost all parameters, namely, number of roots, root length and leaf length were for the variant enlightened with blue light (V_B) (Figure 1 B). The lowest increase was -59.99% for leaf length.

The concept of using sunlight for the micropropagation systems is suggested as a way to reduce tissue culture costs. Significantly, more shoots were produced by the *Musa acuminata* seedlings grown in a sunlit room during summer, with photosynthetic photon flux densities (PPFD) ranging up to 570 µmol m^{-2} s^{-1}, at temperatures of 23 to 30°C and photoperiods of 12 to 16 h, than seedlings under artificial light in a growing room which ensures controlled conditions of a constant PPFD of 65 µmol $m^{-2}.s^{-1}$, at temperatures between 23 and 29°C and 16 h photoperiod (Kodym and Zapata-Arias, 1999).

It is well known that the energy of photons is inversely proportional to the wavelength of light radiation, so that the photons of blue radiation have a higher energy compared to those of red radiation and determine the achievement of a photosynthetic maximum. Red radiation is the complementary colour for chlorophyll green pigments, as the amounts of red radiation that have the lowest mass are better absorbed by the chlorophyll molecules of most plant species (Inada, 1976). By knowing these physiological characteristics, it was expected that the blue light (V_B) would lead to the accumulation of a lower amount of chlorophyll *a*, a phenomenon was found at day 7. A similar situation was identified for the amount of chlorophyll *b* in the control variant and in the blue light, the amount of pigment being approximately 0.134 g. The amount of carotenoids in variant V_B showed a positive increase of 5.23% compared to V_0 (Figure 2C). For the 3 data of observations, at 7, 14, 21 days, natural light (V_N) led to negative accumulation increase in all pigments compared to the values recorded in the control group, on the last experimental date, by 59.53% for chlorophyll *a*, 12.24% for chlorophyll *b* and 82.2% for carotenoids. Due to the fact that overall, the highest quantities of assimilating pigments (chlorophylls and carotenoids) accumulated in the leaves were recorded in seedlings exposed to blue artificial light, it is recommended to use it in the processes of acclimation to the septic environment, as these seedlings may have the greatest adaptability to the change of nutrition from the heterotrophic to the autotrophic type during the acclimation process.

On the last date of the observations, the biometric results showed that the seedlings enlightened with natural light (V_N) had the most numerous roots, the highest number of leaves and coleoptiles. In case it is desired to initiate wheat root crops, along with phytohormones stimulating rhizogenesis, a lighting can be used to increase the stimulation of root proliferation and growth, natural light. The variant enlightened with white artificial light stimulated each experimental date, both the growth and development of the seedlings. Red artificial light (V_R) and blue artificial light (V_B) can be used to maintain these *in vitro* seedlings for a long time *in vitro*. Red light has also proven effective on protoplasts from wheat leaves (*T. aestivum*), as they respond to short irradiation of red light by increasing their number (Fallon et al., 1993).

Similar results on leaf growth, chlorophyll content and root branching were obtained when using LED light. In *Cymbidium*, red light promoted leaf growth, but decreased chlorophyll content. It was reversed by the blue light; root branching was comparable under red plus blue LEDs and in fluorescent lighting systems (Tanaka et al., 1997). The morphometric analysis of the palisade cell micrographs in *in vitro* cultures of *Betula pendula* showed that the area of the functional chloroplast was the largest in the chloroplasts of leaves exposed to blue light and the smallest in those exposed to red light (Saebø et al., 1995), which confirms that vitroplants grown in blue light are more easily adaptable to acclimation.

Conclusion

Overall, the most effective variant on the proliferation and root growth of the coleoptile and leaf in wheat seedlings, also on the quantities of pigments accumulated into the leaves, was represented by the use of white artificial light. For wheat seedlings germinated under these conditions, the high degree of growth and branching recommends the use of white light, mainly for *in vitro* subcultures or for micropropagation, and secondarily in the processes of acclimation to the living septic environment and hydric stress; the seedlings being kept for a short period of time *in vitro*, and the optimal time for mending or acclimation was 14 days.

When the red light was used, two advantages were noted, namely: (1) the possibility of keeping wheat into *in vitro* culture for a longer period of time, as the seedlings did not show any sign of senescence and/or necrosis and (2) economic advantage, because this technique does not require the use of growth inhibitory hormones (that is, abscisic acid) to slow the growth rate of *in vitro* plantlets, while maintaining vitality and thus the proliferative and growth properties.

When the blue light was used, wheat seedlings were obtained with the highest degree of accumulation of

photosynthesizing pigments, which favours the transition from *in vitro* to natural conditions (*in situ*), respectively for the transition from heterotrophic to autotrophic nutrition. From this point of view, it is recommended to use blue light, but not after the 14th day of vitro culture, when the maximum accumulation of pigments with a role in photosynthesis is reached.

Maintaining wheat *in vitro* culture for up to 14 days under natural light has proven to be successful in terms of growing in the direction of obtaining a source of quality germplasm to establish cultures of callus, cells in suspension or protoplasts. Also, natural light most strongly stimulates root and cauline proliferation and growth, but newly developed organs have a lower degree of differentiation than other working variants. The benefits of using this type of light are of economic nature as crops do not require artificial lighting, with a significant reduction in energy consumption.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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