Australian Journal of Crop Science AJCS

AJCS 12(02):318-325 (2018) ISSN:1835-2707
doi: 10.21475/ajcs.18.12.02.p7685

Influence of the quality of artificial light on grafting tomato

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Abstract

To improve the success of grafted tomato plants, the response under different types of lighting has been evaluated. The lights used are compact fluorescent, high efficiency fluorescent, standard fluorescent and pure Blue-Light-Emitting Diodes. The assay was conducted in a culture chamber under the following conditions: temperature (night-day) 17.5/19.8 ºC (0.8 ± 0.2/0.1 ºC), relative humidity 56.44/72.40% (6.65 ± 0.01%) and radiation 92, 70, and 36 Wm^{-2}. The tomato (*Solanum lycopersicum* L.) grafting method used was tube grafting with a small grafting clip using the cultivars Myla as scion and Maxifort as rootstock. Spectral radiation was measured at canopy level. Fresh and dry biomass in partitioning organs (leaves, stems and roots), Indol Acetic Acid (IAA), proline and reducing sugars were quantified. Standard fluorescent lamps have a very interesting spectral quality for grafting applications due to their B:R (blue:red), B:FR (blue:far red), PAR:NIR (Photosynthetically Active Radiation:Near Infrared) and R:FR (Red:Far Red) ratios; grafted plants under standard fluorescent lamps present lower proline concentrations (990µg per g of fresh weight) and higher sugar concentrations (27µg per g of fresh weight) than plants under other treatments. Furthermore, standard fluorescent lamps ensure the highest stem and root growth as well as an adequate water status.

Keywords: auxin; carbohydrates; fluorescent lamps; light emitting diodes; proline; *Solanum lycopersicum* L.
Abbreviations: B_blue light; CF_compact fluorescent lamp; DW_dry weight partition; FR_far red light; FW_fresh weight; G_green; IAA_indol acetic acid; LED_light emitting diodes; LFW_leaves fresh weight; LPSC_leaf proline synthesis capacity; NIR_near infrared radiation; PAR_photosynthetically active radiation; PPF_photosynthetic photon flux; R_red light; T_treatment; TDW_total dry weight; TFW_total fresh weight; TL5_tubular lamp with the diameter of the bulb in eighths of an inch 5/8"; TLD_tubular lamp don's bulb; UV_ultraviolet.

Introduction

Tomato grafting has many potential benefits such as resistance to soil-borne disease, a decrease in nematode damage, superior quality fruit, higher yields, and greater efficiency in water and nutrient use as well as increased tolerance to abiotic stressors (Rivard and Louws, 2008).

Grafting is the union of two pieces of living plant tissue (scion and rootstock). The main goal of employing these agricultural practices is to control soil borne pathogens (Crino et al., 2007). However, the importance of vegetable grafting includes higher crop performance (Colla et al., 2008), higher tolerance to abiotic stresses such as salinity, alkalinity, nutrient stress, drought and heavy metal contamination (Colla et al., 2010, 2011, 2012, 2013; Kumar et al., 2015a,b; Rouphael et al., 2008, 2010; Schwarz et al., 2010; Savvas et al., 2010) as well as improving fruit quality (Proietti et al., 2008; Rouphael et al., 2010).

Auxins are a family of plant hormones which induce new vascular tissue by promoting cell elongation, division and development. They can be synthesized in leaves and travel down by polar transport promoting plant growth (Overvoorde et al., 2010). Light is an environmental factor that greatly effects lateral Indole-3-Acetic Acid (IAA) transport, and thus distribution of auxins in the plant (Sassi et al., 2013). The grafting process is regulated by plant hormones such as auxin and cytokinin (Shanfa, 2001). In addition, auxin levels are related to radiation quality (Kurepin et al., 2007) and intensity (Kurepin et al., 2008).

During the grafting process, the shoot donor (the scion) supports high drought stress. Proline is an amino acid which acts as an osmotic agent, protecting the plant from dehydration. Proline is also involved in multiple roles regarding the plants´ tolerance to stress, acting as a mediator of osmotic adjustment. Under stress, light intensity plays a role in the promotion of proline accumulation (Zadebagheri et al., 2014).

However, photosynthesis is not related to proline concentration (Arora and Saradhi, 2002). Carbohydrates are related to PAR (Photosynthetically Active Radiation), and they are the main known source of energy in plants. Their abundance can be considered a major criterion of cell division activity and region differentiation. The quantity of carbohydrates can be expected to greatly influence the healing of graft wounds. The percentage of “take” in grafting of macadamia was found to be related to the relative amount of carbohydrates in the scions (Fahmy, 1952).

Carbohydrate synthesis and proline are related to dehydration and drought stress; both of them, along with auxin synthesis, are modified by light intensity and spectrum quality (Tofiño et al., 2007). For this reason, artificial light treatments are used to improve grafting success (Shuangxia et al., 2006). The hypothesis of this work is based on how the spectral quality of light modifies grafting quality, related to the physiological changes induced.

The aim of this work is to evaluate the quality of grafting in tomato plants (*Solanum lycopersicum*), based on total and organ partitioning biomass and water status under four treatments, using different lighting lamps: fluorescents and light-emitting diodes.

Results

The relevant values associated with radiation from the agricultural point of view in order to characterize the quality of light (Almansa et al., 2011) are given in Table 1.

The spectrums of different treatments are shown in Fig 1. It is interesting to note that CF (compact fluorescent lamps), TL5 (tubular lamps with a bulb diameter of 5/8 of an inch) and TLD (tubular lamps with a Don bulb) present peaks at 436, 544, 612 nm, but only B-LED presents a wide peak at 470 nm. The lowest total radiation is given off by B-LED.

The UV (ultraviolet) radiation that was measured can be considered in the UV-A (ultraviolet-A) (320-400 nm) range. CF and TL5 have similar characteristics in UV-A, B (blue), R (red), NIR (near infrared) and TOTAL light, with values higher than TLD and B-LED. TL5 shows the highest values in the R (Red) and FR (Far red) regions. PAR (Photosynthetically Active Radiation) values in TLD treatments seem to be very low compared to CF and TL5. The CF treatment lamps are more powerful than the TL5 lamps, CF and TL5 show similar values of total radiation received by the grafts as CF lamps do not have luminaires. The low radiation intensity measured in B-LED, in contrast to TLD, could be due to lamp distribution and polarized light emission.

Table 2 shows the ratios between different spectral radiation ranges. The LED-blue spectrum is limited between 400-500 nm. It has not been possible to estimate the PAR:NIR, B:R, B:FR and R:FR parameters .

The relationship between PAR and Total radiation shows values near to one, corresponding to high photosynthetic efficiency radiation for all treatments (Table 2). The highest PAR:NIR lamp ratio can be seen in TLD (27.94) and this permits the use of this kind of lamp without causing heat damage to the plant. This value is higher than solar radiation which presents a ratio of about one. B-LED does not emit in the NIR range and for this reason it does not dissipate energy as heat. The CF treatment shows the highest B:R (0.90) ratio. In TLD, the B:FR and R:FR parameter values were superior to other treatments. The latter two ratios were related to phytochrome and cryptochrome responses.

Ultraviolet-B (UV-B) radiation can cause a reduction of stem length due to an oxidation mechanism of the phytohormones. These plant hormones cause changes in cell size, such as the IAA, which is likely to be degraded by UV-B (Mark and Tevini, 1996); however, this parameter has not been measured. UV-A and blue light ranges show higher values in the CF and TL5 treatments. According to Kimura et al. (2004), we can expect a positive induction of process tissue repair in grafting plants related to enzymatic photo-reactivation conducted by photolyase, an enzyme sensitive to UV-A -and blue light. Furthermore, blue light (B) is related to photo-morphogenic aspects of plant growth and their development (Sundström, 2000). The TL5 treatment presents the highest values of intensity radiation of the R and FR regions; both of which indicate the extent to which the observed responses are mediated by the phytochrome (Hall and Rao, 1999). PAR values superior to our artificial sources were found in the literature. Nevertheless, in *in vitro* Grape propagation,PPF (photosynthetic photon flux) was fixed at 10.94 W m^{-2} (50 µmol m^{-2} s^{-1}) for all lighting systems (Poudel et al., 2008); artificial light at 10 W m^{-2} PAR also improved lettuce growth and decreased the duration of the production cycle (Gaudreau et al., 1994); photosynthesis of tomato (*Solanum lycopersicum* Mill.) was nearly linear within the photon flux range of 0 to 50 µmol m^{-2} s^{-1} from LEDs (Tennessen et al., 1995). Similar PAR values are found under CF and TL5 treatments. Near Infrared Radiation (NIR) is a useful part of radiation related to heat and the greenhouse energy balance (Castilla, 2005).

Notwithstanding, in a culture chamber, NIR only results in plant over-heating and in possible tissue damage. Spectral ratio, such as R:FR, is related to phytochrome photoequilibrium, and the B-light receptor (cryptochromes) is also involved in the photo-regulation of plant development, either independently or in conjunction with the phytochrome (Poppe et al., 2001). A B:R ratio close to 1 may indicate activation of cryptochromes and phytochromes. For this reason, responses due to cryptochrome activations must be considered in CF. The B:R (0.51) value for TL5 indicates that the plants received a greater amount of red light energy than blue light. Consequently, different responses are expected in tomato plants in relation to other treatments (Demers et al., 1998). With regard to the B:FR relationship, it is expected that the highest value (TLD) induced greater activation of cryptochromes against phytochromes. The values of B:FR and R:FR may be related to stem elongation due to the plants' response to blue and FR light (Park et al., 2015).

Grafting light response

Biomass evaluation

Fresh and dry weights of grafting plants at the end of the trial were evaluated as shown in Fig 2 and 3. Total leaf and stem fresh weight (FW) do not show significant differences between treatments. The root shows less fresh weight in TLD.

Total dry weight (DW) does not show significant differences between treatments. Nevertheless, the leaf, stem and root present differences in dry weight between treatments. CF shows the lowest dry weight of leaves and TL5 presents the highest dry weight of stems and roots.

The water status evaluated as the FW/DW ratio, related to the irradiative component of transpiration, decreases gradually in the following order: root, stem and leaf, in grafting plants for all treatments (Fig 3). CF has the highest

Table 1. Agronomic characterization of light quality (W m^{-2}).

Treatments	CF	TL5	TLD	B-LED
UV-A (320-400 nm)	0.18	0.20	0.04	0.00
B (400-500 nm)	2.81	2.04	1.51	1.22
R(600-700 nm)	3.12	3.97	2.19	0.00
FR(700-800 nm)	0.30	0.36	0.14	0.00
PAR(400-700 nm)	10.06	9.94	5.88	1.28
NIR(700-1100 nm)	0.47	0.47	0.21	0.00
TOTAL(300-1000 nm)	10.68	10.57	6.12	1.28

Radiation: UV-A (ultraviolet-A); B (Blue); R (Red); FR (Far red); PAR (Photosynthetically Active Radiation); NIR (Near Infrared). CF (compact fluorescent lamp); TL5 (Tubular Lamp with a bulb diameter of 5/8" of an inch); TLD (Standard Tubular Lamp with a Don bulb); B-LEDs (Blue Light Emitting Diodes).

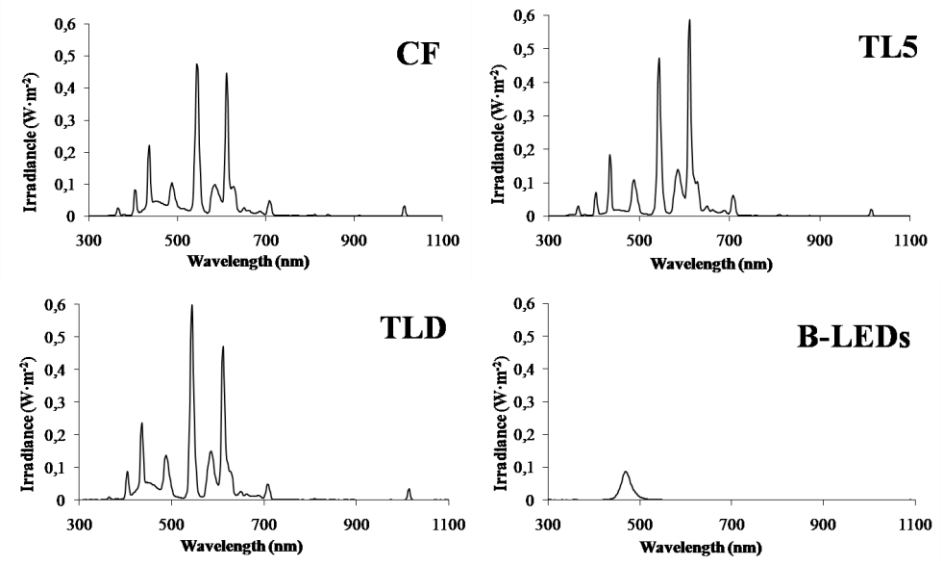

Fig 1. Spectral irradiance received by plants of different lighting treatments, measured at canopy level.

Table 2. Ratios between different spectral radiation ranges.

Spectral Fraction	CF	TL5	TLD	B-LED
PAR:TOTAL	0.94	0.94	0.96	0.99
PAR:NIR	21.34	21.36	27.94	
B:R	0.90	0.51	0.69	
B:FR	9.30	5.70	11.00	
R:FR	10.34	11.13	15.89	

Radiation: UV-A (ultraviolet-A); B (Blue); R (Red); FR (Far red); PAR (Photosynthetically Active Radiation); NIR (Near Infrared). CF (compact fluorescent lamp); TL5 (Tubular Lamp with a bulb diameter of 5/8" of an inch); TLD (Standard Tubular Lamp with a Don bulb); B-LEDs (Blue Light Emitting Diodes).

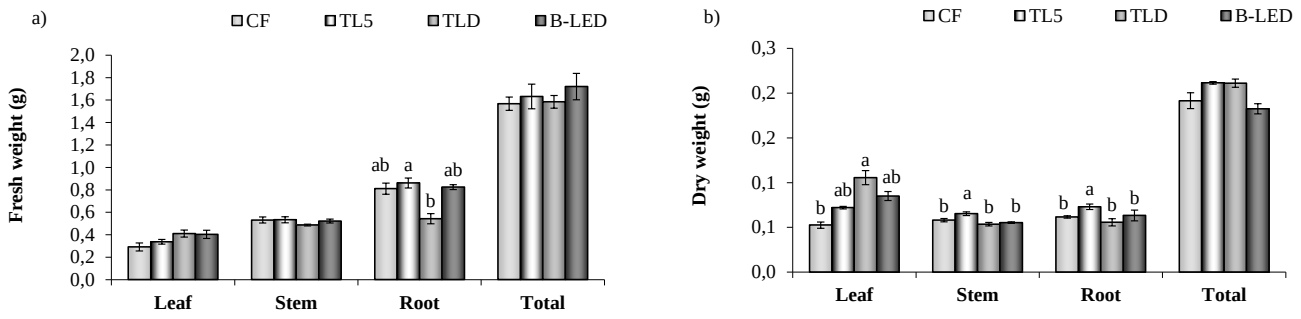

Fig 2. Total and organ partitioning biomass evaluated at the end of the trial on grafting plant a) Fresh and b) Dry weight. All means at the top of each column with the same letters are not significantly different at p≤0.05 by LSD test.

Table 3. Lighting per treatments arranged by level. CF was positioned at the top and the others followed the established order.

Treatments	Lighting	Power (W m^{-2})
CF	Compact fluorescent (CF) 23W (4 lamps)	92
TL5	High Efficiency Fluorescent TL5 35W (1 light MAXOS 4M691 x 2 lamps)	70
TLD	Fluorescent TLD 18W (1 light Philips TCS097 x 2 lamps)	36
B-LED	Pure Blue-Light-Emitting Diodes (B-LEDs) RGB (4 lines ALUM 40*25 LED SMD RGB x 9 W with console DN-RGB FIBER LIGHT)	36

CF (compact fluorescent lamp); TL5 (Tubular Lamp with a bulb diameter of 5/8" of an inch); TLD (Standard Tubular Lamp with a Don bulb); TCS (Transmission Control System); B-LEDs (Blue Light Emitting Diodes); ALUM (aluminum); LED (Light Emitting Diode); SMD (Surface Mount Device); RGB (Red, Green and Blue colors); DN (Denon electronics brand).

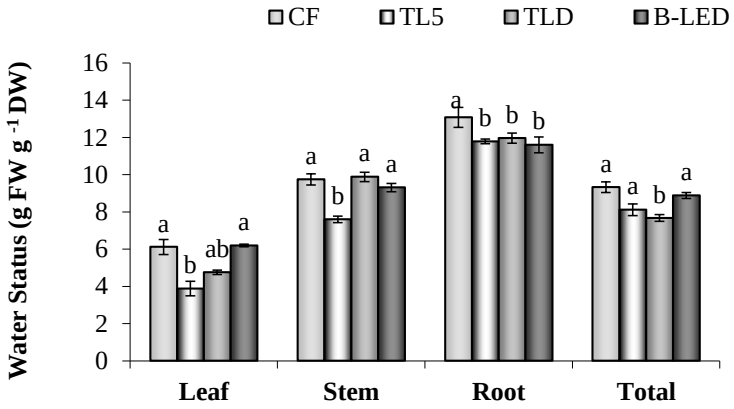

Fig 3. Water status and response to drought stress. Water status was established on fresh weight divided by dry weight (FW:DW ratio) for leaves, stems and roots. All means at the top of each column with the same letters are not significantly different at p≤0.05 by LSD test.

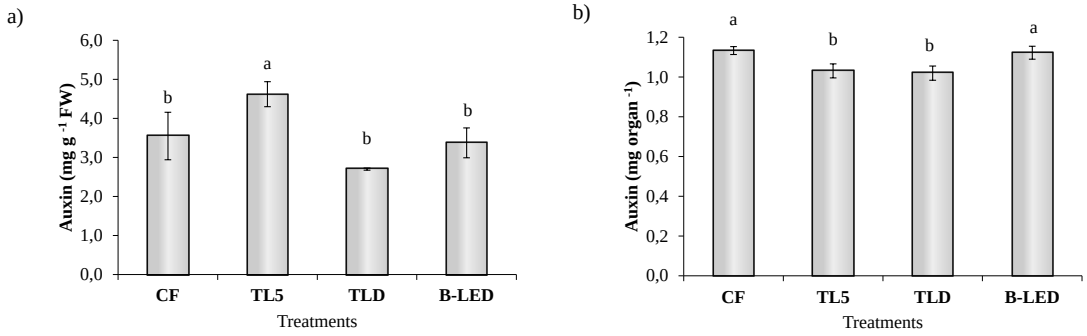

Fig 4. Auxins in grafting plant leaves. Total auxins were measured in supernatant fraction by colorimetry. All means at the top of each column with the same letters are not significantly different at p≤0.05 by LSD test.

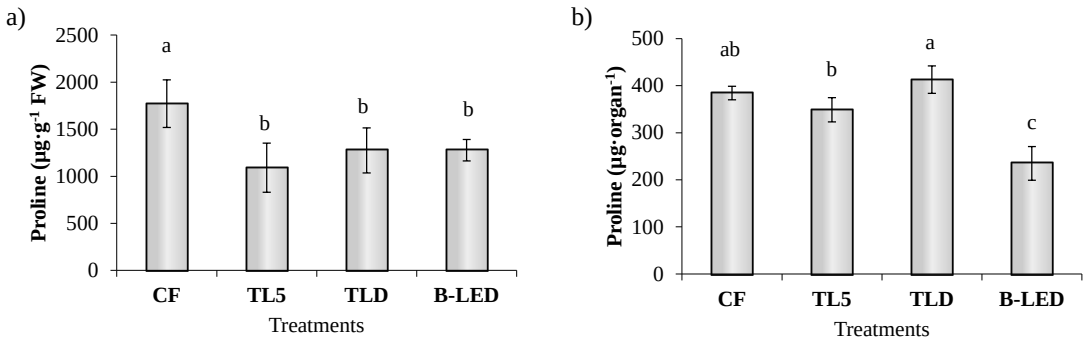

Fig 5. Proline grafting plant leaves. Total proline was measured in supernatant fraction by colorimetry. All means at the top of each column with the same letters are not significantly different at p≤0.05 by LSD test.

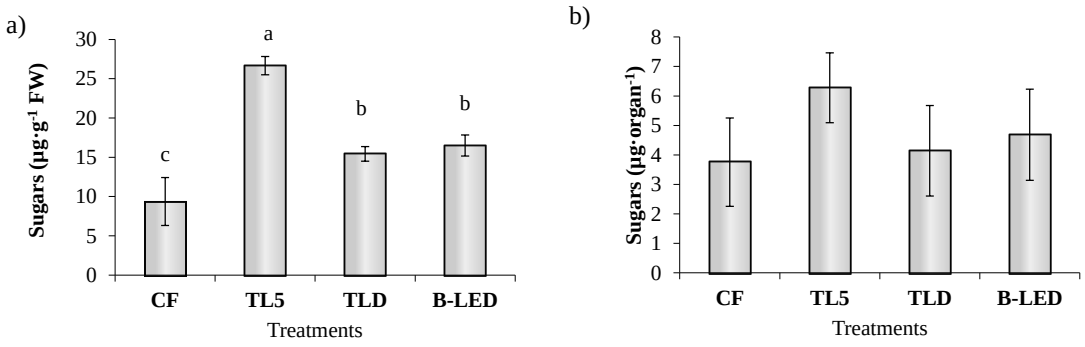

Fig 6. Reducing sugar concentrations in grafting plant leaves expressed in (a) micrograms of glucose by fresh weight, (b) micrograms of glucose by leaf. Total sugars were measured in supernatant fraction by colorimetry. All means at the top of each column with the same letters are not significantly different at p≤0.05 by LSD test.

FW/DW ratio of roots and TL5 has the lowest FW/DW ratio of leaves and stems. The values of stem and root DW in TL5 could be related to PAR, but the lowest value of leaf DW can be not related to PAR. Nevertheless, B-LED provided blue light to increase leaf growth and DW. Blue light inhibited shoot elongation, whereas leaf expansion was negatively affected only at the highest blue level (Rapparini et al., 1999). The TLD treatment provided the highest R:FR ratio related to phytochrome activation and its effects on total biomass accumulation and allocation (Humberto and Wulff, 2003). There are two spectral regions involved in the plant's water status defined by the FW/DW ratio: blue and NIR regions. Phototropins are activated by low blue radiation (Takemiyaa et al., 2005) and induced stomata opening (Briggs and Olney, 2001), with an increase in transpiration and water losses. On the other hand, when NIR increases, energy is converted into heat and, to maintain leaf temperature, water loss occurs through transpiration (Pieruschka et al., 2010). These reasons could explain the behaviour of TL5, but they do not explain the behaviour of CF. Nevertheless, a relationship between B:R and B:FR and auxin concentration may exist , as well as their joint auxin effect on cell turgor (Krieger, 1978).

IAA and plant development

The concentration and Leaf Auxin Synthesis Capacity (LASC), defined as auxin concentration in leaf (µg g^{-1} LFW) per Leaf Fresh Weight (LFW) (g) are shown in Fig 4 a) and b), respectively. The highest auxin concentration occurred in TL5, and similar values were found in the other treatments studied; however, LASC is higher in CF and B-LED than in TL5 and TLD. The lowest values of B:R and B:FR under TL5 treatment may be related to the increase in IAA production, and the fact that blue light stimulates their production. Kurepin et al. (2007) consider that a low R:FR ratio increases levels of endogenous IAA, but in this trial the R:FR ratio is similar in CF and TL5. The production of auxins by B-LED in aerial organs can be put down to cell differentiation (Horwitz, 1994) and growth of secondary roots (Günes, 2000) to ensure the survival of young plants (Geßner et al., 1999).

Proline content in plant

Proline concentration and Leaf Proline Synthesis Capacity (LPSC) are shown in Fig 5 a) and b), respectively. CF shows the significantly highest proline concentration. Similar values were found in the other treatments essayed. Nonetheless, LPSC is higher in CF and TLD than in TL5. B-LED presents the lowest value.

Proline is accumulated in plants during adaptation to environmental stresses (Claussen, 2005). Proline increased in the leaves of the plants when the time under water stress increased (Ismail et al., 1994). In our trial, the lowest FW/DW ratio occurred in TL5, but it does not show a high proline level. However, these values could be related to the highest sugar levels in TL5 as proline acts as an osmolite (Jang and Sheen, 1997). CF presents the highest proline concentration and this could be related to the highest total radiation received according to Zadebagheri et al. (2014). They considered that when light intensity increases, proline accumulation also increases. Relevant literature was found regarding the increase in proline content by UV radiation (Demir, 2000); Rajagopal and Madsen (1981) found unrolling of etiolated barley leaves, and reduction of the free proline under blue (426 nm) and red (658 nm) light: However, leaf unrolling was prevented by FR (728 nm); light does not explain why total proline in leaves is higher in CF and TLD. Nevertheless, B:FR ratios could explain our results.

Sugars and plant growth

The concentration and total reducing sugar in leaves are given in Fig 6 a) and b), respectively. CF shows the significantly lowest value, TLD and B-LED provide intermediate values and TL5 presents the highest value. No differences have been found in total reducing glucose per leaf between treatments.

In short, photosynthesis is the highly efficient conversion of radiant energy into carbohydrates, and it consequently promotes growth (Alves de Alvarenga et al., 2003). Photosynthetically active radiation (PAR) contributes directly to photosynthesis and hence to crop growth. The lowest sugar content provided by the CF treatment may be related

to the highest proline concentration and stress situation accompanied with a high respiration ratio. In terms of the production of sugar, the best treatment may be TL5. Nevertheless, the lowest leaf dry weight occurs in TL5. Kemp and Blacklow (1980) found an increasing over-supply of carbohydrates for growth of the shoot as the plant increased in size, related to the consumption of carbohydrates. TLD and B-LED demonstrate similar sugar concentrations and this could be related to the high dry weight of the stem and root in TLD, along with the consumption of carbohydrates in growth.

Materials and methods

Plant material and growing conditions

The tomato (*Solanum lycopersicum*) grafting methods used were tube grafting with a small grafting clip. A hundred grafts of tomato (cultivar 'Myla' as scion and 'Maxifort' as rootstock) were grown in expanded polystyrene seedling trays. Peat moss covered with vermiculite substrate was used. The plants were hand-watered daily with a Steiner nutrient solution (1961) (EC 1.5 dS m^{-1}, pH 5.5). The density was 421 plants m^{-2}. A Testo 625 Model 05636251 measured the air temperature and relative humidity inside the room. Average values were 17.5/19.8 ºC (0.8 ± 0.2/0.1 ºC) and 56.44/72.40% (6.65 ± 0.01%) night-day for temperature and relative humidity, respectively. Similar conditions were found in commercial greenhouses for the second stage of development in the formation of a graft union (Fernández-García et al., 2004). This period was chosen because the major hydraulic connections within the graft union of tomato become functional over a period of about 48 h from the fifth day after grafting (Turquois and Malone, 1996).

Plant sampling

Experimental design consisted of 4 lighting treatments with 20 replications (one plant per replication), by treatments. Four days after grafting, plants were taken into the chamber. The trial lasted for five days, corresponding to the second stage of development in the formation of a graft union.

The experiment was conducted in a 1.8 m by 1.5 m culture chamber, equipped with different light treatments: compact fluorescents (CF), high efficiency fluorescent (TL5), standard fluorescent (TLD) and pure blue-light-emitting diodes (B-LEDs). Table 3 shows the type of lamps and luminaires used in each treatment. Lamps were chosen taking into account the usual commercial equipment found in greenhouses (Chica et al., 2007) and simulation lighting models (Chica et al., 2008). Continuous lighting was applied following the recommendation of Black et al. (2003).

Light measurements

Spectral radiation was measured from 300 nm to 1100 nm in each shelf-treatment at canopy level with a Licor 1800 (LI-COR inc. P.O. Box 4425 Lincoln, Nebraska 68504 USA).

Plant analysis

At the end of the trial, grafted plants were evaluated. Fresh and dry biomass partitioning between assimilation (leaves), conductive (stems and petioles) and absorption (roots) organs were measured using a precision balance (Mettler Toledo classic PB303-S; CH-8606 Greifensee, Switzerland).
Extraction was carried out by grinding fresh leaves with 95%+70% (1:1 v/v) ethanol. After filtering and centrifuging the samples at 5500 rpm for 10 min, IAA (Tien et al., 1979), proline, and sugars (Irigoyen et al., 1992) were quantified in supernatant fraction by colorimetry with a Spectrophotometer (Shimadzu UV-1201, Shimadzu; Kyota, Japan).

Statistical analysis

Analysis of data was carried out using software packages Excel 7.0 and Statgraphics (Stat- Point, Herndon, VA) plus 4.0. Analysis of variance and the Least Significant Difference (LSD) test for $p \leq 0.05$ were used to assess the significance of treatment means.

Conclusion

The use of standard fluorescent lamps (TLD) is the most successful treatment essayed as the grafted plants showed an adequate stem-root ratio and water status. TLD induced a lower proline level and a higher sugar level in grafted plants, corresponding with medium total radiation. TLD provided a lower PAR:NIR ratio (27.94) and a higher R:FR ratio (15.89). All these reasons lead us to the use of standard TLD type fluorescent lamps in post graft chambers in nurseries.

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