Subtle changes in light intensity affect in vitro responses but not ex vitro performance of *Limonium sinuatum*

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Abstract

Environmental conditions during the in vitro stages of a micropropagation process can further affect the ex vitro plant performance. In this work, the behaviour of four blue cultivars of statice (*Limonium sinuatum*) was studied with respect to four distances (18.0, 12.5, 8.0, or 2.0 cm) between culture tubes and the light source during their in vitro multiplication stage. In vitro shoots of statice were cultured on MS medium supplemented with 4% sucrose, 0.9% agar and 0.2 mg L⁻¹ BA and incubated under four slightly different light intensity treatments (PPFD of 96.9, 99.6, 101.2, or 102.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 35 days at 23 °C with a daily photoperiod of 16 h. The light treatments assayed resulted in no significant differences in in vitro propagation ratio and ex vitro survival and crop production and quality (as judged by the number of inflorescence stems per plant, length of inflorescence stems and number of both spikes and branches per stem produced). However, in vitro growth (estimated as the length of the longest leaf in a shoot at the end of the culture period) and levels of pigments (chlorophylls and carotenoids) and antioxidants (as estimated by the DPPH test) showed variations among cultivars. This indicates that statice cultivars were able to adjust their metabolism in response to the small differences in light intensity of the treatments applied. Results point to the importance of optimization of growth room use to increase profitability of micropropagation processes.

Keywords Statice · Micropropagation · Light intensity · Crop productivity

Introduction

Reduction of costs is a matter of concern for the micropropagation industry. Analysis of expenditures shows that labour constitutes the main direct cost in the whole process of plant propagation. Wearing-out expenses are linked to the depreciation of buildings and laboratory equipment value, and it is worth about 20% overall direct cost (Hempel 1986). In this way, Kaur and Sandhu (2015) reported that non-recurring costs associated with commercial micropropagation of sugarcane accounted for 24% plant's production costs. One way to minimise the charges associated to wearing-out costs is to increase the number of cultured plants in the facility. This

can be accomplished by simply increasing the number of culture flasks per shelf and/or by increasing the number of shelves per rack, the latter resulting in a reduction in the distance between shelves. However, physiological, technical, and operational constraints can make these solutions inadvisable.

Regarding physiological issues, light is a prominent determinant of plant development and it is necessary to optimise both its intensity and spectral distribution to achieve the desired developmental response. Light intensity is usually low in conventional micropropagation practices, therefore increased flask density on shelves could result in undesirable shading effects on development. Some beneficial effects on growth and development upon increasing irradiance on in vitro plant material have been reported (Jorge et al. 2000). Therefore, it seems to be reasonable that a good way to optimise growth room space would consist in reducing the distance between culture tubes and the light source. However, relatively high light intensities can also provoke deleterious effects on in vitro-regenerated plant material. In this sense, failure in in vitro regeneration (Nameth et al. 2013) and reduction in plant material survival (Alvarenga et al. 2015) have

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been observed in some assays. Therefore, optimisation of light conditions would be convenient for a profitable use of the in vitro culture technique.

Limonium sinuatum is a prized species because it has a good market for both dry and fresh flowers. Micropropagation is commonly used for the mass production of statice plants due to the problems associated with conventional propagation practices. In vitro regeneration and micropropagation of *Limonium* species have been carried out by applying several strategies based on both organogenesis and embryogenesis (Bose et al. 2017; Igawa et al. 2002; Jeong et al. 2001; Xiao and Kozai 2006). Despite the undoubted advantages of using micropropagation in *Limonium* plant production, the commercial exploitation of these techniques requires the optimisation of a number of parameters which, in many cases, have to be specifically adjusted for each cultivar.

In this context, and taking into account that information about possible effects of in vitro culture conditions on *L. sinuatum* productivity is scarce, this paper describes a trial of four cultivars of blue statice which was undertaken during the laboratory multiplication stage of propagation, to know how the distance from the light source to the plants affects their development and further ex vitro performance. Morphometrical and biochemical analyses are included to assess the physiological status of micropropagated materials at the end of light treatments. Finally, data on flower production and quality from micropropagated plants are provided with the aim to estimate whether light treatments applied during the in vitro multiplication stage affected plant productivity.

Materials and methods

Plant material and culture conditions

In vitro cultures derived from four cultivars of blue *L. sinuatum* ('SN8925', 'SN8946', 'SN9022', and 'SN89QX'), gently provided by Barberet & Blanc, S.A. by Dümmer Orange (Murcia, Spain), were used in this study. Shoots formed at the base of the initial explants containing 4–6 leaves were used as plant materials for conducting the subsequent assays and for maintenance of in vitro cultures. The shoots were recut from the apex into 1.5–2.0 cm segments (Fig. S1a) and subcultured on Murashige and Skoog (1962) basal medium (Duchefa Biochemie BV) supplemented with 100 mg L⁻¹ myo-inositol, 0.4 mg L⁻¹ thiamine HCl, 4% (w/v) sucrose, and 0.2 mg L⁻¹ 6-benzylaminopurine (Sigma-Aldrich). Subsequently, the pH of the media was adjusted to 5.8 and agar (Conda Pronadisa) was added at a concentration of 0.9% (w/v) prior to sterilization by autoclaving. The medium (10 mL) was distributed in glass culture tubes (20 mm in inner diameter and 145 mm high), capped with

polypropylene caps. All cultures were incubated in a growth room (5.2 × 10.8 × 2.4 m) for 35 days at 23 °C with a daily photoperiod of 16 h. Culture tubes were located on 0.68 m² shelves (440 tubes per shelf, 6 shelves per rack, 120 shelves in the growth chamber).

Four light treatments were established by varying the distance between light sources (OSRAM SYLVANIA Cool White fluorescent lamps) and culture tubes, taking as a reference the top of their caps. In the light treatments applied tube caps were placed at 18.0 (T-1), 12.5 (T-2), 8.0 (T-3), and 2.0 cm (T-4) from fluorescent tubes. Photosynthetic photon flux densities (PPFDs) for the different treatments were calculated from data of light intensity obtained using a lux meter model LX-93 (Nieuwkoop BV). For conversion to PPFD units a conversion factor of 0.013 was applied, according to the specifications provided by the light source manufacturer. Four PPFDs calculated were: 96.9, 99.6, 101.2, and 102.7 μmol m⁻² s⁻¹ for T-1, T-2, T-3, and T-4, respectively. Differences in temperature of the air chamber inside the culture tubes were below 1 °C, as determined by K-type bead probes (thermocouple probe model TP-K01) coupled to a Data Logger Thermometer 4KDATLOG (TC Direct) (Fig. S2).

Experimental design and determinations

Twenty-four explants of each cultivar were incubated under the different light conditions for 35 days. At the end of this period, growth (estimated as the difference between the initial size of a explant and the length of the longest leaf in the culture tube) and multiplication ratio (the number of newly formed shoots around the initial one) were determined in a non-destructive way. Then, the aerial parts of half of the plant materials were frozen and homogenised using liquid nitrogen and stored at –80 °C until used for further analysis. The remaining shoots were rooted and transferred to a greenhouse for ex vitro determinations.

Extracts for spectrophotometric analyses were prepared by mixing frozen shoot powders (0.1 g) with methanol (3 mL) for 60 min in the dark. Photosynthetic pigments contents were calculated according to Lichtenthaler and Wellburn (1983) using the following equations: Chl_a = 15.65 A₆₆₆ – 7.34 A₆₅₃; Chl_b = 27.05 A₆₅₃ – 11.21 A₆₆₆; Cx + c = (1000 A₄₇₀ – 2.86 Chl_a – 129.2 Chl_b)/245, where Chl_a, Chl_b, and Cx + c denote concentrations of chlorophyll *a*, chlorophyll *b*, and total carotenoids, respectively, expressed in μg pigment per mL extract. Values obtained were transformed to express the results on a tissue-weight basis.

Antioxidant capacity of methanolic extracts was determined according to Pérez-Tortosa et al. (2012) using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH; Sigma-Aldrich) as electron acceptor. All the spectrophotometric

determinations were carried out in quadruplicate using a Shimadzu UV-1603 spectrophotometer.

Transfer of plantlets to ex vitro conditions

Plants from light treatments were transferred for rooting to MS medium supplemented with 1.25 mg L^{-1} 1-naphthalene acetic acid (Sigma-Aldrich) for 30 days at 25°C under $96.9 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD for 16 h day^{-1} photoperiod. After this period rooted plantlets were transferred to jiffy pots and incubated for 45 days in a greenhouse provided with a fog system. Finally, plants were transferred to plastic pots (21 cm in diameter) and kept in the greenhouse ($25\text{--}28^\circ\text{C}$, 40–50% relative humidity, 400–450 ppm air CO_2 concentration, as determined using a Temp/RH/ CO_2 Hand-Held Meter, model 3440, provided by Spectrum Technologies Inc.), without artificial light supplementation, until harvesting of inflorescences (70 days after transference to plastic pots). Number of inflorescence stems per plant, length of inflorescence stems and number of both spikes and branches per stem were determined at harvest time.

Statistical analysis

Four independent replicates of every light treatment were carried out and results were expressed as mean $\pm$ standard deviation. Data collected on growth (length of the longest leaf minus the initial size of the explant), multiplication ratio, photosynthetic pigments contents, antioxidant capacities, and parameters related to ex vitro yield and quality were treated statistically using SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA, followed by Tukey's HSD test, was performed to analyze the significant differences between data ($P < 0.05$).

Results and discussion

In vitro growth and multiplication rate

The growth of the four blue *L. sinuatum* cultivars assayed was affected by the proximity to the light source. Increase in light intensity tended to provoke increase in growth, defined as the difference in size between the longest leaf at the end of the treatment and the initial explant (Fig. 1a). From a quantitatively point of view, this effect was observed to be more pronounced for the cultivars SN8925 and SN8946 than for the two others. Thus, the light treatment T-4 resulted in significant increase in growth of about 48 and 35% when compared to the growth observed for T-1 in cultivars SN8925 and SN8946, respectively (Figs. 1a, S1b, and S1c). Regarding the multiplication ratio, no significant differences between light treatments were observed (Fig. 1b).

Fig. 1 Effect of light treatments on in vitro growth (estimated as the difference between the length of the longest leaf at the end of the treatment and the size of the initial explant) (a), and multiplication ratio (b) of the four *L. sinuatum* blue cultivars studied after 35 days of culture. Data are presented as mean $\pm$ standard deviation. For each cultivar, different letters indicate significantly different values following Tukey's HSD test ($P < 0.05$)

It is well-established that quantity of light can affect dramatically plant performance. In this way, Alvarenga et al. (2015) found that a difference of only $8 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in PPFD (from 27 to $35 \mu\text{mol m}^{-2} \text{ s}^{-1}$) resulted in a reduction of about 36% in shoot length of *Achillea millefolium* cultured in vitro, whereas an increase in PPFD of the same order (from 35 to $47 \mu\text{mol m}^{-2} \text{ s}^{-1}$) was demonstrated to significantly increase this parameter. In the present study, despite the fact of using a narrow range of PPFD, increase in growth could be seen upon increasing light intensity for the four cultivars assayed (Fig. 1a). Overall, these observations reinforce the view that it is necessary to accurately set environmental conditions of in vitro culture for each species/cultivar to optimise explant performance.

Light intensity can also determine the regeneration ability of explants. Nameth et al. (2013) showed that the exposure of explants to approximately $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD for only a few hours after excision was enough to impair shoot

regeneration. According to these authors, even inadvertent light exposure (e.g., ambient light or fluorescent tubes in the laminar air flow hood) immediately after explant excision could account for the variability observed in some assays regarding in vitro regeneration rates (Nameth et al. 2013). Since no significant decreases were observed in multiplication ratio, the results shown in Fig. 1b seem to exclude negative effects of the PPFD applied on regeneration ability of the static cultivars used in this study. Therefore, it is possible to reduce the distance between culture tubes and light sources without negatively affecting multiplication ratio of static cultivars. This, in turn, would lead to a more efficient use of the available space in growth rooms.

Levels of photosynthetic pigments and antioxidant capacity

The success of commercial micropropagation is not only a matter of quantity but also, and perhaps more importantly, it is a matter of quality of plants produced. Levels of photosynthetic pigments (chlorophylls and carotenoids) can be considered as reliable markers of plant vitality and give an indirect estimation of photosynthetic performance. Chlorophyll contents in static cultivars are shown in Table 1, where it can be observed that cultivars responded differently to light treatments.

The chlorophyll *a* to chlorophyll *b* (chl *a/b*) ratio has been linked to chloroplast protein composition and structure, nitrogen partitioning between photosynthetic protein complexes, and light environment of the samples analysed (Fritsch and Ray 2007, and references therein). In the present study no statistically significant changes in the chl *a/b* ratio could be observed (data not shown). However, when the chl *a/b* ratio was represented against total chlorophyll contents, a similar decreasing trend for three cultivars (i.e., SN8925, SN8946 and SN89QX) was observed (Fig. 2a, b, d), indicating that the increase in total chlorophyll contents was mainly related to increased sizes of photosystem II antenna in these cultivars. Kitajima and Hogan (2003) also described negative correlations between the chl *a/b* ratio and total chlorophyll contents in four Bignoniaceae species and reported marked differences between species in the

Fig. 2 Relationships between the chl *a/b* ratio and total chlorophyll contents in the four *L. sinuatum* cultivars after 35 days of in vitro culture. **a** cv. SN8925, **b** cv. SN8946, **c** cv. SN9022, **d** cv. SN89QX. The correlation line for all the data in the plot is shown for each cultivar

strength of the correlations found. In the present work cultivar SN8946 showed the strongest, statistically significant ($P < 0.05$), negative correlation between those parameters (Fig. 2b), which could indicate a higher sensitivity of this cultivar to changes in growth irradiation on a long-term basis.

Cultivar SN9022 was the only one that showed a slightly positive slope for the regression line when the chl *a/b* ratio was plotted against total chlorophyll contents (Fig. 2c). Taking into account the low value of chl *a/b* ratio determined and that chlorophyll *a* can be considered as a bottleneck in the use of light for photosynthesis (Blackburn 2007), it is tempting to suggest that the size of photosystem II antenna was large enough to provide excitons to the core complex which would be operating nearly at its maximum capacity. Whatever the case, the chl *a/b* ratio showed relatively small changes in relation to changes in total chlorophyll contents for the cultivar SN9022.

Carotenoid pigments are also associated with the photosynthetic complexes and they participate in photon harvesting and in the dissipation of excess energy, thus contributing to maintain the correct structure and function

Table 1 Effect of light treatments on total chlorophyll contents in the four *L. sinuatum* cultivars after 35 days of in vitro culture

Treatment	Total chlorophyll contents ($\mu\text{g g}^{-1}$ FW)			
	SN8925	SN8946	SN9022	SN89QX
T-1	1272.93 $\pm$ 192.75 ^a	651.13 $\pm$ 51.78 ^{ab}	1001.45 $\pm$ 51.46 ^a	779.21 $\pm$ 18.36 ^c
T-2	972.46 $\pm$ 20.32 ^a	491.28 $\pm$ 9.18 ^b	758.87 $\pm$ 256.86 ^{ab}	1140.22 $\pm$ 42.72 ^b
T-3	834.76 $\pm$ 38.57 ^{ab}	649.97 $\pm$ 94.16 ^{ab}	436.01 $\pm$ 55.47 ^b	1337.45 $\pm$ 46.28 ^a
T-4	625.86 $\pm$ 133.56 ^b	906.78 $\pm$ 2.17 ^a	955.61 $\pm$ 44.01 ^a	1152.90 $\pm$ 41.27 ^b

Data are presented as mean $\pm$ standard deviation. For each cultivar, different letters indicate significantly different values following Tukey's HSD test ($P < 0.05$)

of the photosynthetic apparatus. Total carotenoid levels tended to reach a maximum at light treatment T-3 for the cultivars SN8946 and SN9022, and at treatment T-2 for the cultivar SN8925, whereas the cultivar SN89QX showed practically no variation irrespective of the light treatment applied (Fig. 3a). Increased carotenoid to chlorophyll ratio in response to increased light intensity has been reported to occur in other plant materials cultured in vitro and proposed to be a protective mechanism against excessive light absorption (Jo et al. 2008; Lee et al. 2007). Interestingly, Fig. 3a also shows a decline in the relative concentration of total carotenoids at high light intensities for all the cultivars except SN89QX. This fact is apparently difficult to reconcile with the above-mentioned protective role of carotenoids. However, it must be taken into account that compounds other than carotenoids could also accumulate in chloroplasts under relatively severe stress conditions, contributing to prevent photo-oxidative damage (discussed in Brunetti et al. 2015). Close et al. (2001) reported that carotenoid-based defence mechanisms in

leaves of *Eucalyptus nitens* seedlings subjected to severe photoinhibitory conditions did not have enough capacity to dissipate excess light energy, suggesting the involvement of phenolic compounds. According to Brunetti et al. (2015) there would exist several defence lines in the chloroplast that are being engaged consecutively in photoprotection as the stress level increases. So, the first line of defence would be constituted by ascorbic acid and glutathione and when light stress becomes more severe (or in chronic stress conditions), activation of the corresponding biosynthetic genes would lead to the accumulation of carotenoids and subsequently to the synthesis of other secondary metabolites, including phenylpropanoids and isoprenoid compounds (Brunetti et al. 2015). Thus, the decrease in the levels of carotenoids in the cultivars SN8925, SN8946, and SN9022 observed in this study at relatively high light treatments could be accompanied by an increase of other metabolites, probably resulting in the enhancement of antioxidant capacity of tissues.

Antioxidant capacity can be considered as a marker of stress in in vitro-cultured plant materials (López-Orenes et al. 2013). High levels of this parameter have been thought to be crucial for tolerance of plants to oxidative stress derived from unfavourable growth conditions. In the present work, the observed decrease of both carotenoid to chlorophyll and chl *a/b* ratios for some cultivars at the highest light intensities assayed suggests that those plant materials experienced some degree of stress (Shi et al. 2015). Figure 3b shows the antioxidant capacity values determined in the static cultivars studied. From this figure it can be seen that antioxidant capacity in all the cultivars studied, with the exception of SN89X, showed a similar increasing trend with increasing light intensity.

Antioxidant capacity differed among cultivars, cultivars SN9022 and SN8925 being those in which the highest and the lowest levels, respectively, of this parameter were determined. Relative variation of antioxidant capacity with respect to PPFD between extreme treatments was maximal for cultivar SN8925 (3.2-fold increase in T-4 over T-1), followed by cultivars SN9022 and SN8946 with similar increases (around 1.3-fold), whereas cultivar SN89QX showed a low, non-statistically significant ($P < 0.05$), variation (4% increase). It can be seen from comparison between Fig. 3a, b that the decline in carotenoid concentrations observed in T-4 was associated with an increase in antioxidant capacity (with the exception of SN89QX cultivar). Although phenolic compounds could be the candidates to take over a primary role in the control of redox status in plants under moderate light stress, deeper studies are necessary to confirm this possibility.

Fig. 3 Effect of light treatments on total carotenoid contents and antioxidant capacity in the four *L. sinuatum* blue cultivars after 35 days of in vitro culture. **a** Carotenoids (expressed as total carotenoids to total chlorophyll ratio). **b** Antioxidant capacity (expressed as µmol DPPH reduced per gram fresh weight). Data are presented as mean $\pm$ standard deviation. For each cultivar, different letters indicate significantly different values following Tukey's HSD test ($P < 0.05$)

Productivity parameters of in vitro-derived plants under greenhouse conditions

One of the major concerns associated with the use of micropropagation techniques is its possible negative impact on crop productivity by decreasing the quantity and/or quality of the final product. Survival of plantlets after transfer to ex vitro conditions can be affected by conditions applied during in vitro culture. Lian et al. (2002) reported a slight decrease in ex vitro survival (from 88 to 84%) when in vitro plantlets of a *Limonium* hybrid were grown under high light intensity and heterotrophic conditions. In the present work, survival rate was higher than 85% irrespective of the light treatment applied (data not shown) which was considered to be acceptable from the standpoint of commercial exploitation.

Production parameter, expressed as number of stems per acclimated plant, did not significantly change irrespective of the light treatment applied during the in vitro multiplication stage (Table 2) and it was always above the standard required for commercial purposes (8 stems per plant). Quality parameters also showed no significant differences with respect to light treatment (Table 2). However, differences in stem length, number of spikes per stem, and number of branches per stem could be observed among the cultivars studied. Marketable statice plants are those with a stem length greater than 60 cm and this requirement was fulfilled by practically all the cultivars and treatments (Table 2). From Table 1 it can be seen that SN9022 and especially SN89QX were the cultivars with the longest flower stems. In addition, SN89QX also stood out among cultivars because of its higher number

of spikes per stem and greater stem branching. Even so, all the blue cultivars of *L. sinuatum* studied met the quality requirements for their commercialisation (minimum of 6 spikes per stem and number of branches per stem equal or superior to 3) (Fig. S3). Stem length has been widely recognized as a quality criterion in statice production. The results of this study show that micropropagation of *L. sinuatum* produces flower stems whose marketability is comparable to those produced by conventional techniques (Mellesse et al. 2013; Verlinden and McDonald 2007).

Conclusion

Reduction in distance between light source and culture flasks induced only small variations in the biochemical parameters analysed, but did not affect productivity figures after transfer of micropropagated plantlets to production conditions. Although differences in light intensity between treatments during the in vitro multiplication stage were small, this study shows that the *L. sinuatum* cultivars assayed were able to differentially respond to light conditions by modifying their metabolism. Since no negative carry over effects on statice productivity could be observed, a closer distance between culture tubes and light sources during the in vitro multiplication stage could lead to the optimization of growth room use and hence might help to reduce wearing-out costs associated to the micropropagation of four blue cultivars of *L. sinuatum* studied.

Table 2 Effect of light treatments during in vitro stage of micropropagation on the ex vitro performance of the four *L. sinuatum* blue cultivars after 70 days in the greenhouse

Cultivar	Treatment	Production (stems/plant)	Commercial quality		
			Stem length (cm)	Spikes/stem	Branches/stem
SN8925	T-1	9.73 ± 0.35 ^a	58.33 ± 3.33 ^a	7.33 ± 0.33 ^a	4.67 ± 0.33 ^a
	T-2	10.43 ± 0.73 ^a	61.67 ± 1.67 ^a	7.00 ± 0.58 ^a	4.00 ± 0.58 ^a
	T-3	9.90 ± 0.38 ^a	61.67 ± 3.33 ^a	9.00 ± 0.58 ^a	4.33 ± 0.33 ^a
	T-4	9.60 ± 0.32 ^a	61.67 ± 3.33 ^a	8.00 ± 0.58 ^a	4.67 ± 0.33 ^a
SN8946	T-1	9.17 ± 0.20 ^a	58.33 ± 3.33 ^a	7.00 ± 1.00 ^a	5.00 ± 0.58 ^a
	T-2	9.10 ± 0.41 ^a	58.33 ± 3.33 ^a	7.00 ± 0.58 ^a	5.33 ± 0.33 ^a
	T-3	10.00 ± 0.76 ^a	60.00 ± 2.88 ^a	6.67 ± 0.33 ^a	5.33 ± 0.67 ^a
	T-4	9.37 ± 0.29 ^a	58.33 ± 1.67 ^a	7.00 ± 0.58 ^a	5.67 ± 0.33 ^a
SN9022	T-1	9.30 ± 0.15 ^a	70.00 ± 5.00 ^a	5.33 ± 0.33 ^a	4.67 ± 0.33 ^a
	T-2	9.43 ± 0.37 ^a	70.00 ± 2.89 ^a	6.00 ± 0.58 ^a	4.00 ± 0.58 ^a
	T-3	9.05 ± 0.18 ^a	75.00 ± 0.00 ^a	6.33 ± 0.33 ^a	4.67 ± 0.33 ^a
	T-4	9.80 ± 0.17 ^a	75.00 ± 0.00 ^a	6.00 ± 0.58 ^a	4.33 ± 0.33 ^a
SN89QX	T-1	9.30 ± 0.15 ^a	73.33 ± 0.00 ^a	12.33 ± 0.88 ^a	6.33 ± 0.33 ^a
	T-2	9.30 ± 0.21 ^a	75.00 ± 0.00 ^a	13.33 ± 0.88 ^a	6.33 ± 0.33 ^a
	T-3	9.47 ± 0.29 ^a	75.00 ± 0.00 ^a	14.33 ± 0.88 ^a	6.33 ± 0.58 ^a
	T-4	9.60 ± 0.26 ^a	75.00 ± 0.00 ^a	13.33 ± 0.67 ^a	7.00 ± 0.19 ^a

Data are presented as mean ± standard deviation. For each cultivar, different letters indicate significantly different values following Tukey's HSD test ($P < 0.05$)

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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