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DIFFERENTIAL BEHAVIOR TO DROUGHT OF TWO ELITE POTATO CULTIVARS GROWN IN SOUTHEASTERN PAMPEAN REGION OF ARGENTINA

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ABSTRACT: Potatoes production requires large inputs of water for which development of drought tolerant cultivars is particularly relevant to ensure or even increase its productivity. Two elite cultivars, Newen INTA and Spunta, were evaluated in the field under contrasting water regimes. After drought treatment, the yield of marketable tubers decreased 53% in Spunta and 22% in Newen INTA while the number of marketable tubers decreased 32% in both cultivars. Yield of tuber > 90 mm only decreased in Spunta (62.81%, $p < 0.05$). Newen INTA showed a greater reduction in the shoot and root dry biomass than Spunta, while the root/shoot ratio was higher in Newen INTA. Proline content increased 61% in both cultivars under drought treatment. Drought tolerance indices (GMP and DTI) were higher in Newen INTA, and the drought susceptibility index was higher in Spunta. Proline content was correlated with root dry biomass and the marketable tuber number in both cultivars. Dry root biomass correlated with the yield of marketable tubers and the number of marketable tubers. In drought conditions, Newen INTA showed greater tolerance to drought. Therefore, Newen INTA emerges as a useful genotype to contribute to increasing crop productivity in areas subject to water stress.

KEYWORDS: drought tolerance; Spunta; Newen INTA; drought stress indices; proline content

INTRODUCTION

Potato (*Solanum tuberosum ssp. tuberosum* L.) is a crop with high to moderate sensitivity to drought (Hijmans, 2003; Monneveux *et al.*, 2013) showing limited yield in arid and semi-arid regions. In 2019, potato world production was around 380 million tons, on a planted area of 19.3 million hectares (FAOSTAT, 2023). Its susceptibility to water stress makes that a regular supply of water is necessary to achieve acceptable yield and quality (Boguszewska-Mańkowska *et al.*, 2020; Ierna *et al.*, 2011; Iwama, 2008; Levy *et al.*, 2013; Wang *et al.*, 2006) for which the development of drought-tolerant cultivars is a highly valued objective to achieve an efficient and sustainable production. Among the main biochemical mechanisms involved in drought tolerance, the accumulation of osmotic compounds such as free proline was described to play a relevant role (Evers *et al.*, 2010; Rai *et al.*, 2011). For instance, the variation in proline content described in plants with contrasting drought tolerance was associated with a differential osmoregulation capacity (Schafleitner *et al.*, 2007; Szabados and Savouré, 2010). Tagliotti *et al.* (2020) reported a negative correlation between yield and proline content working with a very diverse potato panel, showing that the response to water stress was also influenced by the genetic background (Hassanpanah, 2010; Sprenger *et al.*, 2018).

Potato is the most consumed vegetable in Argentina, with 40.8 kg per person, concentrating 55% of the national production

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in the southeastern (SE) Pampean region where the crop is mostly grown under irrigation (MAGYP, 2021). *S. tuberosum* ssp. *tuberosum* cv. Spunta is the most produced cultivar in Argentina, representing 90% of the local consumption (Huarte and Capezio, 2013; MAGYP, 2021). Newen INTA is a promising cultivar developed by INTA (*Instituto Nacional de Tecnología Agropecuaria*, Argentina) for fresh consumption and food industry (Variety Register Number 12126; https://gestion.inase.gob.ar/consultaGestion/gestiones). Under water stress conditions Spunta was described as sensitive or moderately sensitive (depending on growing conditions) when compared with Newen INTA cultivar (Bedogni *et al.*, 2018; Lerna and Mauromicale, 2006). To confirm this observation, drought responses of Spunta and Newen INTA were compared in the field under differential water regimes focusing on yield, tuber quality and the proline-mediated osmoregulatory response.

MATERIALS AND METHODS

Experimental design and measurement of soil and climate parameters

Field trials were conducted during 2019-2020 growing seasons in Balcarce, Argentina (37° 45'32'' S, 58° 17' W, 100 m.a.s.l.). Spunta and Newen INTA cultivars were planted in October 2019 and harvested in March 2020 (123 days of crop cycle). The average relative humidity was 72.4 ± 9.3%, and the mean temperature was 19.23 ± 3.38 °C. The minimum and maximum temperatures were 12.2 ± 4.3 °C and 27.2 ± 4.6 °C, respectively. Cumulative solar radiation ranged from a minimum of 6.9 W m^{-2} in November and March and a maximum of 7.8 W m^{-2} in January. The soil was a typical Argiudoll (Mar del Plata soil serie; Soil Survey Staff, 2006) with 5% organic matter and a pH of 5.7 (https://zenodo.org/records/6353509; 8/10/2023). Five virus-free tuber seeds per meter were planted in 5 m rows spaced by 0.85 m. The experimental design was a complete randomized block with four replicates and four rows per block. At planting time, plots were supplemented with 200 kg ha^{-1} of diammonium phosphate. To minimize biotic stresses, fungicides and insecticides were sprayed (Infinito™ [Fluopicolide 6.25% + Propamocarb hydrochloride 62.5%], Bayer CropScience; Poliram DF™ [Metiram 70%], BASF Chemistry) following the supplier's recommendations. Weeds were manually controlled.

Field capacity (FC, %), and permanent wilting point (PWP, %) and bulk density were measured to evaluate available water in the soil. These values were used as input variables to estimate the soil water holding capacity (SWHC) and establishing the irrigation protocol. Therefore, at planting time, soil water conditions at depths between 0 and 20 cm, SWHC was 33.7 mm, between 20 to 40 43.5 mm. The irrigation protocol was divided into "irrigated treatment" and "drought treatment". Irrigation treatment was applied by sprinkling when the 60% of the SWHC was reached and consisted in twenty doses of water of average 16.1 mm each added throughout across the crop cycle. In drought treatment, plants grown with no irrigation (dry-farming conditions) during the whole crop cycle. The total water supplied under irrigation was 571.0 mm and under drought treatment 248.9 mm. The daily water balance at 40 cm depth of soil was calculated according to:

$$SWC_r = SWC_i + NP + NI - RET*Kc$$

Where SWC_r is the soil water content on day r, SWC_i is the soil water content on the previous day i. NP is the net precipitation, NI is the net irrigation and RET is the evapotranspiration reference, and Kc is the crop coefficient on day r (Allen *et al.*, 2006). The NP was calculated with a run-off coefficient of 0.1, and to calculate the NI an efficiency of 0.6 was assumed (Della Maggiora, 1996). The chart in Fig. 1 shows the soil and climate parameters collected during the assay. SWC_r values below the 60% SWHC were not recorded.

Sampling and measurement of proline content

Proline content was measured at 46, 60, 75 and 90 days after planting in fully expanded leaves of the third level relative to the top of the plant. Each sample consisted of four plants harvested from the outer rows. Leaf tissues were collected between 9:00 a.m. and 10:00 a.m. taking 0.1 g fresh tissue which was immediately frozen in liquid nitrogen and stored at –80°C until processing following Bates' protocol (Bates *et al.*, 1973) modified by Carillo and Gibon (2011). The sap of each frozen leaf tissue was collected with an electric roller press (Sew-Eurodrive D752) and diluted 20-times with a 70:30 ethanol/water mixture (v/v) (Hummel *et al.*, 2010). A 1:1 mixture of extraction homogenate and reaction mix (ninhydrin 1% [w/v] in acetic acid 60% [v/v] and ethanol 20% [v/v]) was poured into 2-ml Eppendorf tubes and heated at 95°C in a water bath for 20 min. After cooling to room temperature, each tube was spun down (1 min, 500*g*) and 100 µl of supernatant was transferred to a polypropylene microplate to measure the absorbance at 520 nm. The proline content was quantified by a calibration curve and expressed in µg per g of fresh tissue (FW) (Mohamed *et al.*, 2010).

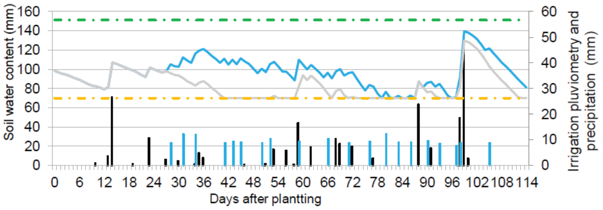

Fig.1. Soil water content and climate parameters measured during the assay: net precipitation (black columns); net irrigation (blue columns); water content of irrigated soil (solid blue line); water content of non-irrigated soil (solid grey line); water content in field capacity (doted green line); water content at the permanent wilting point (doted yellow line).

Yield and tuber evaluation

For dry biomass measurements, fully mature plants were removed from inner rows keeping the surrounding soil within a radius of 20 cm and a depth of 40 cm (Lahlou and Ledent, 2005). Whole plants were immersed in water during 30-45 min to remove adhered soil and shoot and root parts (without tubers) were separated (Man-Hong *et al.*, 2020). Each fresh tissue was weighted (FW) and then oven-dried at 60° C until constant weight to obtain dry biomass values. Each replicate measured the dry biomass of roots, leaves, stems, and fruits (including dead material). The dry root/shoot biomass ratio (R/S) was calculated.

Total yield, marketable yield and tuber number were recorded. The tubers were classified by diameter (<50 mm - undersized; 50-75 mm - intermediate; 70-90 mm - upper intermediate; >90 mm - oversized), counted and weighted. Tubers without diseases or defects and larger than 50 mm were recorded to calculate marketable yield. A subset of the total harvest was used to measure the dry matter content, according to Cacace *et al.* (1994). The following drought tolerance indices were calculated: drought susceptibility index (DSI) (Fischer and Maurer, 1978), and geometric mean productivity (GMP) and drought tolerance index (DTI) (Fernandez, 1992):

$$\mathrm{DSI} = [1- (Y_s \;/\; Y_p)] \;/\; [1 - (\mathrm{SI})]; \text{ where stress intensity } (\mathrm{SI}) = 1 - (\hat{Y}_s \;/\; \hat{Y}_p)$$

$$\mathrm{GMP} = (Y_p \times Y_s)^{0.5}$$

$$\mathrm{DTI} = (Y_s \times Y_p) \;/\; (\hat{Y}_p)^2$$

Where Y_s is the marketable yield of a given genotype under drought, Y_p is the marketable yield of this genotype under irrigation treatment, and $\hat{Y}_s$ and $\hat{Y}_p$ are the mean marketable yields of all genotypes under drought, and irrigation treatments, respectively.

Statistical analysis

To calculate the marketable yield, marketable tuber number and dry matter content, the fitted model was:

$$y_{ijp} = \mu + v_i + t_j + (vt)_{ij} + b_p + e_{ijp}$$

Where y_{ij} is the trait value, μ is the overall mean, v_i is the fixed effect of the *i*th potato cultivar, *tj* is the fixed effect of the *j*th treatment, $(vt)_{ij}$ are the interaction effects between the previously fixed effects, b_p is the random effect of the *p*th block and e_{ijp} is the random error of the (*ijp*)th observation. This model assumes a distribution explained by $b_p \sim N(0; \sigma^2_b)$ where σ^2_b is the variance of the random effect of the block and $e_{ijp} \sim N(0; \sigma^2_e)$ where σ^2_e is the variance of the error.

For the shoot and root dry biomass, and proline content the fitted model was:

$$y_{ijsp} = \mu + v_i + t_j + m_s + (vt) + (tm)_{js} + (vm)_{is} + (vtm)_{ijs} + b_p + e_{ijsp}$$

Where y_{ijsp} is the trait value, and μ, v_i, t_j, $(vt)_{ij}$, and b_p were defined above. The m_s is the fixed effect of the *s*th day of treatment, $(tm)_{js}$, $(vm)_{is}$, and $(vtm)_{ijs}$ are the interaction effects between the previously fixed effects and e_{ijsp} is the random error of the (*ijsp*)th of the *s*th repeated observation (Ashraf and Foolad, 2007). This model assumes distributions $bp \sim N(0; \sigma^2_b)$ and $e_{ijsp} \sim N(0; \sigma^2_e)$ which were verified by graphical exploratory analysis of residuals and restricted likelihood ratio test. In the case of violation, alternative assumptions were proposed, evaluated, and checked. In each mixed linear model, ANOVA tests were performed for the main factors and their interactions. When statistically significant differences ($p < 0.05$) were detected, a *post-hoc* pairwise comparison across each factor was performed by using the Tukey test.

The correlation between the traits studied was assessed using the Pearson correlation coefficient (*r*) approach. All statistical analyses were performed using InfoStat software (Di Rienzo *et al.*, 2017) and R Project software (R Core Team, 2019).

RESULTS AND DISCUSSION

Yield performance and drought indices

Under drought treatment, Newen INTA presented a higher marketable yield (59.46 t ha^{-1}) than Spunta (29.9 t ha^{-1}). The percent reduction in marketable yield was 22.64% in Newen INTA and 53.39% in Spunta (Fig. 2a). The dry matter content of the tuber was not significantly affected under contrasting water regimes ($p = 0.67$). The number of marketable tubers under drought decreased 31.07% in Newen INTA and 33.66% in Spunta (Fig. 2b).

The effect of drought treatment on yield was evaluated differentially according to the tuber size category (Table 1). In Newen INTA, drought treatment decreased yield respect to the irrigated for most size categories ($p < 0.05$). Yield reduction of 29.51% was observed in tubers with diameter smaller than 50 mm,

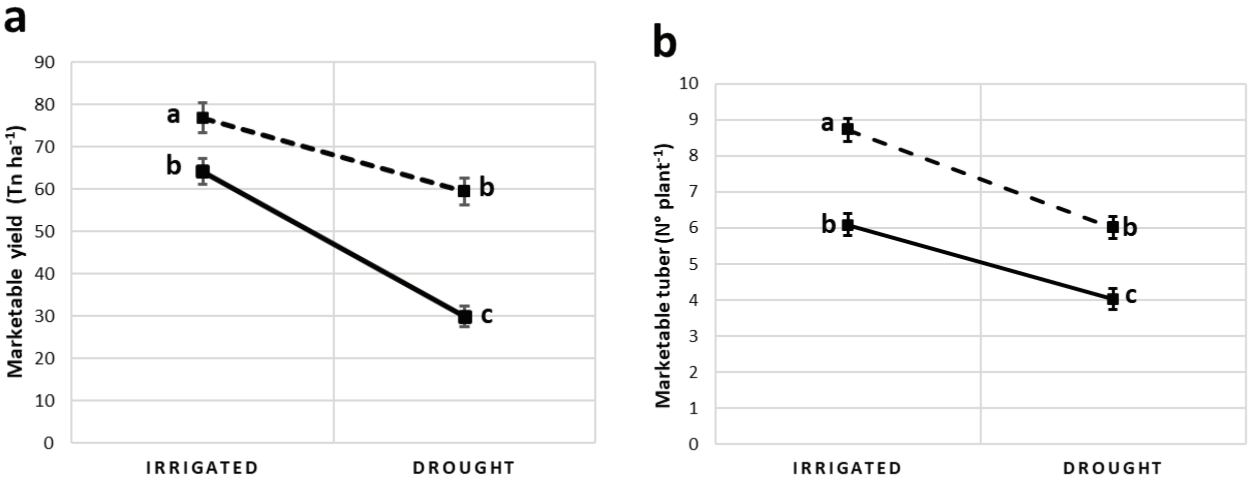

Fig.2. Mean yield performance of potato cultivars grown under irrigated and drought regimes. The marketable yield (A) and marketable tuber number (B) were measured at harvest. Newen INTA (dotted lines) and Spunta (solid lines). The thin bars represent the standard error. Values with the same letters indicate non-significant differences among mean values (P = 0.05) according to a protected LSD test.

Table 1. Effect of contrasting water regimes on the yield of tuber size categories. Overall means ± standard deviation. Means within the same tuber size category and with the same letters are not significantly different at $P = 0.05$ according to a protected LSD test (ns: non-significant). YR: yield reduction.

Cultivar	Treatment	Yield of each tuber size category (t ha^{-1})			
		< 50 mm	50 - 75 mm	75 - 90 mm	> 90 mm
Newen INTA	Irrigated	6.10 ± 0.61 b	19.44 ± 1.7 a	25.43 ± 0.82 a	32.60 ± 2.74 b
	Drought	4.30 ± 0.56 a	7.32 ± 0.76 b	15.13 ± 1.01 b	36.42 ± 0.82 b
	YR	29.51%	62.34%	40.50%	ns
Spunta	Irrigated	6.63 ± 0.64 bc	2.86 ± 0.60 c	8.40 ± 1.16 c	52.90 ± 0.82 a
	Drought	7.94 ± 0.77 c	3,27 ± 0.61 c	6.78 ± 1.20 c	19.67 ± 0.82 c
	YR	ns	ns	ns	62.81%

62.34% in diameters between 50 - 75 mm, and 40.50% in diameters between 75 - 90 mm. Newen INTA tubers larger than 90 mm did not significantly modify their yield under drought. In Spunta, drought treatment significantly reduced the yield only for tubers with diameter larger than 90 mm, showing 62.81% reduction (Table 1).

The stress intensity (SI) for both potato cultivars was 0.36. For such condition, three drought tolerance indices were evaluated by measuring the marketable yield. Thus, Newen INTA showed higher GMP and DTI, and lower DSI values when compared with Spunta (Table 2)

Growth parameters

Under drought treatment shoot dry biomass decreased 31.09% in Newen INTA

Table 2. Drought tolerance indices of potato cultivars under contrasting water regimes. MY: marketable yield expressed as overall means ± standard deviation.

Cultivar	Index	MY (t ha^{-1})
Newen INTA	GMP	67.52 ± 1.94
	DTI	0.91 ± 0.05
	DSI	0.37 ± 0.06
Spunta	GMP	43.56 ± 2.17
	DTI	0.38 ± 0.04
	DSI	0.84 ± 0.07

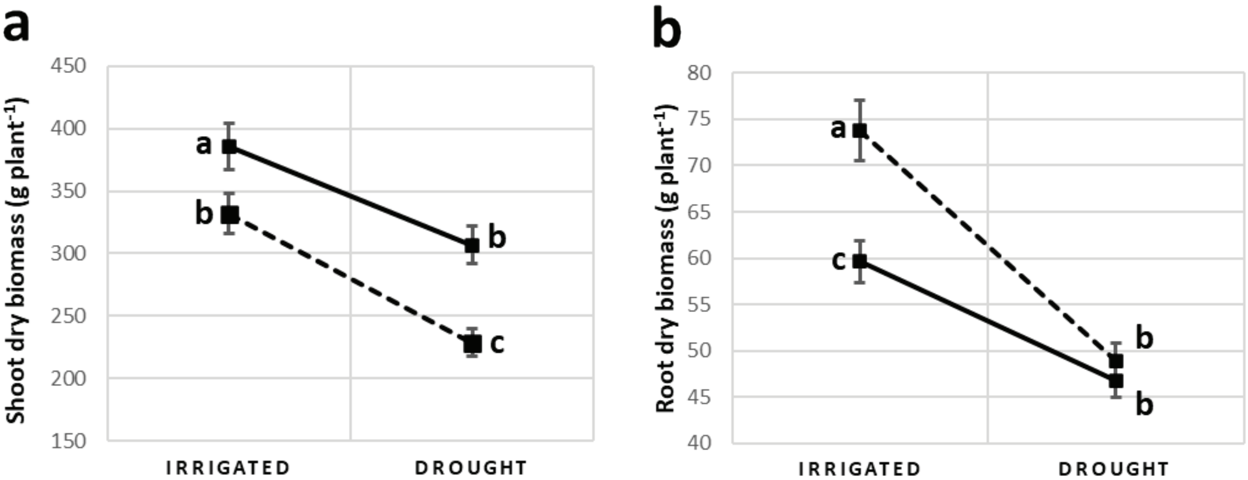

Fig.3. Growth parameters of potato cultivars under contrasting water regimes. S. tuberosum cv. Newen INTA (dotted lines) and cv. Spunta (solid lines) were cultivated under irrigation and drought regimes. The shoot dry biomass (a) and root dry biomass (b) were measured throughout the whole crop cycle. Thin bars represent the standard error. Means within the same trait with the same letters are not significantly different at P = 0.05 according to a protected LSD test.

and 20.46% in Spunta (Fig. 3a). When dry root biomass was measured, drought treatment produced a decrease of 33.77% in Newen INTA and 21.58% in Spunta (Fig. 3b). The R/S ratio in Newen INTA was 0.20 and 0.15 in Spunta, regardless of the water regime; however, the difference was not significant.

Proline content

The mean proline content between treatments was 4.68 ± 1.44 µmol g^{-1} FW in Newen INTA and 8.89 ± 1.42 µmol g^{-1} FW in Spunta (Fig. 4). The percentage of proline under drought increased 31.09% in Newen INTA and 20.46% in Spunta. The lowest proline content in Newen INTA was found 90 days after planting (Fig. 4).

Joint analysis

Pearson correlation coefficients were calculated for marketable yield, tuber dry matter, proline content, shoot dry biomass, root dry biomass and marketable tuber number (Table 3). Marketable yield, root dry biomass and the number of marketable tubers correlated positively. Proline content, dry root biomass and the number of marketable tubers correlated negatively. The calculation of correlation matrix coefficients for each individual cultivar was consistent with the analysis grouping data from both cultivars.

Table 3. Pearson correlation coefficients (r) between the different parameters measured grouping data from both potato cultivars.

	TDM	PC	SDB	RDB	TN
MY	0.43ns	- 0.46ns	0.25ns	0.74**	0.91***
TDM		- 0.19ns	- 0.58*	0.33ns	0.53*
PC			-0.18ns	-0.57*	- 0.51*
SDB				0.45ns	0.21ns
RDB					0.87***

MY, marketable yield; TDM, tuber dry matter content; PC, proline content; SDB, shoot dry biomass; RDB, root dry biomass; TN, tuber number; ns: non-significant; ***significant at 0.001; **significant at 0.01; *significant at 0.05.

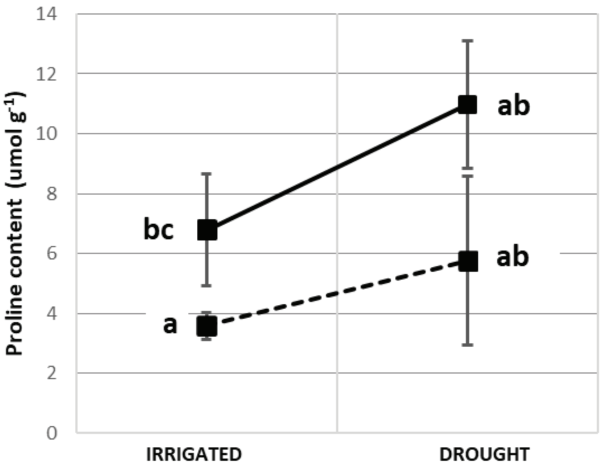

Fig.4. Proline content of potato cultivars under differential water regimes. Newen INTA (dotted lines) and Spunta (solid lines). Means represent the average proline content across the crop cycle. Thin bars represent the standard error. Means with the same letters are not significantly different at P = 0.05 according to a protected LSD test.

Water deficit is the main factor affecting potato production and is responsible of significant reductions in yield, tuber size, and tuber number (Aliche *et al.*, 2019; Ierna and Mauromicale, 2006; Ierna *et al.*, 2011; Onder *et al.*, 2005). Therefore, identification of cultivars with tolerance to drought is highly valuable. Newen INTA and Spunta exhibited differential responses to water stress arising as a promising model to characterize the mechanism involved in. Spunta was confirmed as a drought susceptible cultivar, showing a greater reduction in marketable yield (53.39%). GMP and DTI were the parameters exhibiting the better ability to identify genotypes combining high yield potential and high yield under drought condition. Therefore, genotypes exhibiting higher GMP and DTI and lower DSI might be identified as drought-tolerant (Cabello *et al.*, 2013; Saravia *et al.*, 2016; Schafleitner *et al.*, 2007; Sharma *et al.*, 2011). Under the same stress intensity (0.36) Newen INTA presented higher GMP, DTI and lower DSI indices than Spunta. Saba *et al.* (2001) proposed that narrow-sense heritability for

GMP and DTI were higher compared to other drought tolerance indices, suggesting that these indices could be useful for breeding selection of drought tolerant genotypes. Dry matter content in tuber of both cultivars was not affected by drought treatment, in agreement with previous results (Cabello *et al.*, 2012; Deblonde and Ledent, 2000). Under the drought conditions applied in this study, Spunta was the cultivar showing the higher shoot dry biomass and while Newen INTA exhibited the higher root dry biomass. Increased foliage development was described in drought-sensitive potato cultivars (Aliche *et al.*, 2018). On the other hand, a large root system is likely a mechanism of adaptation to drought in potato (Boguszewska-Mańkowska *et al.*, 2020). Hence, R/S ratio is a suitable indicator of drought tolerance since a higher R/S ratio improves soil water uptake and reduces water losses for transpiration (Zhang *et al.*, 2018). When comparing Spunta and Newen INTA, R/S was not modified significantly under drought stress. Root biomass was associated with potato yield under drought (Zarzynska *et al.*, 2017), and the correlation between marketable yield and dry root biomass observed in Newen INTA and Spunta agrees with this idea.

Drought treatment increased proline content in both cultivars. However, the proline content in Spunta was higher than in Newen INTA across the whole cycle. Claussen (2005) described that the content of foliar proline might be considered as an indicator of drought stress. However, the role of proline content in potatoes under drought conditions was controversial (Ashraf and Foolad, 2007; Schafleitner *et al.*, 2007). The proline accumulation might contribute to drought tolerance or indicate stress intensity. According to Tagliotti *et al.* (2020) under moderate drought stress, proline accumulation was not associated with drought tolerance. The observed negative correlation between proline content and the number of marketable tubers suggests that proline content could be an indicator of stress, rather than a stress protector (Schafleitner *et al.*, 2007; Tagliotti *et al.*, 2020). Even significantly higher proline level was observed in the leaves of different species, proline deposition in the root-tip of maize, barley and watermelon (among others) was described under low water potential. However, this increase in proline root content was not due to the proline biosynthesis in the roots but rather its increased transport from leaves (Verslues and Sharp, 1999; Raymond and Smirnoff, 2002; Shelden *et al.*, 2016; Wang *et al.*, 2022). Probably, the negative correlation observed in this study between proline content and the number of marketable tubers could be due to most of the proline synthesized in leaves was already mobilized to root at the time of sampling.

Evaluation of the effect of drought on physiological, growth and yield parameters suggested that Newen INTA is a drought tolerant cultivar respect to Spunta, for which can be considered a good genotype for improving crop production in semi-arid regions.

CONCLUSIONS

Two elite potato cultivars in Argentine showed a differential drought tolerance. Newen INTA presented an improved drought tolerance respect to Spunta. The drought tolerance indices used (GMP, DTI, and DSI) were suitable to address drought tolerance. The role of proline content was as an indicator of non-specific stress, rather than an indicator of drought tolerance. The present results propose Newen INTA as a promising potato cultivar to improve potato production and to save irrigation resources.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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