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The Effect of Light Quality and Intensity on *in vitro* Potato Cultures

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ABSTRACT

Purpose: *The study investigates the effect of light quality and intensity factors on in vitro potato meristem cultures and multiplication for optimization of pre-basic seed potato productions of some potato varieties.*

Research Method: *In vitro experiments were conducted to study the response of meristems and nodal cuttings of five potato varieties, i.e., Cara, Hermes, Lady Rosetta, Santana, and Spunta, to four LED light qualities (blue, red, red+blue, or white) for five potato varities. Also, the response of nodal cuttings was examined under three light intensities (50, 75 and 100 µmol m^{-2} s^{-1}).*

Findings: *Significant differences were obtained between the four tested light qualities. Red LED gave the best meristem survival rates of the Cara, Hermes, Lady Rosetta, and Spunta potato varieties. In the multiplication phase, a significantly ($p \leq 0.05$) higher plantlet length was obtained from nodal cuttings under red light quality. Also, white and red light produced vigorous plantlets, expressed as higher significant dry weight (82.2 and 80.4 mg/plantlet, respectively). Increasing light intensity from 50 to 75 and 100 µmol m^{-2} s^{-1} resulted in increases in leaf number, stem diameter, root length, leaf area, chlorophyll content, fresh weight, and dry weight.*

Originality/ Value: *White LED light quality enhanced in vitro initiation of potato meristems. Furthermore, Light intensity of 75 µmol m^{-2} s^{-1} gave better performance of potato plantlets in vitro.*

Keywords: *in vitro, LED, light intensity, light quality, Potato*

INTRODUCTION

Potato is the fourth food crop in area cultivated after rice, wheat, and maize; potato is considered the largest non-cereal food crop worldwide. Furthermore, for human consumption, potatoes are the world's third most important food crop after wheat and rice. Also, the potato is the world's most important tuber vegetable. In Egypt, in recent years, the growing area cultivated with potatoes reached about 235544 ha in 2020, producing 6.8 million tonnes (FAO, 2022). Potato is also considered one of the most important food crops for local consumption and exportation. However, in recent years, the problem of seed potatoes and their quality have grabbed the attention of the developing governmental and private sectors and focused efforts on the establishment of local seed potato production systems based on tissue culture techniques and minituber production. The production of high-quality and healthy plantlets from the *in vitro* phase is the first step in modern seed potato program (Forbes *et al*., 2020; Oves, 2021).

Light quality as a tool for enhancement of the production of healthy plantlets with good physiological performance has been the focus of

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many tissue culture research studies recently; the emerging results are promising for the utilization and manipulation of light quality for plant production *in vitro* and in greenhouses. The use of Light Emitting Diodes (LEDs) technology in biological studies and horticultural applications has recently been the focus of scientific interest (Nhut and Nam, 2010; Dutta Gupta and Jatothu, 2013); LEDs are cheap, easily available, low heat energy emitting and very efficient. Many studies indicated that LED is better than fluorescent and incandescent lamps as source of lighting in growth chambers (Nhut and Nam, 2010) and plant factories (Goto, 2012). Light quality manipulation using LEDs offers an alternative tool for adjusting plant responses (Hernández and Kubota, 2016; Sabzalian *et al*., 2014; Choi *et al*., 2015; Pattison *et al*., 2018; Xu *et al.,* 2018). Light quality shortens the production cycle of *in vitro* potato plantlets and increases the number of microtubers (Li *et al*., 2020). Although the important role of light quality and intensity on the performance of *in vitro* culture is well established, there is a lack of literature on the optimum light quality and intensity for *in vitro* propagated potato varieties. Furthermore, to our knowledge, no study has manipulated the role of light in the meristem culture phase, which is a critical phase in free virus seed potato production. Nevertheless, there is a need for to determine the optimal light intensity for *in vitro* potato multiplication by stem cuttings. In the current study, the effect of different LED light qualities on potato meristem culture was studied. Also, light qualities and intensities were examined on potato nodal cutting cultures *in vitro* of five potato varieties of major concern under Egyptian conditions.

MATERIALS AND METHODS

This study was performed in a tissue culture laboratory located in the Potato and Vegetatively Propagated Vegetables Research Department, Horticulture Research Institute, Agricultural Research Center, Egypt. The study contained three experiments.

First Experiment

Plant material was taken from the sprouts of seed potato tubers of five imported potato varieties. Meristem cultures were obtained by excision of the sprout apical meristem from sprouts, which were surface sterilized with sodium hypochlorite (1.5 %) and two drops of Tween 20 for 15 minutes, then rinsed with sterile distilled water three times. Under sterile conditions (laminar flow hood) using a stereomicroscope, meristem tips were obtained by removing the outer leaves and leaf primordia of sprouts apical tips, then excising meristem tips with two leaf primordia (0.1 mm width and 0.25 mm length), and after that, inoculated in a culture tube containing MS medium (Murashige and Skoog, 1962) salts and vitamins (Caisson Laboratories Inc. USA) supplemented with 10 mg/l adenine sulphate, 5 mg/l calcium pantothenate, 0.1 mg/l GA_3, 30 g/l sucrose, and 7 g/l agar. The pH was adjusted to 5.7 before autoclaving at 1.45 Kg/cm^2 for 20 min. The first experiment was conducted to study the meristem growth response of five potato varieties (Cara, Hemes, Lady Rosetta, Santana, and Spunta) to four light qualities (white, blue, red, red+ blue). The light quality was from the same source, i.e., a Light Emitting Diode (LED) with a 50 µmol m^{-2} s^{-1} light intensity measured with the HPL-220P, quantum light meter (Hopoocolor Technology Co. Ltd., China) in the range of Photosynthetic Active Radiation (PAR). Data of survival meristems percentage were collected after 30 days from culture.

Second Experiment

The second experiment was conducted to examine the response of nodal cutting cultures of five potato varieties (Cara, Hermes, Lady Rosetta, Santana, and Spunta) to different light qualities (white, blue, red, or red+ blue) in multiplication stage. Plant material (Nodal cuttings) for the second experiment were excised from the middle nodes of virus-free plantlets produced from meristem cultures, which were virus detected using the Double Antibody Sandwich Enzyme

Linked Immunosorbent Assay (DAS-ALISA) to test samples for viruses against PVX, PVY, and PLRV, according to Clark and Adams (1977). The same medium was used (MS salts and vitamins supplemented with 5 mg/l calcium pantothenate, 0.1 mg/l GA3, 30 g/l sucrose and 7 g/l agar). Also, the same light sources and qualities (White, blue, red, or red+blue) used in the first experiment with a 75 µmol m^{-2} s^{-1} light intensity. Data on plantlet growth (stem length, leaf number, and fresh and dry weight) were recorded after 4 weeks of culture.

Third Experiment

This experiment was conducted to examine the response of five potato varieties (Cara, Diamant, Hemes, Lady Rosetta, and Spunta) to three white light intensities of 50, 75, and 100 µmol. m^{-2} s^{-1}. Nodal cuttings were excised from the middle nodes of virus free plantlets produced from meristem cultures. Nodal cuttings were cultured on the same medium used in the first and second experiments. Data on stem length, leaf number, stem diameter, chlorophyll content (as SPAD, using a chlorophyll meter, TYS-B, Hinotek, China), leaf area (cm^2, using Easy Leaf Area software), and fresh and dry weights were measured after 4 weeks from culture.

Experimental Design Statistical Analysis

The experiments were arranged in a factorial complete randomized design with three replications (Compton and Mize., 1999). Data were analyzed using analysis of variance (ANOVA), and the difference between means was compared using the LSD (least significant difference test; and differences at $P < 0.05$ were considered significant). Each experiment was repeated twice.

RESULTS AND DISCUSSION

First Experiment: Effect of Light Quality on Meristem Culture

The obtained results confirmed a varietal difference between the five tested varieties in the survival rate of meristem cultures (Figure 01). Also, the obtained results showed that the highest survival rate of meristems after one month of culture *in vitro* was obtained by Cara variety meristems, followed by Hermes variety. On the other side, the lowest survival rate of meristems was obtained in the Santana variety. However, concerning light quality effects (White, Red, Blue, and red+ blue) on meristem survival rate; significant differences between light qualities were obtained, with the uppermost meristem survival rate resulting when red LED light quality was used (Figure 02). However, no differences were recorded between the white, blue, and red plus blue light qualities in meristem survival rate. The effect of red light could be attributed to the enhancement of higher gibberellins (Potter *et al.*, 1999; OuYang *et al*., 2015).

Second Experiment: Effect of Light Quality on Potato Stem Cuttings Culture

Significant differences were recorded between tested varieties in stem length, leaf number, root length, and plantlet dry weight (Table 01.). The Spunta variety gave the tallest plantlets (9.6 cm). While the Santana variety gave the highest average leaf number (6.1). Furthermore, the highest root length was recorded in the Cara, Hermes, and Spunta varieties. In the same respect, the lowest root length was recorded by the Lady Rosetta and Santana varieties. However, Cara, Hermes, and Lady Rosetta gain the highest plantlet dry weight. On the other hand, the lowest dry weight was recorded by the Santana and Spunta varieties.

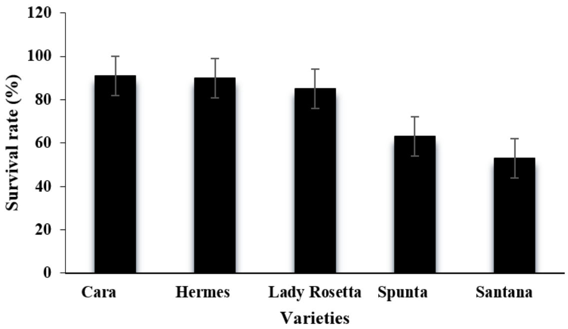

Figure 01: **Difference between five potato varieties in meristem survival rate. Bar indicate LSD at 0.05.**

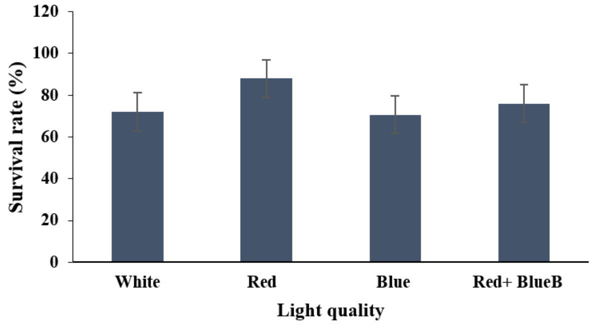

Figure 02: **Difference between light qualities (white, red, blue or red+ blue) in survival rate percentage of potato meristems Survival rate. Bar indicate LSD at 0.05.**

Table 01: **Varieties response to four light qualities after 4 weeks from in vitro culture of potato nodal cuttings.**

Variety	Light quality	Stem length (cm)	Leaves N0.	Root length (cm)	DW (mg/plantlet)
Cara	White	6.9	5.6	7.9	89.2
	Blue	4.2	4.6	0.4	43.8
	Red	12.7	6.4	3.1	79.3
	Red+ Blue	6.7	5.6	7.6	60.8
Hermes	White	6.4	5.6	7.2	87.0
	Blue	3.4	4.6	0.1	30.7
	Red	10.1	6.0	2.7	83.1
	Red+ Blue	6.3	5.6	7.1	57.7
Lady Rosetta	White	10.1	5.6	7.4	90.0
	Blue	5.4	5.3	0.3	27.3
	Red	10.2	4.8	1.3	85.5
	Red+ Blue	4.7	4.9	3.2	58.6
Santana	White	7.5	6.7	3.2	68.7
	Blue	4.3	5.3	0.2	41.2
	Red	9.7	5.9	3.0	77.5
	Red+ Blue	8.4	6.7	2.9	55.5
Spunta	White	11.9	4.6	5.9	76.1
	Blue	5.4	6.2	0.5	31.1
	Red	13.4	5.3	4.0	76.4
	Red+ Blue	7.4	4.6	5.9	57.0
LSD at 0.05*		2.3	NS	1.6	7.8
	White	8.6	5.6	6.3	82.2
	Blue	4.6	5.2	0.1	34.8
	Red	11.2	5.7	2.8	80.4
	Red+ Blue	6.7	5.5	5.3	57.9
	LSD at 0.05	1.0	NS	0.7	3.5
Cara		7.6	5.6	4.7	68.3
Hermes		6.6	5.4	4.3	64.6
Lady Rosetta		7.6	5.2	3.0	65.3
Santana		7.5	6.1	2.3	60.7
Spunta		9.6	5.2	4.0	60.2
LSD at 0.05*		1.2	0.6	0.8	3.9

**LSD at 0.05: least significant difference at 0.05*

Concerning the main effect of light qualities, significant differences were recorded in stem length, root length, and plantlet dry weight. The tallest stem length was shown under red light, followed by white then, red, and blue light qualities, while the blue light quality produced the shortest plantlets. The effect of red light caused by longer internodes (Rocha *et al.,* 2015), IAA (Aksenova *et al*., 1994) or enhancing gibberellin biosynthesis (OuYang *et al*., 2015). The effect of blue light has been reported previously (Seabrook and Douglass, 1998; Aksenova *et al.,* 1994; Rocha *et al.* 2015). Also, blue light reduces GA_1 content in dark-grown rice seedlings through phytochrome-mediated suppression of genes, while up-regulation or other genes by blue light is mediated by cryptochromes (Hirose *et al*., 2012). In the same respect, Omar (2017) indicated that red LEDs gave the best plantlet vegetative development.

The light qualities did not cause any significant differences in leaf number. On the other hand, root length differs significantly according to the change in light quality. The uppermost root length is obtained under white light, followed by red and blue. However, the blue light quality resulted in the lowest root length. Furthermore, blue light qualities gave the lowest dry weight. On the other side, white and red light quality gave the highest dry weight, followed by RB. In this respect, Chen *et al.* (2020) mentioned that the combined spectrum contributed more to biomass accumulation in potato plantlets *in vitro* than monochromatic light blue or red. On the other side, Chang *et al*. (2009) indicated that blue light gave the highest dry matter of potato plantlets, while white gave the lowest. However, the effect of white light quality could be interpreted by the fact that typical white LEDs emit 25, 45, and 30% of blue, green, and red light, respectively (Nozue *et al*., 2017).

The interaction between varieties and light qualities had significant effects on stem length, root length, and dry weight. Spunta and Cara varieties under white or Cara under red light produced the tallest plantlets. However, the shortest plantlets formed by Hermes under blue light. Furthermore, Cara, Hermes, and Lady Rosetta under white light or Cara and Hermes under red+ blue light gave the highest root length. Concerning plantlet dry weight, Cara, Lady Rosetta, and Hermes under white light quality, or Lady Rosetta and Hermes under red light, formed the highest plantlet dry weight.

Third Experiment: Effect of Light Intensity on Potato Stem Cuttings Culture

Light intensity effects on the growth behavior of the five selected potato varieties (Figure 03) revealed obvious and significant differences between the light intensity levels (50, 75 and 100 $\mu mol\ m^{-2}\ s^{-1}$). Increasing light intensity from 50 to 75 and 100 $\mu mol\ m^{-2}\ s^{-1}$ resulted in increases in leaf number, stem diameter, root length, leaf area, chlorophyll content, fresh weight, and dry weight. However, the tallest shoots are obtained under lower light intensities and the stem length is reduced with increasing light intensities. The highest dry matter percentage was obtained with 75 $\mu mol\ m^{-2}\ s^{-1}$ Concerning the main effect of varieties (Figure 04); Diamant produced the shortest shoots, while Diamant produced the highest leaf number, root length, leaf area, and fresh and dry weight. Contrariwise, Spunta gave the tallest shoots with the lowest fresh and dry weight. Moreover, the interaction between the varieties and light intensity factors recorded significant differences in the tested trait variables (Table 02.); the highest shoot length obtained by Lady Rosetta and Spunta under the lowest light intensity. However, the highest values of stem diameter were obtained in Diamant under any of the three light intensities and in Hermes under100 $\mu mol\ m^{-2}\ s^{-1}$. Furthermore, Diamant and Cara under 100 $\mu mol\ m^{-2}\ s^{-1}$ formed the highest overall chlorophyll content. Diamant under the highest light intensity (100 $\mu mol\ m^{-2}\ s^{-1}$) recorded the highest fresh and dry weight per plantlet.

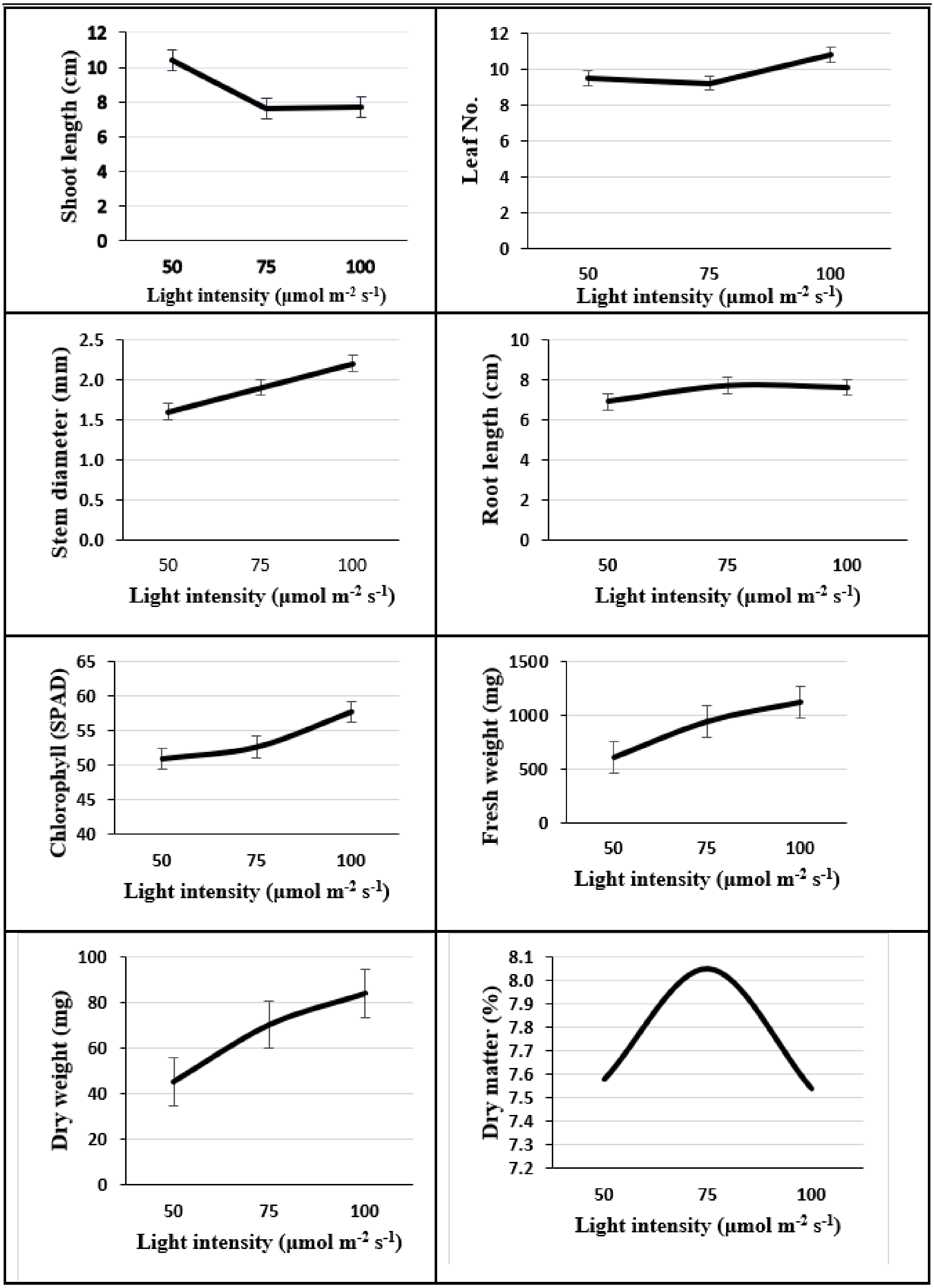

Figure 03: **Light intensity effects on potato plantlet growth.**

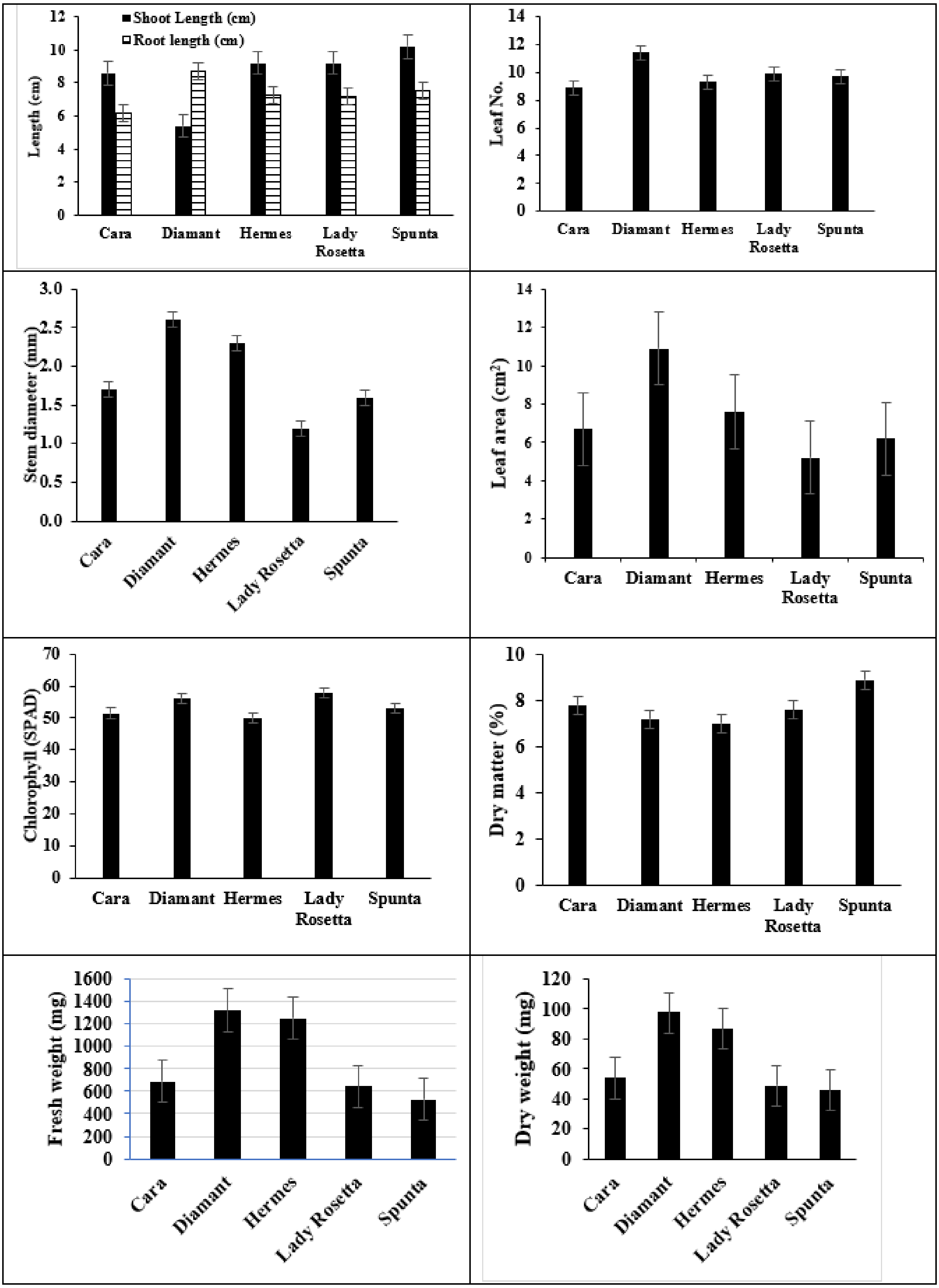

Figure 04: **Main effect of varieties on potato plantlet growth under different light intensities.**

Table 02: Effects of interaction between varieties and light intensity on potato plantlet growth.

Variety	light intensity (µmol m-^{2} s^{-1})	Shoot Length (cm)	Leaf No.	Stem diameter (mm)	Root length (cm)	Leaf area (cm^{2})	Chlorophyll (SPAD)	Fresh weight (mg)	Dry weight (mg)
Cara	50	10.4	8.9	1.5	4.9	4.9	41.5	423	31.1
Cara	75	7.5	8.0	1.5	6.7	5.2	51.5	561	45.0
Cara	100	8.0	10.0	2.2	7.0	10.0	61.6	1081	85.0
Diamant	50	5.2	10.0	2.0	9.0	9.3	55.1	779	55.0
Diamant	75	6.5	10.0	3.3	8.5	12.3	50.4	1321	91.7
Diamant	100	4.5	14.3	2.5	8.6	11.1	63.1	1856	145.7
Hermes	50	10.5	9.2	1.7	5.9	4.5	45.3	751	55.0
Hermes	75	8.2	10.1	2.2	7.4	10.9	52.5	1480	101.0
Hermes	100	8.9	8.7	2.9	8.6	7.4	52.3	1517	103.7
LR	50	13.0	9.6	1.6	7.0	6.1	53.3	632	47.0
LR	75	7.6	9.0	1.1	7.7	5.1	60.4	858	65.7
LR	100	7.0	11.0	1.0	7.0	4.5	60.2	445	34.0
Spunta	50	12.7	10.0	1.1	7.5	5.5	59.5	437	37.7
Spunta	75	8.0	9.0	1.5	8.0	5.8	48.1	464	48.0
Spunta	100	10.0	10.0	1.5	7.0	7.2	51.4	680	51.7
LSD at 0.05*		1.2	0.8	0.1	0.8	3.3	2.7	328	23.5

**LSD at 0.05: least significant difference at 0.05*

Similar results according to light intensity, shoot length, and fresh and dry weight were reported by Kitaya *et al.* (1995). Furthermore, Kulchin *et al.* (2018) reported varietal differences in light intensities and recommended 75 or 135 µmol m^{-2} s^{-1} for micropropagation according to two different varieties. The increase in stem length is related to higher endogenous gibberellin content at low light intensities (Potter *et al.*, 1999).

Principal component analysis (PCA) was carried out to discover the relationship between different parameters and treatments (Figure 05) based on the Pearson's correlation and biplot distribution showing variables and observations showing strong positive loading for the tested characters.

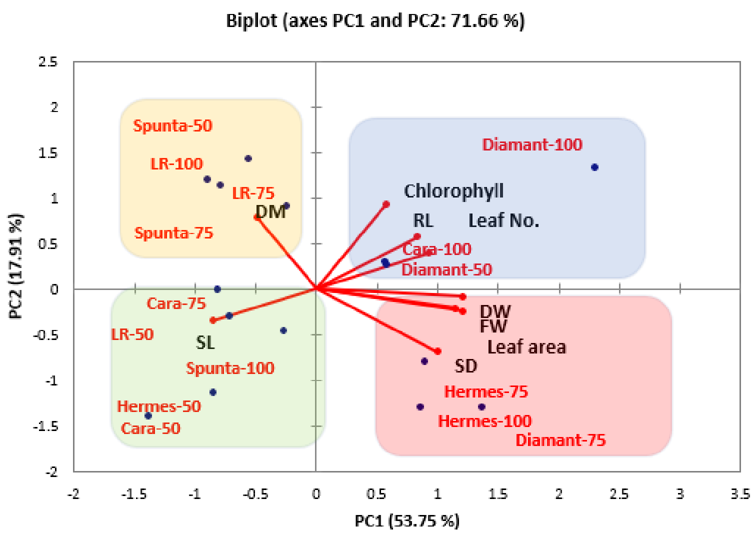

Figure 05: Principal component analysis (PCA) analysis based on the Pearson's correlation and biplot distribution showing variables (black) and observations (red).

CONCLUSIONS

The results of the study recommend the use of red LED light in potato meristem culture and white LED with 75- 100 µmol m^{-2} s^{-1} light intensity for potato nodal cultures for the best vegetative growth response for *in vitro* pre-basic seed potato production. Also, to consider the varietal differences in environmental requirements, i.e., light quality and intensity, during the *in vitro* phase of pre-basic seed potato production.

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Conflicts of Interest

The authors declare no conflict of interest.

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