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GROWTH IN LENGTH OF THE EMBRYONIC RADICLES OF SWEET SORGHUM PLANTLETS (SORGHUM BICOLOR SUBSP. BICOLOR), RESULTED FROM CARYOPSIS GERMINATION ON PAPER FILTER SUBSTRATE, EXPOSED TO DIFFERENT TYPES OF LIGHT

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ABSTRACT. In this study, we used sorghum caryopsis (*Sorghum bicolor* subsp. *bicolor*), to investigate the radicle system formation developming of plantlets, as their germination and growth occurred on filter paper, iluminated with natural, or different colors of fluorescent light. Sorghum plantlets, generated from zygotic embryos, in the first 10 days of germination, can be used as experimental models in a series of studies regarding the plant physiology. The development of embryonic radicles exposed to natural light, continuous darkness or in fluorescent light - different colors - different wavelengths, mark an increase of radicles length, which is dependent on the applied light regime. The most significant increase of radicles length had occurred in samples kept in continuous darkness, or illuminated 16h/day with green or blue fluorescent light. Percentage values recoreded for this parameter (compared to white fluorescent light samples, considered as reference lot) are higher with 75, respectively 45%, exceeding the average increase of embryonic radicles in samples exposed for 10 days to continuous darkness. Obviously, radices dry weight biomass of these variants register an increase, but only by 27-28%.

Keywords: *Sorghum,* germination, radilcles, lenght, light

INTRODUCTION

Considering that sweet sorghum (*Sorghum bicolor* subsp. *Bicolor*) is a very valuable plant for farming, in arid and semiarid lands from the tropic areas, more than half of the sorghum crop in the world is cultivated in underdeveloped regions of India and Africa (*Mehmood* et al., 2008; *Iqbal*, et. al., 2010). This plant is well adapted to drought conditions, because this kind of plants species presents a highly developed radicle system. Such a radicle system allows plants to absorb water and mineral salts from relatively large soil surfaces and at considerable depths, which represents an advantage especially when environmental conditions are unfavorable (*Robinson*, 1994, *Lynch* 1995, *Lynch* and *Brown*, 2001).

Development of sorghum radicle system was examined, in recent years (*Singh* et al., 2008, 2010, 2012), right from the first two days of germination, it's architecture dependent finally on the origin of roots and their emergence point, from the moment in which they are formed at embryonic radicle or coleoptile levels.

In order to analyze organs reaction to osmotic stress *Rajendran* et *al*. (2011) have used sorghum plantlets, derived from 19 genotypes, investigating caryopsis germination percentage, radicle, coleptile and the stem lenght. The method was used to select genotypes that might be more tolerant to different type of stress, like humidity or drought, extrapolating the obtained responses to understand the mechanisms involved in acquiring such tolerances.

Similar to our research, experiments made by *Rajendran* (2011) where sorghum caryopsis were put to germinate on filter paper, moistened with solutions of polyethylene glycol (PEG) of various concentrations, were plantlets development, from embryos, was monitored for 10 days.

The goal of our research was to study germination and growth of sorghum plantlets, that have been kept in different lighting regimes, under natural light or white light, emitted by fluorescent tubes, or which were iluminated with different wavelengths, of light produced by neon tubes.

MATERIALS AND METHODS

Our research was based on nine experimental variants (Table 1). The experiments were performed in summer (June-August). First experimental variant consisted of caryopsis germinated in natural light, 16 hours per day (variant V_0 - control group 1), at a window with northern exposition, then these samples were covered and were uncovered in the following morning.

Another variant (V_{00}) consisted of sorghum cariopse exposed during the whole experimental period- to continuous darkness, opposed to this, we mounted

a variant to which germination and plantlets growth generated from embryos was held to continuous white fluorescent light (V_{0A1} - variant) or 8h light/24h - to V_{0A} variant. In pararell there were also variants exposed to 16h\24h - fluorescent light, of different wavelengths like red (V_2), yellow (V_3), green (V_4) or blue (V_5) (Table 1). Light intensity at germination surface was 1300 lux, regardless of the wavelength used. Within the growth chambers where the experiments were conducted the temperature was kept constant at 23 ° C.

Table 1

The experiments organization scheme

EXP. VAR.		ILLUMINATION
EXP. 1	V_0	***- 16h/24h natural light - Control 1 -***
	V_{00}	- continuous darkness
	V_{0A1}	- 24h continuous white fluorescent light
	V_{0A}	- 8h/24h white fluorescent light
EXP. 2	V_1	***- 16h/24h white fluorescent light - Control 2-***
	V_2	- 16h/24h red fluorescent light: 610-780 nm
	V_3	- 16h/24h yellow fluorescent light: 575-590 nm
	V_4	- 16h/24h green fluorescent light: 510-560 nm
	V_5	- 16h/24h blue fluorescent light: 452-470 nm

Experiments from this paper consisted in observing the germination of sweet sorghum (*Sorghum bicolor* subsp. *bicolor*) caryopsis and the plantlets growth. The grains were put on filter paper soaked with bidistilled water, the paper was horizontally positioned in transparent, and colorless containers, with the size of 30 cm long, 15 cm wide and 10 cm high, covered with plastic lids. In the basal part of the containers were the filter paper was applied, are grooves that preserving permanent water reserve, media that ensure optimal humidification which prevented the water excees and the installation of anaerobiosis within caryopsis embryo.

Parameters monitored:

- *caryopsis germination* percentage at 24 hours and at 48 hours;
- *length of the embryonic radicle*, by measurements made in the 2nd, 4th and 10th day from caryopsis germination;
- *total dry mass* of root mass/plantlet, determined in the 10th day of germination.

The method for determination of radicles dry mass was made according to AOAC 925.23 standard, and it consists in samples of plant material dried at 105 º C, at least for three days, until the dry mass obtained was constant in weight. In a weighing bottle with cap are put 3g of freshly harvested plant material, then, the jar container was placed in oven to dry the plant material. After 3 days, the vials were closed with lids and were removed from the oven and put in a desiccator. After cooling, samples were re-weighed and the value obtained was introduced in the following formula:

$$DW\ \% = \frac{a \cdot 100}{b}$$

a - weight of sample before drying;
b - weight of the dry sample.

The entire experiment was conducted in three replicates.

For each experiment, the results were statistically processed using the STATISTICA Release 7 program as follows:

· Standard deviation of parameter values within each experimental variants;

· Variance in the string, present in the experimental groups;

· Statistical significance threshold was determined by the T test.

Evaluation of caryopsis germination percentage - at 24 and 48 hours after germination - was performed according to "International Seed Testing Association" (ISTA, 2006), 100 caryopsis were used for each variant.

Data concerning the number of normal caryopsis germinated from each experimental variant was expressed in percentage (%). The biometric results obtained were reported to the control group 1, reprezented by V_0 - caryopsis germinated in natural light - or to the average of biometric measurements recorded in plantlets derived from germinated caryopsis, iluminated with white fluorescent light (variant V_1) (control group in experiment 2) in regime of 16/24h, were considered as reference, 100% value.

The main aspects resulted from our experiments are presented in table 2 and in the images from figures 1 to 3.

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 22, issue 3, 2012, pp. 323-329

RESULTS AND DISCUSSIONS

Sorghum caryopsis germination under different lighting conditions

Analyzing, the data presented in table 2 shows that after 24 h from experiment initiation, that 100 -sorghum caryopsis put to germination/experimental variant, shows a variable reaction; depending on lighting conditions on which grains were subject.

At 24 h after sorghum caryopsis germination, as shown in table 2, the highest percentage of germination is 88%, was recorded in samples exposed to blue light (variant V_5) emitted by fluorescent tubes under a regime of 16h light /24h iluminaíon period.

Table 2

Sorghum (Sorghum bicolor subsp. Bicolor) caryopsis germination percentage, compared with 100 caryopsis per experimental variant at 24 and 48 hours after germination,

EXPERIMENTAL VARIANTS	PERCENTAGE OF GERMINATION	
	at 24 h	at 48 h
EXPERIMENT- 1		
V_0 - *16h/24h natural light- Control 1-*	76	92
V_{00} - continuous darkness	76	**96**
V_{0A1} - 24h continuous white fluorescent light	**80**	88
V_{0A} - 8h/24h white fluorescent light	68	**96**
EXPERIMENT- 2		
V_1 - 16h/24h white fluorescent light- Control 2	76	92
V_2 - 16h/24h red fluorescent light	80	96
V_3 - 16h/24h yellow fluorescent light	84	92
V_4 - 16h/24h green fluorescent light	76	**100**
V_5 - 16h/24h blue fluorescent light	**88**	96

As concerning the percentage of germination recorded at 48h after germination, we observed that at the variant V_4, caryopsis illuminated with fluorescent green light (Table 2), 100% germination was obtained, exceding with 4% the parameter values registred at samples illuminated with blue light (V_5).

After 48 hours from germination, samples illuminated with continuous white fluorescent light (V_{0A1} variant), have shown an inhibition by 12% of germination. At 16 hours lighting/24h (V_1) values of this parameter decreased by 8% at samples illuminated with natural light as well at the variants iluminated with fluorescent white or yellow light (V_3).

Only red (V_2) and blue fluorescent light (V_5), under a regime of 16 hours lighting/24h, or white fluorescent light (V_{0A}) functional for 8 hours/24h, or continuous darkness (V_{00}) induced a 4% inhibition of caryopsis germination.

We concluded that the continuous white fluorescent light (V_{0A1}) had an inhibitory effect on germination at 24 hours by 20%, respectively by 12% at 48 hours after germination. Green light (510-560 nm) (V_4 variant), under regime of 16 lighting hours/24h has stimulated caryopsis germination to 100% after 48 hours.

Growth in length of the embryonic radilcles of sorghum plantlets, in the first 10 days of germination.

Figure, 1A and 1B are showing, as histograms, with the percentage values, resulting from biometry made in the 2nd, 4th and 10th day of germination, in terms of growth in length of embryonic radicels. The images from figure 2, are showing relevant aspects of sorghum plantlets, captured in different lighting conditions, during the experiments, attention being given to the growth of embryonic radicles.

As shown in figures 1 and 2, elongation of embyonic radicles was strongly influenced by lighting regime in which germination and plantlets growth took place, both the 2nd day (48 hours) from putting caryopsis to germination and in the 4th and 10th day of germination.

In the case of the first experiment, percentage values made were reported to averages of biometric growth in length of the embryonic radicles, measurements considered 100% to the variant (variant V_0) at sorghum grain germinated and plantlets grown on filter paper, humidified with bidistilled water, the samples were maintained throughout the experiment under natural light (Exp. 1).

In the second experiment, the control variant - was V_1, in which samples were illuminated with white fluorescent tubes, for a photoperiod of 16h light/day as in samples exposed 24h to natural light (V_0). Experimental variant V_1 was plotted as a histogram in the first experiment, as in the second; results were compared with those obtained for the other experimental variants (V_{00} and V_2 - V_5).

As compared with the natural light (variant V_0), to which caryopsis were exposed during their germination,

size of sorghum embryonic radicles was considered 100% for control variant (Fig. 1A).The measurments made in the 2nd day of caryopsis germination revealed that the longest embryonic radicles was marked on sorghum seeds germinated under continuous darkness (V_{00}). In the others exerimental variants (V_{0A1}, V_1 and V_{0A}), moderate increases in length of embryonic radicles were recorded. At 4 days of germination (Fig. 2A), and even after 10 days, the white fluorescent light, regardless of itsduration of action - in relation to continuous dark variant (V_{00}), inhibited radicles growth by up to 32%.

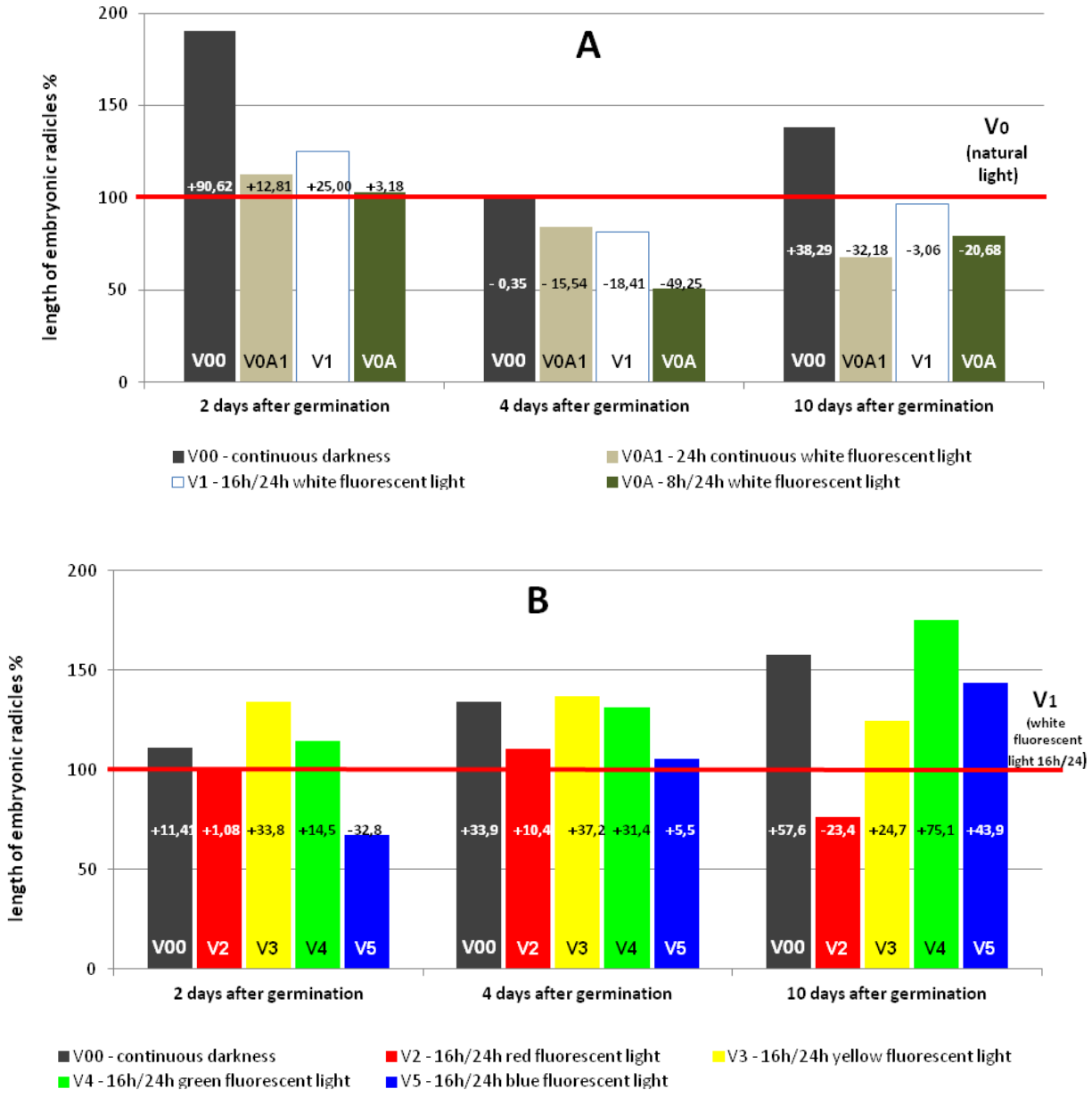


Fig.1 - Lenght increase of the embryonic radicles from sorghum (*Sorghum bicolor* subsp. *bicolor*) plantlets, subjected to the influence of *natural light* or light emitted by fluorescent tubes of *various colors*, grown on filter paper, soaked with bidistilled water. Ilumination regime: **A** - V_0 - *natural light - control group (exp.1);* V_{00} - continuous darkness; V_{0A1} - 24h *white* fluorescent continuous light; V_1 - 16h/24h *white* fluorescent light; V_{0A} - 8h/24h *white* fluorescent light; **B** - V_1 - *16h/24h white fluorescent light - control group (exp. 2);* V_2 - 16h/24h *red* fluorescent light; V_3 - 16h/24h *yellow* fluorescent light; V_4 - 16h/24h *green* fluorescent light; V_5 - 16h/24h *blue* fluorescent light.

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 22, issue 3, 2012, pp. 323-329

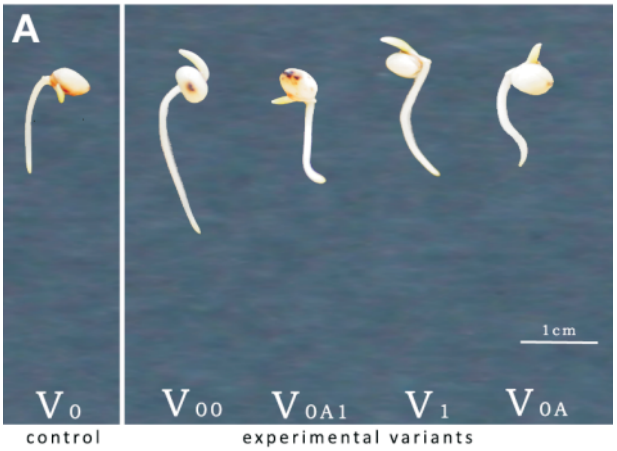
Plantlets after two days of germination - *including sorghum ones* - are undergoing a process of "conversion" from heterotrophic to mixotrophic metabolism, as they are becoming photosynthetisant after the leaflets penetrate the coleoptile (Fig. 1B and Fig. 2C), and the caryopsis endospermum nutrient reserves are about to get exhausted. On the other hand, the young leaflet stomatic apparatus, and probably also the constructive elements of chloroplasts, compared with fully photoautotroph plants, are not yet fully functional.

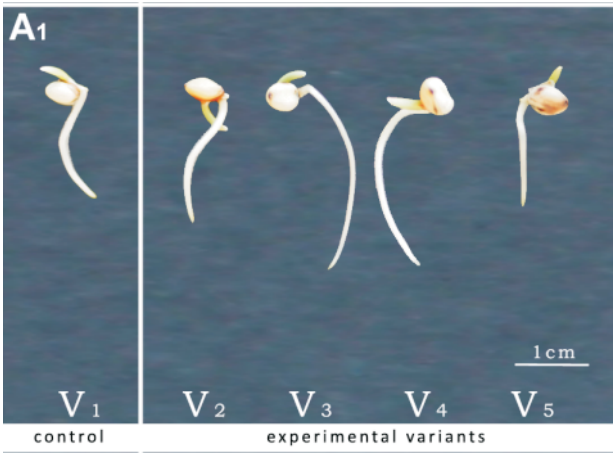
In figure, 1B and $2A_1$ - C_1 can be seen that in the 2nd day of germination in fluorescent yellow light (V_3), growth in length of embryonic radicles was stimulated by 16% and with 37% in the 4th day. After 10 days of germination, when plantlets develop secondary roots, on the embryonic radicles, and adventives roots at the first node wrapped by the coleptile, growth in length of embryonic radicles was stimulated by 18%.

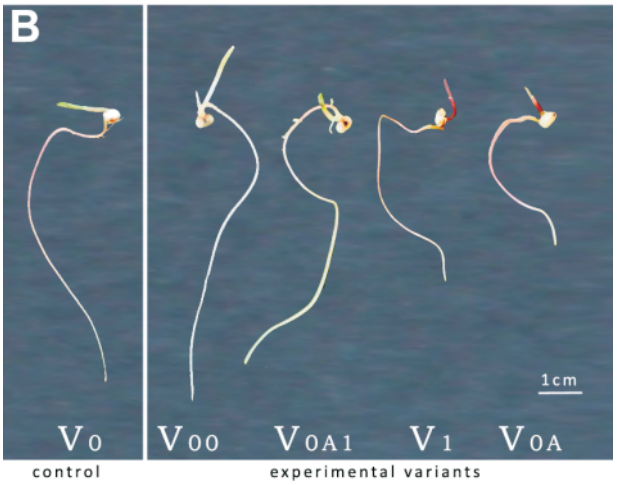
In figure, 1B and $2A_1$ - C_1 can be seen that in the 2nd day of germination in fluorescent yellow light (V_3), growth in length of embryonic radicles was stimulated by 16% and with 37% in the 4th day. After 10 days of germination, when plantlets develop secondary roots, on the embryonic radicles, and adventives roots at the first node wrapped by the coleptile, growth in length of embryonic radicles was stimulated by 18%.

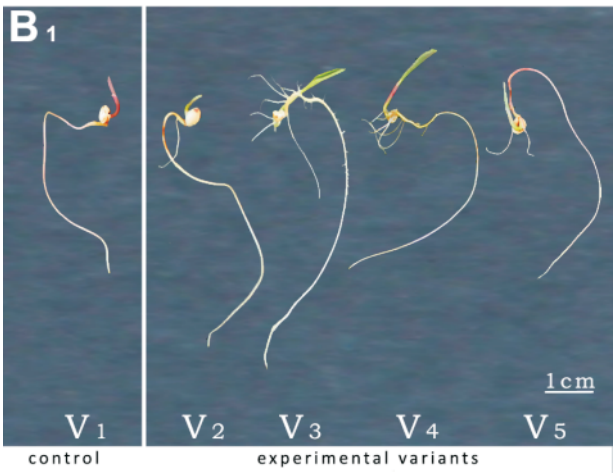
The strongest stimulation of embryonic radicles growth was observed for to samples illuminated with light emitted by green fluorescent tubes (V_4), that increased in the 4th day with 43.8%, and with 87.7% in the 10 th day of germination. In addition, an increase of 43.9% was observed in embryonic radicles and to the root system, in samples illuminated with blue fluorescent light (V_5) in the 10th day of germination, aspects possible to be seen also in figure $2C_1$.

Statistical analysis, clearly show that the nature of light on which sorghum caryopsis and plantlets were exposed, in first 10 days of germination, strongly influenced growth in length, but also the root system formation, by generation of secondary and adventive roots.









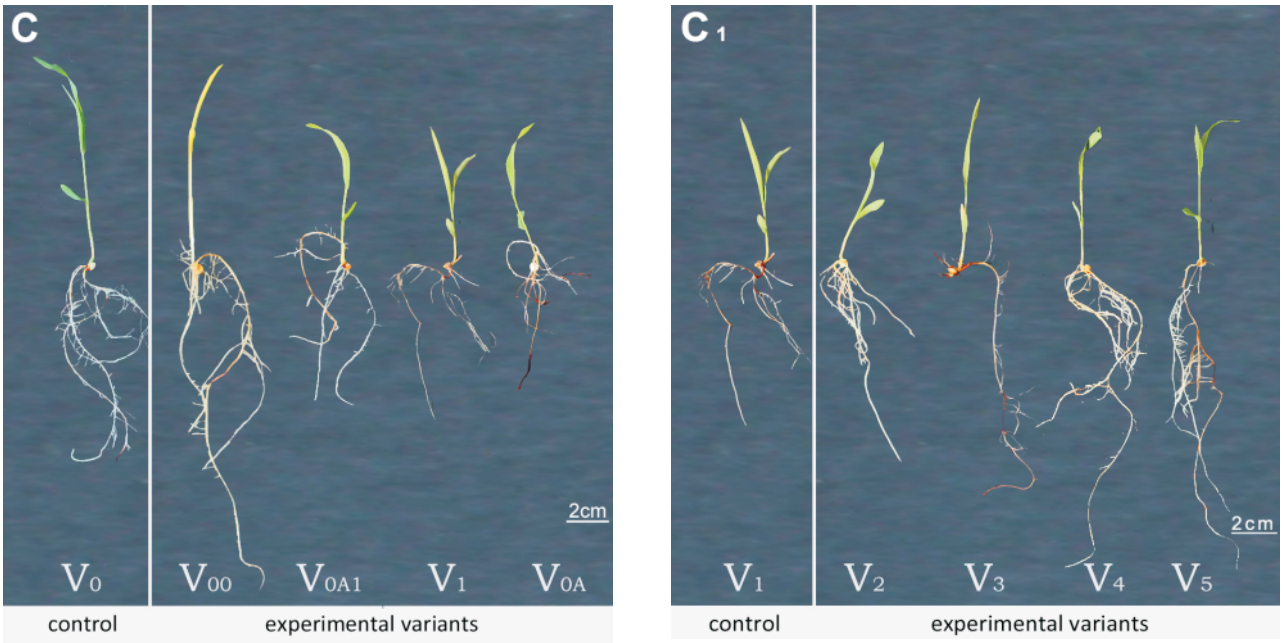


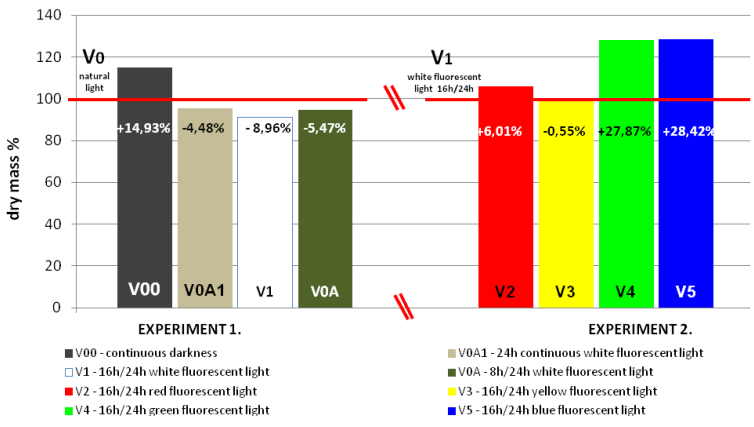
Fig. 2 - Increase in lenght of the sorghum (*Sorghum bicolor* subsp. *biolor*) plantlets: A and A_1 - in the 2nd day of germination, B and B_1 - on the 4th day of germination, and C and C_1 in the 10 th day of germination, grown on filter paper moistened with bidistilled water. **A-C Series**: V_0 - *natural light - control group;* V_{00} - continuous darkness; V_{0A1} - 24h *white* fluorescent continuous light; V_1 - 16h/24h *white* fluorescent light; V_{0A} - 8h/24h *white* fluorescent light; **A_1-C_1 series**: V_1 - *16h/24h white fluorescent light - control group (exp. 2);* V_2 - 16h/24h *red* fluorescent light; V_3 - 16h/24h *yellow* fluorescent light; V_4 - 16h/24h *green* fluorescent light; V_5 - 16h/24h *blue* fluorescent light.

Root dry mass

As shown in the histograms presented in figure 3 (Exp.1) *continuous darkness* (version V_{00}) stimulated the growth of root system, as number of roots and their length, the increase in dry mass of the root level was 14.9%. In contrast, samples exposure to *continuous light* regime (version V_{0A1}) had inhibited with 4.48% dry mass accumulation in radicles biomass and with 8.96% for V_1 variant iluminated 16h/24h with white fluorescent light, used as reference group of in experiment 2.

In the 10th day of germination an increases in total dry root mass by 27-28% was obtained for plant material exposed to 16h *green light* (variant V_4), or *blue light* /24h (variant V_5). Samples illuminated 16h/24h with *red fluorescent light* (variant V_2) were stimulated, but only with 6% in comparison with dry root mass accumulation parameter determined in samples exposed to 16h/24h *white fluorescent light* (variant V_1), considered reference values.

Fig.3 - Root dry mass, generated in sorghum (Sorghum Sorghum bicolor subsp. Bicolor) plantlets, subjected to the influence of *natural light* or light emitted by fluorescent tubes of *various colors*. Caryopsis were germinated on filter paper, soaked with bidistilled water, determinated in day 10 of germination. (Exp.1. - V_0 - *natural light - control group (exp.1);* V_{00} - continuous darkness; V_{0A1} - 24h *white* fluorescent continuous light; V_1 - 16h/24h *white* fluorescent light; V_{0A} - 8h/24h *white* fluorescent light; Exp. 2. - V_1 - *16h/24h white fluorescent light - control group (exp. 2);* V_2 - 16h/24h *red* fluorescent light; V_3 - 16h/24h *yellow* fluorescent light; V_4 - 16h/24h *green* fluorescent light; V_5 - 16h/24h *blue* fluorescent light.



Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 22, issue 3, 2012, pp. 323-329

CONCLUSIONS

Generally in the first 48 hours of germination, the nature of light under which sorghum caryopsis were exposed, did not affect germination. Exception from this behavior was to the variant in which caryopsis were placed under *continuous illumination* with *white* fluorescent ligh. After 48 hours of germination, there was a percentage of 88% germination, compared with the 96% germination, achived in samples exposed to *continuous darkness*, to red and blue light, and of 100% to treatments made by *green light*.

Examination of embryonic radicles growth of sorghum plantlets, compared with samples exposed to *natural light* (reference values considered 100%), showed that *continuous darkness* stimulated their elongation by 90% in the second day of germination, and by 38% in the 10th day of germination. During the period in which sorghum plantlets switch from heterotrophic nutrition regime to photoautotrophic, even in *continuous darkness*, there is a delay in growth of this organ, because only after this threshold, the recovery of the growth happens only from the 10-th day from germination.

Exposing the samples to light emitted by fluorescent tubes of different colors, opposed to the sorghum plantles obtainded by caryopsis germination in fluorescent light, white (16h/day)(growth parameters considered as reference, as 100%), has given the following results: *green light* in the tenth day of germination has stimulated with 75% at the most the radicles growth, and the *blue light* with 43.9%.

A stimulating effect concerning the growth in length of embryonic radicles in samples exposed to *yellow light,* the measurements made in the 2nd, 4th, and 10th day of germination, recorded increases exceeding the percentage value of 24.7%. Instead, in day 10, red lights have inhibited growth of embryonic radicles by 23.4%.

Regarding the root mass, determined in the 10-day of germination, a stimulation by 27-28% to sorghum plantlets germinated and exposed to a lighting system with fluorescent lights, either *green* or *blue*, for 16h/day, increases at this parameter exceedind beyond the results marked at the *continuous dark* samples, and the weight values of root mass was increased by only 14.9%.

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