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Effects of Polyploidy on Physiological Performance of Acclimatized *Solanum betaceum* Cav. Plants under Water Deficit

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Abstract: The urgent need to identify stress-tolerant genotypes and understand their inherent genetic plasticity is one of the major targets of research and breeding programs. Species that are cultivated in areas that are prone to drought need to be able to tolerate water stress (WS) while still displaying features that are economically valuable. Tamarillo (*Solanum betaceum*) is a solanaceous fruit crop with increasing agronomic interest due to the nutritional properties of its edible fruits and its biotechnological potential. Several protocols have been established for the in vitro culture of this species and controlled hybridization, as well as for the induction of tetraploidy. Nevertheless, the impact of WS on *S. betaceum* performance has been poorly studied, and nothing is known about the role of ploidy status on this response. Since no morphological differences were noticed between diploids and tetraploids at the end of the acclimatization period, we hypothesized that ploidy level may have a role in plant drought responses. Thus, micropropagated and acclimatized tamarillo diploid (2n = 2x = 24) and tetraploid (4n = 4x = 48) plants were exposed to WS, and several physiological parameters were evaluated, such as plant growth, water potential, photosynthetic performance, sugars, proline, and MDA levels. Water stress did not affect plant growth in both diploids and tetraploids, but it induced stomatal closure and reduced the net CO_2 assimilation rate. Water stress also reduced the photosynthetic efficiency of PSII, but no differences were found in the total chlorophyll content. From all the parameters analyzed, tetraploid plants showed a better response under water shortage conditions when considering water potential (WP). Metabolite analysis indicated no significant differences in the accumulation of soluble sugars and MDA in WS plants but a significant increase in proline accumulation in diploids exposed to WS. These observed differences in parameters such as WP and proline accumulation point to mechanisms of osmoregulation and stress signaling that differ between diploid and tetraploid plants, particularly in WS conditions, demonstrating that tetraploids can adapt better to water shortage conditions than their diploid counterparts.

Keywords: fruit crop; ploidy; proline; sugars; water deficit

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1. Introduction

Tamarillo (*S. betaceum* Cav.) is a small solanaceous tree, also commonly known as tree tomato or 'tomate de arbol', which produces edible and highly nutritious fruits from which derived products might be produced, such as smoothies and jams. Originating in the Andean regions of Argentina and Bolivia, it has spread worldwide, including to the Portuguese islands of the Azores and Madeira [1]. The high nutritional value of its fruits, with significant concentrations of vitamins, minerals, and bioactive components, such as anthocyanins, carotenoids, and flavonoids [2,3], makes this species an important resource whose beneficial properties for human health should be explored [4]. Nevertheless, tamarillo is still considered a NUC (Neglected and Underutilized Crop).

Seed germination is the easiest way to get tamarillo plants, and this is one of the most often used techniques of propagation. However, the genetic heterogeneity of these plants

makes this approach inappropriate when the objective is to propagate selected genotypes. Tissue culture includes several in vitro methods for the propagation (micropropagation) of selected plants in an artificial medium and under aseptic conditions. The need to rapidly multiply important tamarillo cultivars can be overcome by this technique, which has great potential to facilitate rapid propagation or support and accelerate breeding programs for this species. Micropropagation of tamarillo through axillary shoot proliferation was the first method to be applied [5] and is the most suitable method for large-scale production of superior genotypes. Shoot regeneration has also been observed from hypocotyls, cotyledons, root explants, leaf discs, and petioles [6] and somatic embryogenesis induction has been reported from several initial explants (reviewed by ref. [7]).

The potential gains of in vitro propagation will be negligible if micropropagated, acclimatized plantlets are not resilient enough to further cope with field conditions. During transplantation, and even after establishment, they must tolerate sub-optimal growth conditions such as water deficit. In fact, though several reports on micropropagation state that hardened plantlets are transplanted to potting mixtures, only a few tested their performance under conditions that they may encounter in the field [4]. Drought is the most significant environmental stress in agriculture worldwide [8], and therefore, the question of tolerance to water deficits is an important attribute of propagules and a key question that deserves intensive research. The risk of summer drought is likely to increase in Central Europe and in the Mediterranean area [9], which poses significant threats to plant field establishments and productivity. More than ever, improving yield and selecting for drought tolerance are major goals in plant breeding programs, which rely on better understanding the impact of drought on plant morpho-physiological attributes [8].

The induction of polyploidy is reported as an efficient way to improve drought stress tolerance in plants by producing differences in plant morphological and anatomic characteristics, physiology, biochemistry, and gene expression [10,11]. For example, polyploid plants of *Coccinia palmata* [12], *Dendranthema nankingense* [13], *Citrus* sp. [14], *Arabidopsis thaliana* [15] and *Oryza sativa* [16] are more drought-tolerant than their diploid counterparts. Triploid and tetraploid seedlings have been rarely found in commercial orchards of diploid tamarillo plants and are thought to have arisen from the union of unreduced gametes [6]. Induction of tamarillo tetraploids by applying colchicine to germinated seeds was also first reported by Pringle and Murry [17], but polyploids, both spontaneous and induced, showed low fertility [6]. Previous experiments carried out in our laboratory allowed for the production of tamarillo tetraploid plants through the in vitro culture of nodal segments in the presence of c-mitotic agents [18].

In this context and considering the significant challenges humanity is facing in relation to food supply and climate change, understanding the role that polyploidy plays in enhancing plant tolerance to various types of stresses and in expanding the range of conditions for plant establishment may lead to better breeding and crop-improvement strategies. However, several questions remain, and the knowledge available is not applicable to all experimental systems [19].

The results obtained so far in Tamarillo enabled the development of a well-established procedure for mass micropropagation of various genotypes and varieties, including induced autotetraploid plants, through axillary shoot proliferation. The objective of this work was to evaluate the physiological performance of tamarillo diploid and tetraploid micropropagated acclimatized plants under water stress conditions. We hypothesized that the ploidy level may have a role in plant drought responses despite no morphologically noticeable differences between two plant types at the end of the acclimatization period (e.g., height, rooting performance). The physiological implications of water limitation on tamarillo plant responses with different ploidy levels were studied in this study, which included the monitoring of key plant functions and cellular processes targeted by stress, such as changes in water plant status, photosynthetic performance, photosynthetic pigments, oxidative status, and osmoregulation, as well as morphometric parameters.

2. Materials and Methods

2.1. Establishment of Shoot Cultures

Shoot cultures were established and propagated from one in vitro germinated seedling (a diploid line) and from one tetraploid line. The tetraploid plants were obtained in previous work at the Plant Biotechnology Laboratory at the University of Coimbra by the in vitro culture of nodal segments in the presence of c-mitotic agents [18]. Each of the lines was obtained from a single genotype through the successive culture of shoot tips (1.5 cm) in test tubes containing Murashige and Skoog (MS) medium [20] supplemented with 0.07 M sucrose, 0.8 µM benzyladenine (BA), and 0.6% (*w*/*v*) agar. Ploidy levels were confirmed by Feulgen staining and flow cytometry, as described in ref. [18]. Shoot cultures were subcultured monthly on a fresh medium of the same composition. Shoots (3 cm) were rooted in an MS medium supplemented with 0.07 M sucrose and 0.6% (*w*/*v*) agar, without plant growth regulators (PGRs). All the cultures were kept in a growth chamber at 24 °C under a 16h photoperiod at 15–20 µmol m^{-2} s^{-1} (cool-white fluorescent lamps). The ploidy of the plants was confirmed by flow cytometry before the assay (data not shown).

2.2. Plant Material and Experimental Design

The experiment was conducted in a controlled climatic chamber with a temperature of 24 °C, a 16/8 h (day/night) photoperiod, 70% relative humidity (RH) and a photosynthetic photon flux density (PPFD) of app. 15–20 µmol m^{-2} s^{-1}. Twelve rooted diploid (2n) and tetraploid (4n) plants of tamarillo with 5–8 cm length were grown in 1L plastic pots filled with 3:2 (*w*/*w*) peat:perlite. The potted plants were acclimatized for 8 weeks inside the chamber while being watered with tap water. The pots were randomly arranged, periodically moved to the neighboring position during the whole experiment and well-watered to 70% of field capacity (FC), prior to application the water limitation treatment. Then two groups for each plant propagation type were established. One control group of well-watered (WW) plants was kept at 70% FC, and a second group was randomly assigned to water deficit conditions (WS) by water withholding for 4 days. After this period (4 days) of experimentation, the following parameters were recorded: growth, leaf gas exchange, chlorophyll a fluorescence, and water potential. Leaves were harvested and immediately frozen in liquid nitrogen for further biochemical analysis (estimation of lipid peroxidation, photosynthetic pigment content, total soluble sugars and starch, proline).

2.3. Growth and Plant Water Potential

To assess plant growth, the shoot and root lengths, plant height, and total dry biomass of six plants were recorded for each treatment. For biomass measurement, the dry weight (DW), was determined after drying the entire plant at 80 °C for 3 days). Midday shoot water potential (WP, Ψmd) was measured with a Scholander-type pressure chamber (PMS Instrument Co., Albany, OR, USA) in six plants per treatment at 12 h 30 m (solar time) as described by Correia et al. [21] to control the plant water status. Measurements were performed on five individuals per treatment. Four leaf discs (diameter = 11 mm) per individual (six individuals per treatment) were also collected to determine relative water content (RWC), by using the following equation: RWC = (FW-DW)/(TW-DW) × 100, where FW is the fresh weight, TW is the turgid weight after rehydration of the leaf discs for 24 h at 4 °C in the dark, and DW is the dry weight after oven-drying the leaf discs at 70 °C until constant weight.

2.4. Leaf gas-Exchange Measurements

Net CO_2 assimilation rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration content (Ci, vpm) were measured in six plants per treatment using a portable infrared gas analyser (LCpro-SD, ADC BioScientific Ltd., Hoddesdon, UK) equipped with the broad leaf chamber. To find out the saturation light intensity, A/PPFD (photosynthetic photon flux density; light response curves of CO_2 assimilation) curves were performed

with the following PPFD: 2000, 1500, 1000, 750, 500, 250, 100, 50, and 0 μmol m^{-2} s^{-1}. After A/PPFD data analysis, punctual measurements at saturation light intensity were performed at 750 μmol m^{-2} s^{-1}. The following conditions were maintained inside the chamber during all the measurements: an air flux of 200 mol s^{-1}; a block temperature of 25 °C; and atmospheric CO_2 and H_2O concentrations. Data were recorded when the measured parameters were stable (2–6 min). Water use efficiency (WUEi) was calculated as the ratio between CO_2 assimilation and transpiration (ratio A/gs).

2.5. Photosynthetic Pigments and Chlorophyll a Fluorescence Analysis

Total chlorophyll and carotenoid content were quantified according to Sims and Gamon [22]. Pigments were extracted with an acetone/Tris (50 mM) buffer at pH 7.8 (80:20) (*v*/*v*). After homogenization and centrifugation, the supernatants were used to read absorbance at 663, 537, 647, and 470 nm (Thermo Fisher Scientific Spectrophotometer, Genesys 10-uv S), and the pigments' content was determined.

Steady-state modulated chlorophyll fluorescence was determined with a portable fluorometer (Mini-PAM; Walz, Effeltrich, Germany) on the same leaves as used for the gas-exchange measurements. Light-adapted components of chlorophyll fluorescence were measured: steady-state fluorescence (F), maximal fluorescence (F'm), variable fluorescence F'v (equivalent to F'm-F), and the quantum yield of PSII photochemistry (ϕPSII), which is equivalent to (F'm-F)/F'm. Leaves were then dark-adapted for at least 20 min to obtain F0 (minimum fluorescence), Fm (maximum fluorescence), Fv (variable fluorescence, equivalent to Fm-F0) and Fv/Fm (maximum quantum yield of PSII photochemistry).

2.6. Lipid Peroxidation and Proline Content

The extent of lipid peroxidation in leaves was estimated by measuring the amount of malondialdehyde (MDA), following the procedure described by Hodges et al. [23] with small adaptations [21]. About 100 mg of leaves were ground in 2.5 mL cold 0.1% trichloroacetic acid (*w*/*v*) and centrifuged. 250 μL of supernatant was then added to 1 mL of 20% TCA (*w*/*v*) in 0.5% TBA (*w*/*v*) (positive control) and 250 μL of supernatant to 1 mL of 20% TCA (*w*/*v*) without TBA (negative control). Both aliquots were heated at 95 °C for 30 min, and the reaction was stopped immediately by putting the tubes on ice. After centrifugation, absorbance was read at 440, 532, and 600 nm, and MDA content was estimated by the formulae [23].

Proline content was determined according to Khedr et al. [24]. About 0.1 g of frozen leaf was homogenized in 1.5 mL of 3% sulphosalicylic acid, and the mixture was centrifuged (10,000× *g*, 10 min, 4 °C). Ninhydrin and glacial acetic acids were added to the supernatant and incubated for 1 h at 100 °C. Toluene was also added to the mixture, and the formation of two phases was observed. The chromophore-containing toluene phase was read at 520 nm, and proline concentration was determined from a L-proline standard curve (0–50 μg/mL).

2.7. Total Soluble Sugars and Starch

Total soluble sugars (TSS) were determined by the anthrone method, as described by Irigoyen et al. [25]. Briefly, TSS were extracted from 50 mg of frozen leaves using 80% (*v*/*v*) ethanol at 80 °C for 1 h. After centrifugation, the supernatant was mixed with 1.5 mL of anthrone and incubated at 100 °C for 10 min. Absorbance was read at 625 nm, and TSS content was calculated against a D-glucose standard curve. The pellet resultant from the centrifugation was used to quantify starch, as described by Osaki et al. [26]. The pellet was incubated with 30% (*v*/*v*) perchloric acid at 60 °C for 1 h. The mixture was centrifuged, and anthrone was added to the supernatant. After heating the mixture at 100 °C for 10 min, absorbance was read at 625 nm, and starch content was determined according to a D-glucose standard curve.

2.8. Statistical Analysis

Values were given as means ± standard deviations of 6 replicates for drought stress experimental parameters. Data were analyzed by two-way ANOVA (GraphPad Prism v. 6.01), followed by a Tukey's multiple comparison test ($p < 0.05$).

3. Results

3.1. Acclimatization of Micropropagated Plants

Tamarillo plantlets regenerated in vitro (Figure 1A–C) were successfully acclimatized in the climatic chamber (100% survival). After 8 weeks, the regenerated plants looked healthy and uniform in growth (Figure 1D), with no visible differences between diploid and tetraploid plants.

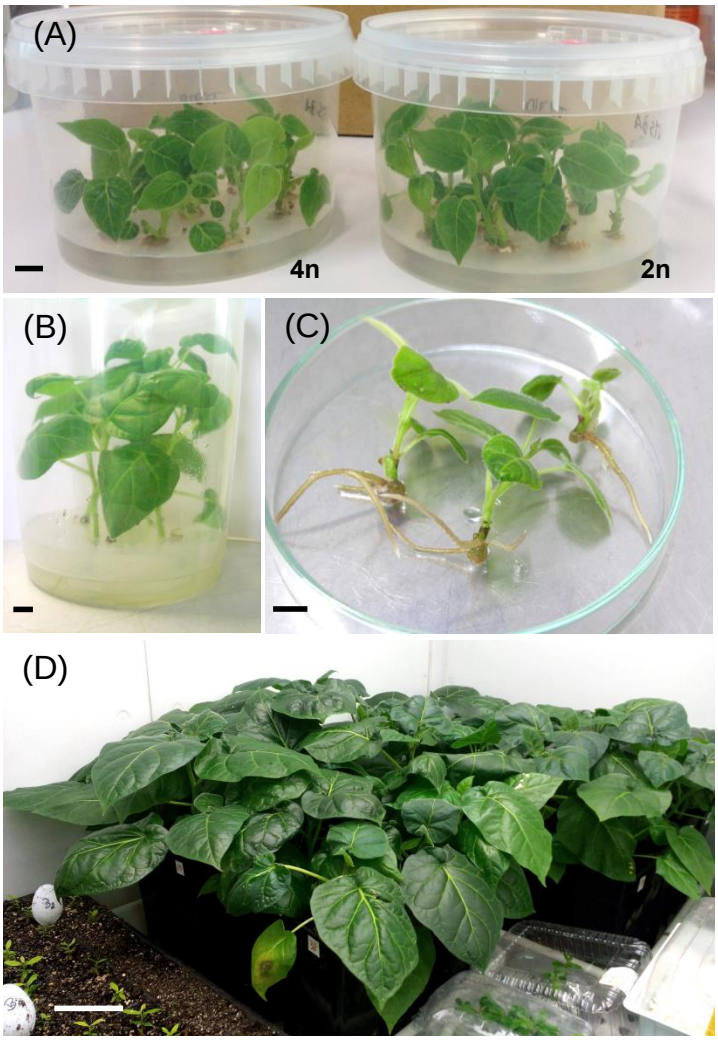

Figure 1. Micropropagation of tamarillo tetraploid (4n) and diploid (2n) plants by axillary shoot proliferation (**A**) and rooting on solid medium (**B**); rooted plants before soil transfer (**C**) (bar = 1 cm) and acclimatized plants after 8 weeks in ex vitro conditions, before the water stress assay (**D**) (bar = 10 cm).

3.2. Responses of Diploid and Tetraploid Plants under Water Deficit

Following the 4 days of water withdrawal, it was already possible to observe clear differences between the behavior of diploid (2n) and tetraploid (4n) acclimatized plants (Figure 2).

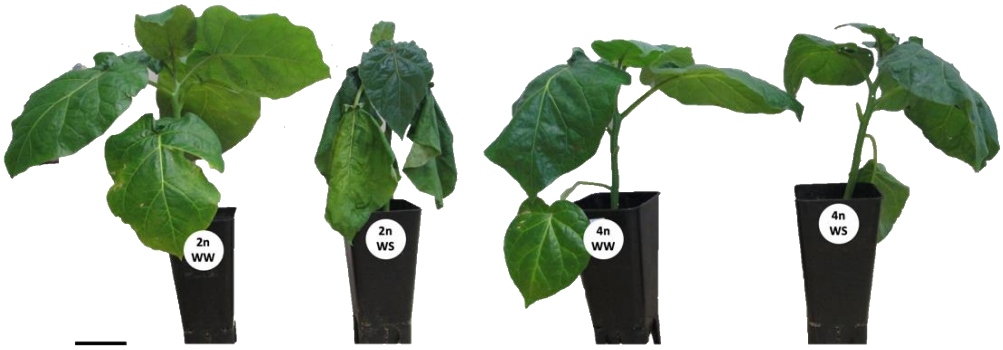

Figure 2. Representative plants of the different treatment groups defined for the water stress experiment: diploid well-watered (2n WW) and water-stressed (2n WS) plants; tetraploid well-watered (4n WW) and water-stressed (4n WS) plants (bar = 10 cm).

For the plant growth parameters (Table 1), total dry biomass, root and shoot length ratio, and plant height were assessed. For these parameters, plant height and shoot length differ significantly, with the diploid plants being taller than the tetraploid plants, particularly in the WW group. Tetraploid plants showed a tendency toward lower dry masses for plants in the WS group when compared with WW plants, which was not the case for diploid plants.

Table 1. Effects of water stress (WS) on growth parameters, total biomass, root and shoot length and plant height of diploid (2n) and tetraploid (4n) acclimatized tamarillo plants in comparison with well-watered (WW) plants. Means ± SDs, n = 6, different letters indicate significant differences between treatments at $p \leq 0.05$.

Treatment	Ploidy	Total Biomass (DW, g)	Root Lenght (cm)	Shoot Lenght (cm)	Plant Height (cm)
WW	2n	5.15 ± 1.18 ^{a}	23.63 ± 4.44 ^{a}	23.00 ± 2.63 ^{a}	46.63 ± 5.34 ^{a}
	4n	4.77 ± 1.18 ^{a}	20.82 ± 1.27 ^{a}	18.58 ± 1.17 ^{b}	39.40 ± 1.10 ^{b}
WS	2n	5.48 ± 1.12 ^{a}	20.37 ± 0.84 ^{a}	20.50 ± 1.51 ^{a,b}	40.87 ± 1.63 ^{b}
	4n	4.12 ± 0.84 ^{a}	21.73 ± 2.24 ^{a}	18.50 ± 0.96 ^{b}	40.23 ± 2.46 ^{b}

To estimate water status under drought conditions, the RWC and WP were also measured. For both parameters, diploid plants submitted to WS differed from those submitted to WW, showing significantly lower values for relative water content and significantly more negative values for water potential (Figure 3). Regarding leaf gas-exchange computed parameters, net photosynthetic rate, transpiration rate, stomatal conductance, the intracellular CO_2 concentration (Figure 4), and intrinsic water use efficiency were analyzed (Figure 3). The net CO_2 assimilation rate (A, Figure 4) was significantly different between WS (lower CO_2 assimilation) and WW plants, for both tetraploid and diploid plants, but with higher values for the tetraploid ones. Such observations were in accordance with the E measurements for both groups and treatments (Figure 4). Transpiration rates were significantly higher in WW plants, but within these groups there were significant differences between diploid and tetraploid plants, the latter showing higher E values. Nevertheless, WUEi showed no significant differences between the WW and WS groups, yet the WW group showed slightly higher WUEi than the WS group (Figure 3). WUEi differences were not significant between WS diploid and tetraploid plants. This behavior of WS tetraploid plants was matched for gs values registered (Figure 4). Stomata were open in WW diploid and tetraploid plants, but gs was significantly reduced in WS groups.

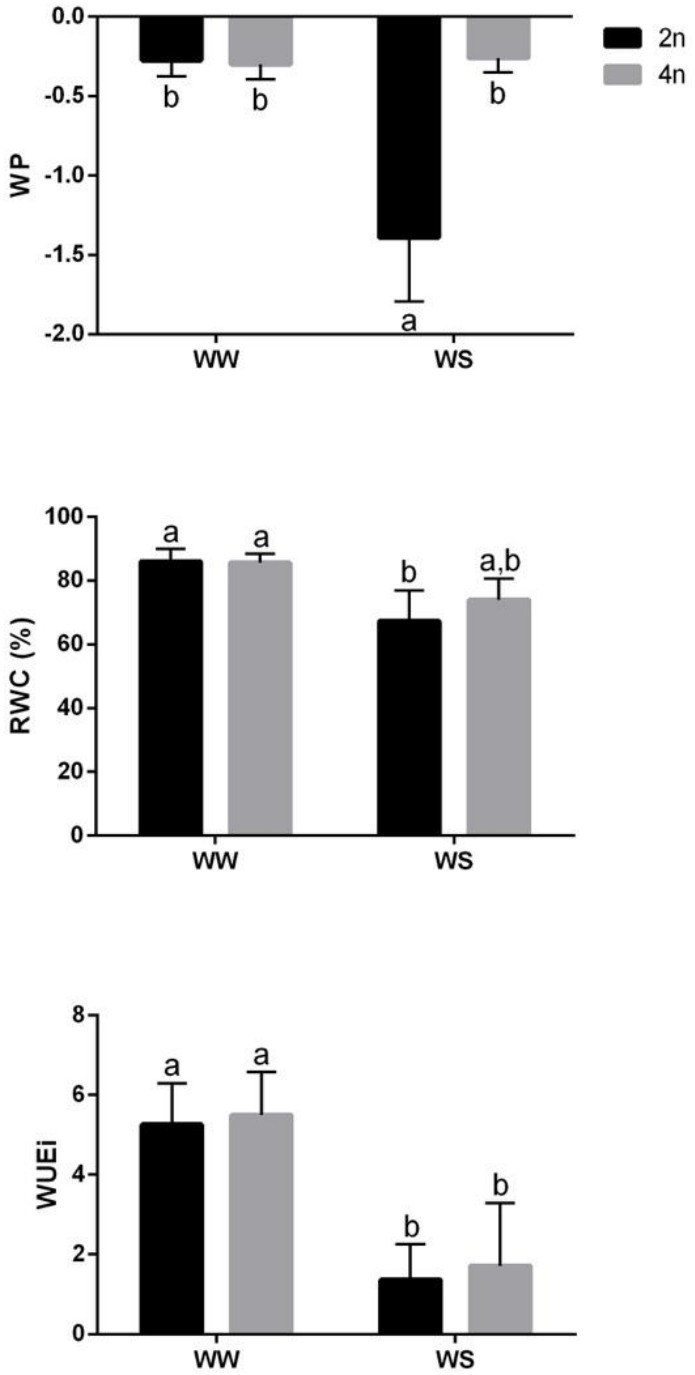

Figure 3. Effects of water stress (WS) on water status parameters, water potential (**WP**, Ψmd) and relative water content (**RWC**) and water use efficiency (**WUEi**) of diploid (2n) and tetraploid (4n) acclimatized tamarillo plants in comparison with well-watered (WW) plants. Means ± SDs, n = 6, different letters indicate significant differences between treatments at $p \leq 0.05$.

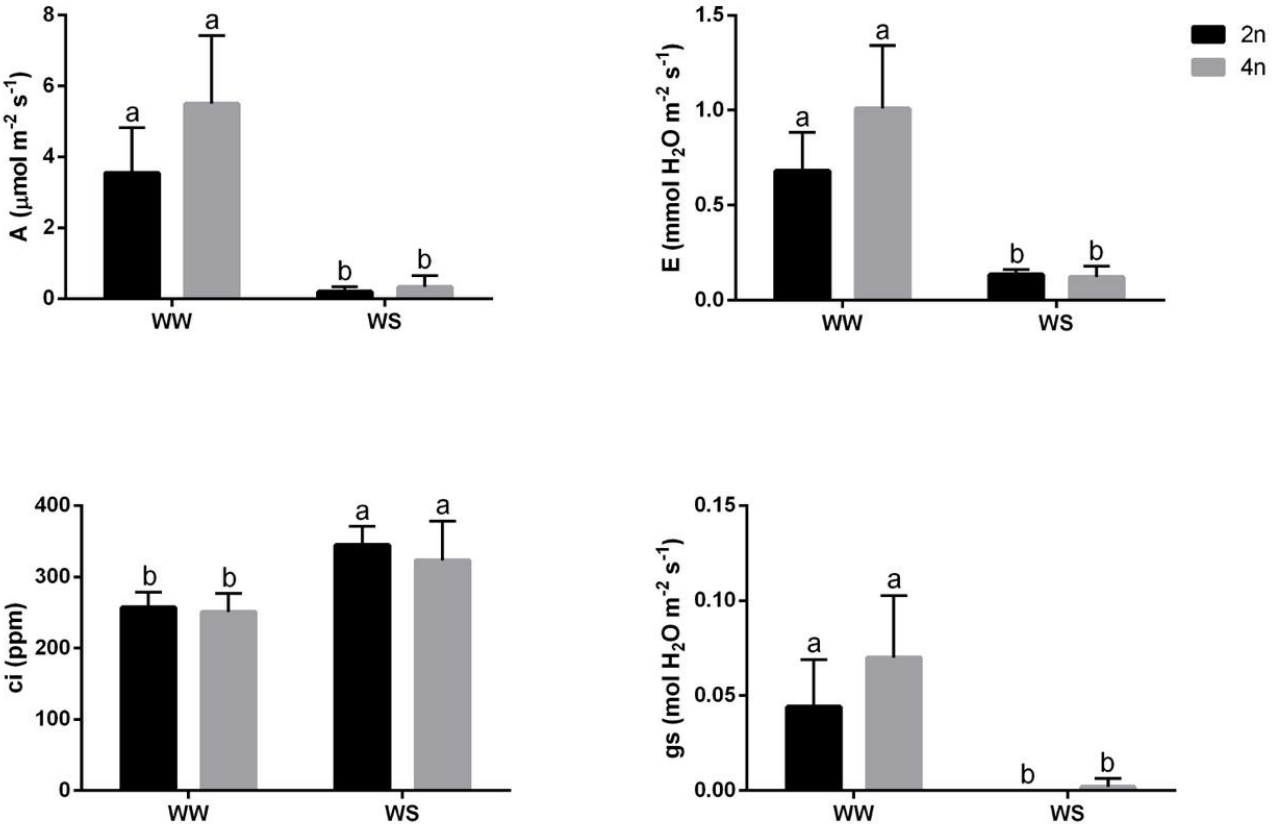

Figure 4. Effects of water stress (WS) on leaf gas exchange parameters of diploid (2n) and tetraploid (4n) acclimatized tamarillo plants in comparison with well-watered (WW) plants. Net CO_2 assimilation rate (**A**), transpiration rate (**E**), intercellular CO_2 concentration content (**Ci**) and stomatal conductance (**gs**). Means ± SDs, n = 6, different letters indicate significant differences between treatments at $p \leq 0.05$.

For intracellular CO_2 concentration (Figure 4), even though no significant differences were registered between 2n and 4n plants, there were significant differences between the WW and WS groups. WS plants showed significantly higher intracellular CO_2 contents.

The quantum yield of photosystem II, ϕPSII (Table 2), was significantly different between the WW and WS groups. As for Fv/Fm, no significant differences were registered between 2n and 4n plants in both WW and WS treatments. Nevertheless, the quantitative analysis of photosynthetic pigments showed no significant differences in pigment levels between plants of different ploidies or between different treatments (Table 2).

Table 2. Effects of water stress (WS) on chlorophyll fluorescence parameters—quantum yield of PSII photochemistry (**ϕPSII**) and maximum quantum yield of PSII photochemistry (**Fv/Fm**) and on the concentration of chlorophyll a (**Chl a**), chlorophyll b (**Chl b**) and carotenoids (**Car**) of diploid (2n) and tetraploid (4n) acclimatized tamarillo plants in comparison with well-watered (WW) plants. Means ± SDs, n = 6, different letters indicate significant differences between treatments at $p \leq 0.05$.

Treatment	Ploidy	Chlorophyll a Fluorescence		Pigment Content (μmol mL^{-1})		
		ϕPSII	Fv/Fm	Chl a	Chl b	Car
WW	2n	0.69 ± 0.02 ^{a}	0.80 ± 0.01 ^{a}	0.0095 ± 0.0015 ^{a}	0.0033 ± 0.0006 ^{a}	0.0043 ± 0.0007 ^{a}
	4n	0.58 ± 0.10 ^{a}	0.82 ± 0.01 ^{a}	0.0093 ± 0.0013 ^{a}	0.0033 ± 0.0004 ^{a}	0.0042 ± 0.0005 ^{a}
WS	2n	0.49 ± 0.10 ^{b}	0.79 ± 0.01 ^{a}	0.0102 ± 0.0008 ^{a}	0.0035 ± 0.0003 ^{a}	0.0049 ± 0.0004 ^{a}
	4n	0.46 ± 0.09 ^{b}	0.80 ± 0.02 ^{a}	0.0084 ± 0.0004 ^{a}	0.0030 ± 0.0002 ^{a}	0.0041 ± 0.0004 ^{a}

3.3. Changes in Proline, MDA and Carbohydrates Content

The diploid plants under WS showed the highest quantities of proline when compared with all the others in the WW and WS groups (Figure 5). There was a slight, but not significant, increase in proline levels between the WW and the WS tetraploids. MDA contents differ significantly between both groups of plants, increasing from the irrigated treatment (WW) to the water stress (WS) conditions (Figure 5).

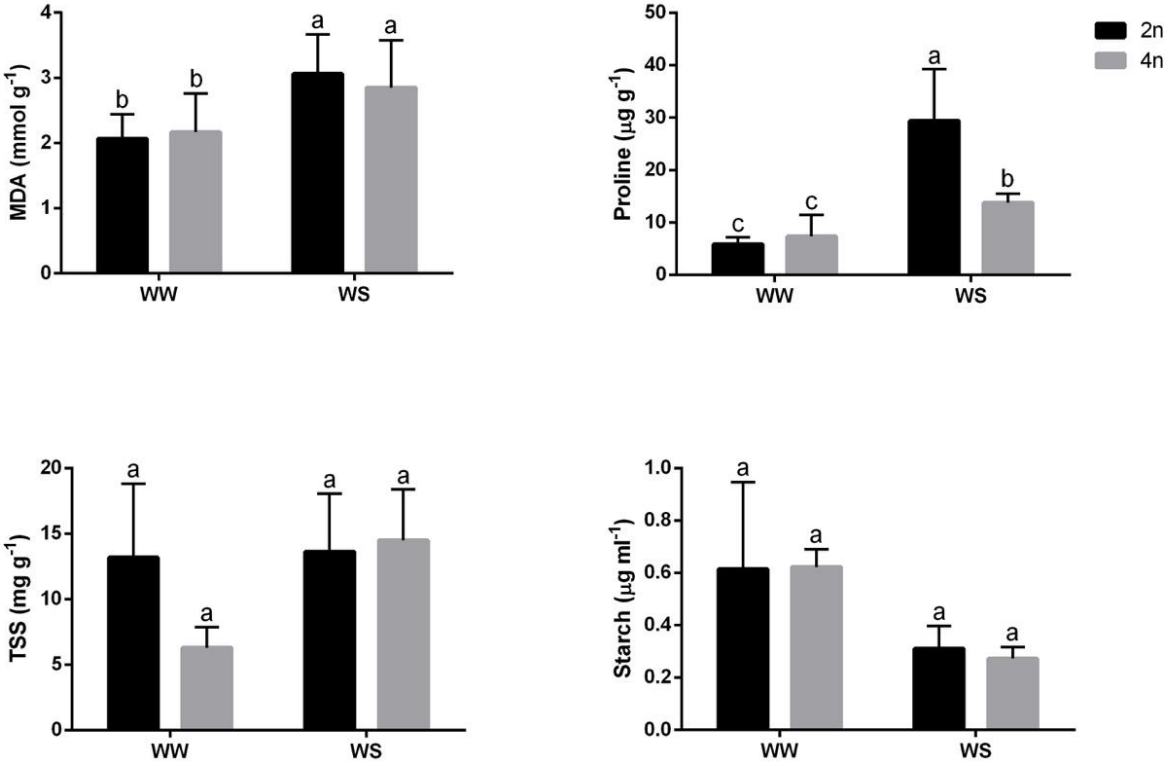

Figure 5. Effects of water stress (WS) on **MDA**, **proline**, total soluble sugars (**TSS**) and **starch** contents in diploid (2n) and tetraploid (4n) acclimatized tamarillo plants in comparison with well-watered (WW) plants. Means ± SDs, n = 6, different letters indicate significant differences between treatments at $p \leq 0.05$.

There was a tendency for tetraploid plants to contain lower amounts of soluble sugars in WW treatments. A slight increase in TSS content was observed in the WS tetraploid plants when compared with the WW ones (Figure 5). The amount of starch, on the other hand, was very similar in tetraploids and diploids with watering. There was a considerable, although not significant, increase in starch levels in the WW groups. In the WS groups, tetraploid plants showed reduced starch accumulation when compared with diploids.

4. Discussion

The close relationship between abiotic stress and polyploidy is well recognized in nature, as wis the role of polyploidy in conferring a selective advantage under stressful or changing environmental conditions (for reviews, see refs. [10,19,27]).

In terms of adaptation to drought stress, genome doubling has been shown to lead to changes in transpiration, water use efficiency, photosynthetic rate, phenology, antioxidant response, and morphology [11,28]. However, the underlying physiological and molecular bases of such mechanisms remain poorly understood. The results obtained in this study showed that polyploidization confers better performance in acclimatized plants under water deficit than their diploid counterparts, which may be related to differential defense strategies to cope with drought. This is also the first time that the effect of water stress on the

physiological performance of acclimatized in vitro micropropagated and then acclimatized tamarillo plants is reported.

In previous reports for *Lonicera japonica*, WS decreased the net photosynthesis rate, stomatal conductance, and transpiration rate of both diploid and tetraploid cultivars. WS also decreased electron transport rate, effective quantum yield of photosystem II, photochemical quenching, and starch content, but increased nonphotochemical quenching and contents for total soluble sugars, proline, and malondialdehyde [29]. However, tetraploid *L. japonica* showed higher resistance to water stress and less affected physiological responses than diploid species [29] which was attributed to morphological and anatomical features, as the tetraploid had a smaller total plant leaf area, higher leaf mass per unit area, thicker epidermis, and palisade tissue, as well as denser pubescence.

Together with changes in hormonal dynamics and water potential, such responses to WS are well reported for other woody plants, yet they can change depending on species/genotype and stress intensity or duration [21]. In fact, one anatomical characteristic of polyploids is their larger size, both in general terms and at the level of the different organs, such as flowers, fruits, roots, or seeds. This results from the increased cell volume of the tetraploid cells. The leaves are generally wider, thicker, and characterized by an increased mass per unit area [30,31]. They also show an increase in stomata size and in the number of chloroplasts per guard cell [29]. Regarding cereals, Huang et al. [32] observed that as ploidy increased (from diploid to tetraploid or hexaploid) in wheat, the amount of water used in transpiration and the growth period decreased while WUE and nutrient use efficiency increased.

In the present work, we start from a point where no apparent morphological differences were noticed within diploid and tetraploid acclimatized tamarillo plants. No differences in biomass between plants of different ploidies, nor between treatments were found. However, there was a slight decrease in biomass from WW to WS tetraploid plants not observable in diploids. This result may reflect a better and faster stress adaptation of tetraploids, through the reduction of photosynthetic rates and consequent decrease in biomass production, compared with diploids.

Marked differences were found regarding the water status of plants with different ploidies under stress. Tamarillo tetraploid plants were able to maintain water potential and RWC values near those from WW. Only significant differences were observed in the behavior of diploid plants under WS. An expressive difference indicated the lower water potential of the WS diploid plants when compared with the other groups under analysis. The changes in water status under water limitation scenarios agree with what has been observed in other studies [21].

It was found that both tamarillo plants subjected to WS conditions showed reduced rates of transpiration and CO_2 assimilation. Although there were no statistically significant differences, tetraploid plants tended to show higher rates of both parameters when compared with diploids, and these values were reflected in the WUEi obtained. In the case of gs there was a sharp decrease when comparing the plants under WW to those in the WS treatment, with a null value of gs in WS diploids and a very low value in tetraploids. It is known that water stress is a factor that significantly affects stomatal conductance [21,33] and this seems to be closely linked with the factors of transpiration and CO_2 assimilation that, in the face of the impact of stress, showed similar results of a decrease.

Photochemical performance analysis showed a significant decrease in the effective quantum yield of photosystem II (PSII) in both diploid and tetraploid plants when subjected to water stress, with the decrease being slightly steeper in diploid plants. The reduction in PSII may be largely related to a decrease in the proportion of photosystem II reaction centers in the "open" state due to limited NADPH consumption or to the reduced efficiency in the capture of excitation energy by the reaction of PSII [34], considering that chlorophyll a values did not decrease significantly. In agreement with other results obtained, these values confirm that WS affects photosynthetic capacity, leading plants to reduce the rate of photosynthesis as a response to low water availability [21,35]. These results are reinforced

by the quantification of chlorophyll a, chlorophyll b, and carotenoids for diploid and tetraploid plants under WW and WS, in which no significant differences were observed. Only a slight decrease in all pigment content was observed in the tetraploids when subjected to WS, whereas in the diploids the content of all types of pigment increased.

Another common plant protective mechanism against water deficit is the accumulation of soluble sugars and other osmoprotectants, such as amino acids (e.g., proline). These osmotically active compounds stabilize proteins and membranes and reduce osmotic potential, aiming to prevent cellular dehydration [36,37]. Apart from the fact that no significant differences were observed regarding TSS between diploid and tetraploid plants, regardless of water treatment, it is worth mentioning that sugar concentration was higher in tetraploids under WS. This slight increase could be biologically relevant to assure the higher tolerance of tetraploids under WS and reinforce the key role of sugars as carbon sources and osmoregulatory agents. Furthermore, it can suggest a reduced consumption of organic solutes as an adaptive measure to stress [38].

Analysis of proline provided very interesting results, with a significant increase in diploid plants under water limitation. In the case of tetraploids, this increase was slight. Proline plays a central role in the ability of plants to react to abiotic stress [39] since it is an amino acid that mediates osmotic balance, protecting macromolecules in periods of dehydration and acting as a protector of oxidative stress. Considering the differential behavior of leaf soluble sugars and amino acids between diploid and tetraploid plants and their potential role in abscisic acid regulation, a key player mediating drought responses [40], further research should be done to quantify phytohormones, as suggested in cold tolerance in tetraploid *Fragaria moupinensis* [41].

Considering the levels of starch and soluble sugars, the fact that there were no significant decreases in their contents between treatments may indicate an inhibition of the hydrolysis of starch into simple sugars under stress conditions [33]. In terms of MDA, a general marker of oxidative stress, there were no differences noticed with either ploidy plant, although its increase with water stress imposition is commonly reported for other species [42]. It is important to stress that the differences found between WS diploid and tetraploid plants were observed after a 4-day water stress period, which was enough to induce leaf wilting, and a dramatic reduction in photosynthetic performance, particularly in terms of gas exchange parameters, but it might not have been long enough to cause damage related to oxidative stress.

5. Conclusions

In the face of rapid climate changes at the global scale, understanding the impact of polyploidization on plant fitness and environmental interactions is an urgent topic. This knowledge may help us design new ways for harnessing more efficient uses of artificial polyploidization to obtain genotypes with increased tolerance to diverse biotic and abiotic stresses and explore this as an efficient tool for the breeding of crop species with intrinsic agronomic value.

This work was designed to explore if polyploidization has a role in plant drought tolerance, and we describe the main physiological traits triggered under stress in tamarillo micropropagated and acclimatized plants. Rather than confirming the drought-resistant nature of tetraploid plants, we aimed to show that early selection of plant stock material is crucial and should be performed under less optimal conditions. Furthermore, the results presented consist of a first report on a drought assay for tamarillo plants and new insights on the effect of abiotic stresses on polyploid *versus* diploid plants. The obtained results showed tetraploid plants displayed traits related to a superior drought tolerance than their diploid counterparts and differential mechanisms underlying this physiological performance may be involved. However, the anatomical/histological differences inherent to that physiological behavior, as well as the molecular basis of this increased tolerance must be further analyzed.

It can be concluded that studies and advances in the improvement of tamarillo, associated with efficient in vitro plant regeneration protocols, can play a decisive role in the development of new clones resistant to abiotic stresses, viruses, pests, and diseases that, through conventional crosses, are difficult to obtain. Nevertheless, it will be important to monitor the field development of the tetraploids obtained and quantify the advantages of the process developed.

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