



Responses of *Solanum tuberosum* L. to Water Deficit by Matric or Osmotic Induction



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Received: 16 June 2020 / Accepted: 4 December 2020 / Published online: 25 January 2021
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Abstract

To select potato genotypes tolerant to water deficit, systems to simulate this stress have been used. Polyethylene glycol (PEG) is the main osmotic agent used for this purpose, but it causes an excessively severe stress. However, it is difficult to carry out an experiment that aims to compare plant responses under water deficit by osmotic or matric induction, and, thus, few studies compare these stress-inducing mechanisms. Therefore, the objective of this study was to compare the responses of Agata, BRS Clara, C2406-03 and Cota genotypes to water deficit in both induction methods (matric or osmotic). The tests were carried out in a greenhouse, one using hydroponics (osmotic induction) and the other in pots with soil (matric induction). In both tests, the application of stressful conditions occurred at the beginning of tuberization. Assessments of gas exchange and shoot temperature were made throughout the exposure to stress. Also, samples were collected from leaves for analysis of osmotic potential and leaves and tubers for analysis of metabolite content. At the end of the potato plant cycle, the number and weight of tubers were evaluated. In both stress conditions, there were significant reductions in photosynthesis and transpiration rate compared to the respective normal hydration conditions. In addition, indicators such as metabolite levels (proline and soluble sugars) were significantly altered in plants exposed to different stress inductions. These data, together with the significant limitations in the growth of stressed plants, indicate that the experimental models induce similar responses. However, the water deficit by osmotic induction was more severe for the potato plants when compared to stress due to matric induction, mainly affecting tuber production. Therefore, the water deficit osmotic induction model can be recommended for phenotyping tolerance to this stress, due to the hydroponic system inducing greater tuber production per plant under optimal cultivation conditions.

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Keywords Metabolite content · Polyethylene glycol · Potato genotypes · Water stress

Introduction

Studies focused on the selection of potatoes for drought stress tolerance have used systems that simulate this stress. Many studies use Polyethylene Glycol (PEG) to promote water deficit by osmotic induction (Azeredo et al. 2016; Carvalho et al. 2016; Meng et al. 2016; Al Atalah et al. 2019).

However, most studies that show the PEG effect on physiological and biometric parameters in potatoes bring results that indicate an excessively severe stress (Meng et al. 2016; Rohr 2016; Silveira 2018). While studies showing the effect of drought stress by matric induction in potatoes indicate a rapid response in physiological parameters, it strongly depends on the phenological stage and time of exposure to this stress (Banik et al. 2016; Aliche et al. 2018).

In addition, the potato can be grown in soil, without a significant difference in productivity, with up to 80% of the necessary irrigation and with an interval of up to 6 days between irrigations (Mantovani et al. 2013). This shows the capacity to use water efficiently. Due to the characteristics of each experimental model, be it water restriction or using PEG, it is difficult to carry out an experiment that compares plant responses in one system or another. For this reason, few studies compare these stress-inducing mechanisms.

Furthermore, previous studies show differences in the responses of potato plants exposed to water stress by osmotic induction, due to phenological characteristics (Rohr 2016; Silveira 2018). In these studies, it was evident that plants with different times of tuber initiation may present differences in tolerance to water stress. Secondly, these studies demonstrated that the sensitivity to stress differs according to the phenological stage of the plant (Banik et al. 2016; Aliche et al. 2018).

When PEG is used to characterize the tolerance of plant genotypes to drought stress the effect of drought is mainly due to effects on gas exchange parameters such as photosynthesis, stomatal conductance and water use efficiency, besides inducing the gene expression linked to the response to drought (Reisser Júnior et al. 2011; Zhang et al. 2014). Also, the consequence of stress caused by PEG leads to significant reductions in tuber production (Rohr 2016).

However, the stress induced by PEG, despite inducing responses like those observed in drought stress, is just osmotic stress, but receives the classification of “Physiological Drought” (Tobe et al. 2000; Pelegriani et al. 2013). Thus, the objective of this study was to compare the responses of potato plants to water deficit by osmotic or matric induction.

Materials and Methods

This experiment was conducted during the spring (15/08/18 to 4/11/18) at Embrapa Temperate Agriculture, Pelotas, RS (31° 40' 34" S 52° 26' 28" W). Genotypes that differ in tuber initiation and maturity were used, one early (Agata), two intermediate ones (BRS Clara and C2406-03) and one late (Cota).

Phenotyping tuber initiation was performed in previous experiments by Rohr (2016) and for tolerance to water deficit by osmotic induction by Rohr (2016) and Silveira (2018). In these studies, the influence of the phenological stage on the responses of plants to water stress was clear; therefore, in the present study, this factor was defined as the starting point for the application of both types of water stress induction.

Seed tubers of each genotype were grown in phenolic foams until the shoots reached a uniform size (5 cm). After this period the plants were transferred to hydroponics or pots as described below.

Osmotic Induction

After reaching a uniform size the plants were transferred to a gutter hydroponic system (Medeiros et al. 2002). In order to induce genotypes to osmotic stress, the polyethylene glycol 6000 (PEG) nutrient solution was added, providing a condition of -0.129 megapascals (MPa), following the methodology described by Reisser Júnior et al. (2011) and confirmed with the use of the VAPRO 5520 osmotic pressure meter (Wescor, Logan, UT).

This stress was applied during the beginning of tuberization when the tip of the stolon reached more than 1 mm in diameter. The application of PEG was carried out in two stages: in the first stage, half the dosage was applied (-0.064 MPa), and after 24 h, the rest was applied (-0.129 MPa), totalling 30 g L^{-1} of solution. The control plants were maintained with a nutritive solution without changes throughout the experiment. The nutrient solution was only replenished every 3 days.

Matric Induction

The plants were transferred to pots with a capacity of five litres containing 1 kg of crushed stone and 6.3 kg of soil type moderate red-yellow eutrophic abrupt 'A' soil, collected in a field area of Embrapa Temperate Agriculture. The soil had the pH corrected to 6.0 using dolomitic lime and nutrients adjusted [100 ppm Nitrogen (Urea), 100 ppm Phosphorus (Triple Superphosphate) and 100 ppm Potassium (Potassium Sulphate)]. Thirty days after transferring the plants to the pots, nitrogen was replenished. The pots were kept under 80% of the water holding capacity, using the pot weight as a reference. Tensiometers were installed in six pots, two with intermediate genotypes, one with an early genotype and the other with a late genotype, as well as in two control pots where there were no plants. This monitored the tension generated by the lack of water supply during the period of exposure to stress due to water deficit.

To obtain the water deficit condition, irrigation was stopped at the beginning of the tuberization stage, which was verified by observing the plants in the hydroponic system. Also, to ensure that the tuberization in both conditions coincided, surplus pots were made with plants of each genotype. These surplus plants had their soil removed to verify the beginning of tuberization in the plants of this cultivation condition. The plants under stress remained without irrigation for 7 days, the same time as the osmotic stress in the hydroponic system. After the stress period, the plants were irrigated until 80% water retention capacity in the pot was reached and the plants grown in hydroponics had their nutrient solution replaced, resuming normal osmotic conditions. For

comparison, control plants were kept under optimal irrigation conditions throughout the experiment (80% of the pot's capacity). This was monitored according to the pots weight in addition to Hg tensiometers.

Evaluations

Gas Exchanges and Thermographic Evaluations

Twenty-four hours after applying both stress-inducing treatments, evaluations were started with IRGA (LI-6400XT, LI-COR) and a thermal camera (Flir SC660, Flir Systems Inc., USA, 7–13 μm , 640 \times 480 pixels) every 3 days, when climatic conditions were favourable. Thus, the CO_2 fixation (A — $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (G_s — $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and water use efficiency (WUE — $\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$) were evaluated. Besides, through the images captured by a thermographic camera, the canopy temperature (heat) of the plants was obtained.

The gas exchange evaluations were carried out between 10:00 am and 12:00, allowing the evaluated plants to be at homogeneous conditions of light, temperature and relative humidity. Thermographic evaluations were carried out between 1:00 pm and 2:00 pm, maintaining the uniformity of conditions for all treatments in both cultivation methods.

Growth Parameters

At the end of the experiment, approximately 90 days after planting, the following variables were assessed: tuber numbers per plant (TN); tuber fresh weight (TFW) with the aid of a precision analytical balance; and percentage of tuber dry weight: 30 g of tubers were cut into small cubes and placed in aluminium plates and dried in a 65 $^\circ\text{C}$ oven with forced ventilation until a constant weight was obtained. After that, the tuber dry weight percentage was calculated.

Metabolite Content

For metabolites analysis (total soluble sugars (TSS), sucrose (SUC) and proline (Pro)), at the end of the period of exposure to stress (168 h), samples of leaves (S_TSS, S_SUC and S_Pro) and tubers (T_TSS, T_SUC and T_Pro) were immediately frozen in liquid nitrogen and stored in an ultra-freezer at -80 $^\circ\text{C}$. The extracts for determining these metabolite contents were obtained according to the methodology of Bielecki and Turner (1966).

Approximately 250 mg of fresh weight were macerated with liquid N_2 and homogenized with 10 ml of extraction solution MCW (methanol: chloroform: water; ratio 12:5:3, respectively). After 24 h, the extracts were centrifuged at 600 G for 30 min, then the supernatant was recovered and for every 4 ml, 1.0 ml of chloroform and 1.5 ml of water were added followed by new centrifugation to separate phases. The supernatant containing the metabolites was collected and transferred to a water bath at 38 $^\circ\text{C}$ for 30 h to eliminate the chloroform residue to concentrate the samples for the analysis of sugars and proline. For the measurement of TSS, SUC and Pro, the methodologies

described by Graham and Smydzuk (1965), Van Handel (1968), and Rena and Masciotti (1976), respectively, were used.

Osmotic Potential

Apical leaflets of the third expanded leaf were collected, from the apex, always in the morning, and packed in plastic bags with moist paper towels. Subsequently, these leaves were placed in a humid chamber for 3 h, until they reached the maximum turgor state. Then, leaf discs of approximately 8 mm were collected, disregarding the central nervure, and stored in an ultra-freezer at $-80\text{ }^{\circ}\text{C}$.

To make the osmolality reading, the leaf disks were macerated, centrifuged and $10\text{ }\mu\text{l}$ of supernatant was collected. The readings were performed using the VAPRO 5520 osmotic pressure meter (Wescor, Logan, UT), following the manufacturer's recommendations. To calculate the osmotic pressure (Ψ) the Van't Hoff equation was used as described in Silveira (2018):

$$\Psi = -MRT^*$$

M = is the value of the osmolality obtained, expressed in mmol/kg;

$R = 0.0083$ (MPa);

T = (K) is the absolute temperature in Kelvin.

*The equation values were expressed in MPa.

In addition, the osmolality of nutrient solutions, with and without PEG, and soil solution was measured. The soil solution was collected with a Soil Control® extractor, according to the method described in Marques (2016). The soil matric potential was estimated by converting millimetres of mercury, observed in tensiometers, to MPa (Azevedo and Silva 1999; Libardi 2004).

Statistical Design and Data Tests

The two main factors investigated were cultivar (Agata, BRS Clara, C2406-03 and Cota) and water deficit induction methods (osmotic, matric and control). There were six replicates (plants) for each combination. The gas exchange and thermographic evaluations were done for three periods of exposure to stress (24, 72 and 168 h). Metabolic content was determined separately for shoots and tubers. Appropriate analyses of variance and either Tukey tests ($P \leq 0.05$) or Scott and Knott (1974) tests ($P \leq 0.05$) on differences between treatment means were done using Sisvar software (Ferreira 2011). Pearson's correlation analysis was performed between the variables studied using PAST software in each induction model (Hammer et al. 2018).

Results

Stress conditions in both cultivation conditions were similar (Fig. 1). Also, the soil solution osmolality value (-0.04 MPa, data not shown) was similar to that observed in the nutrient solution without PEG (-0.05 MPa). This indicates that under control conditions in which

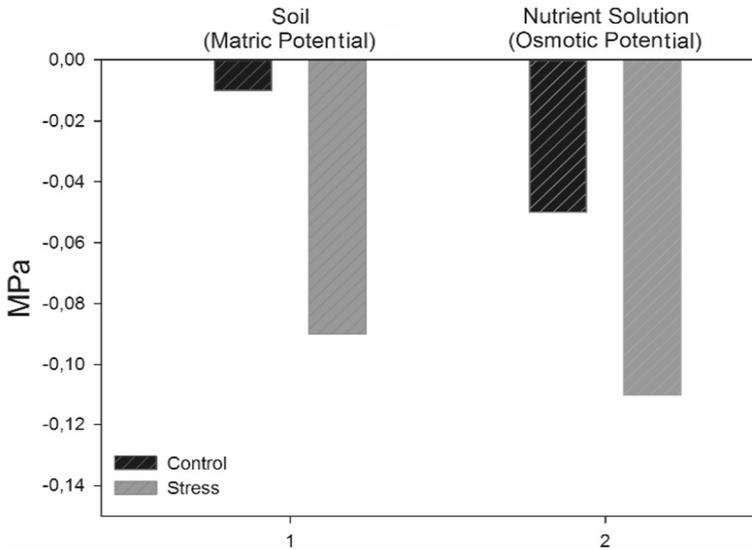


Fig. 1 Soil matric potential used in the pots and nutrient solution osmotic potential in hydroponics conditions, during the stress exposure

the soil matric potential in the pots was less negative, the water availability was similar. It is because the only relevant factor in that condition is the solution osmolality.

The plants tuberization was the standardized signal for stress application in both cultivation conditions. Thus, the beginning of tuberization occurred at 47 DAP in the early genotype, at 52 DAP in the intermediate genotypes, and 57 DAP in the late genotype. The stress conditions were maintained for 7 days. From this point on, gas exchange and shoot temperature assessments were initiated.

All genotypes showed a significant reduction in net CO_2 fixation in both cultivation conditions from the beginning of the stress exposure (Fig. 2). Plants grown under water deficit by osmotic induction showed a gradual reduction in CO_2 fixation, although there was also a significant reduction compared to control plants. Cota showed the most abrupt reduction in CO_2 fixation among plants exposed to PEG, reaching low values from the first evaluation (Fig. 2E).

Under water deficit by matric induction, the plants in pots showed a more abrupt reduction in CO_2 fixation rates, reaching close to zero from the first assessment. The BRS Clara was the most affected, with CO_2 fixation values practically zero from the first assessment (Fig. 2D).

There was a significant reduction in stomatal conductance (GS) when the plants were exposed to both water deficit inductions (Fig. 3). Plants exposed to water deficit by osmotic induction showed a gradual reduction for up to 72 h of exposure, except for Cota, which had its GS reduced to zero in the first 24 h of exposure (Fig. 3E). Plants of BRS Clara cultivated in pots, even under optimal conditions of irrigation, presented GS values statistically equal to plants under water deficit by matric induction in the last evaluation (Fig. 3D). However, all plants grown in pots had their GS reduced to zero in the first 24 h in exposure to water deficit (Fig. 3).

The water use efficiency (WUE) of all plants was affected by both stress conditions (Fig. 4). Only Cota under water deficit by osmotic induction showed an increase in

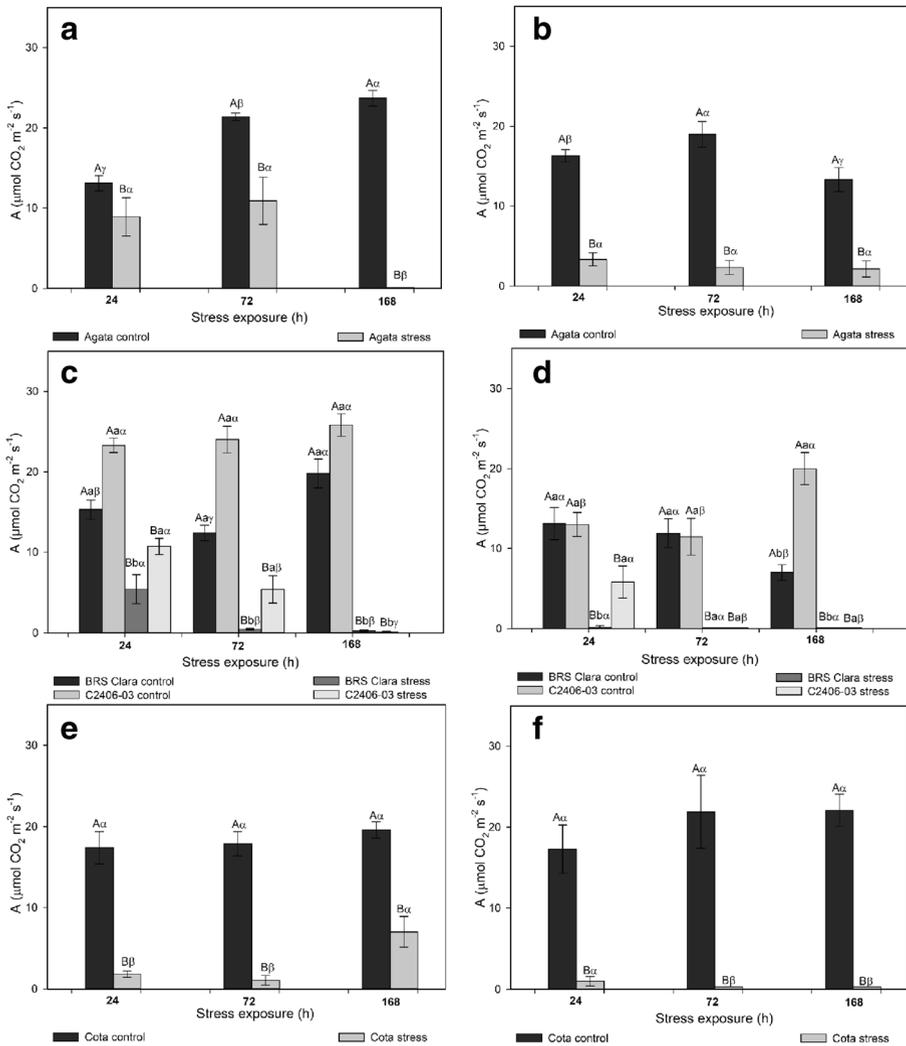


Fig. 2 Net CO₂ fixation in genotypes with early (**a** and **b**), intermediate (**c** and **d**) and late (**e** and **f**) tuberization, under water deficit by osmotic (**a**, **c** and **e**) and matric (**b**, **d**, and **f**) induction. Means followed by different letters, uppercase between osmotic conditions within the same genotype, lowercase between genotypes within the same osmotic condition and Greek between times of exposure to stress within the same genotype and treatment, indicate a significant difference according to the Tukey test ($P \leq 0.05$)

WUE in the last evaluation (Fig. 4E). Plants C2406-03, under water deficit by osmotic induction, kept their WUE superior to control plants for up to 72 h of exposure to stress. However, in the final evaluation, they showed a significant reduction in the WUE when exposed to PEG (Fig. 4C). Agata under water deficit showed a significant increase in the WUE at 72 h of exposure (Fig. 4B). However, all the genotypes evaluated in this work behaved similarly in conditions of water deficit by matric induction, in the last evaluation (Fig. 4).

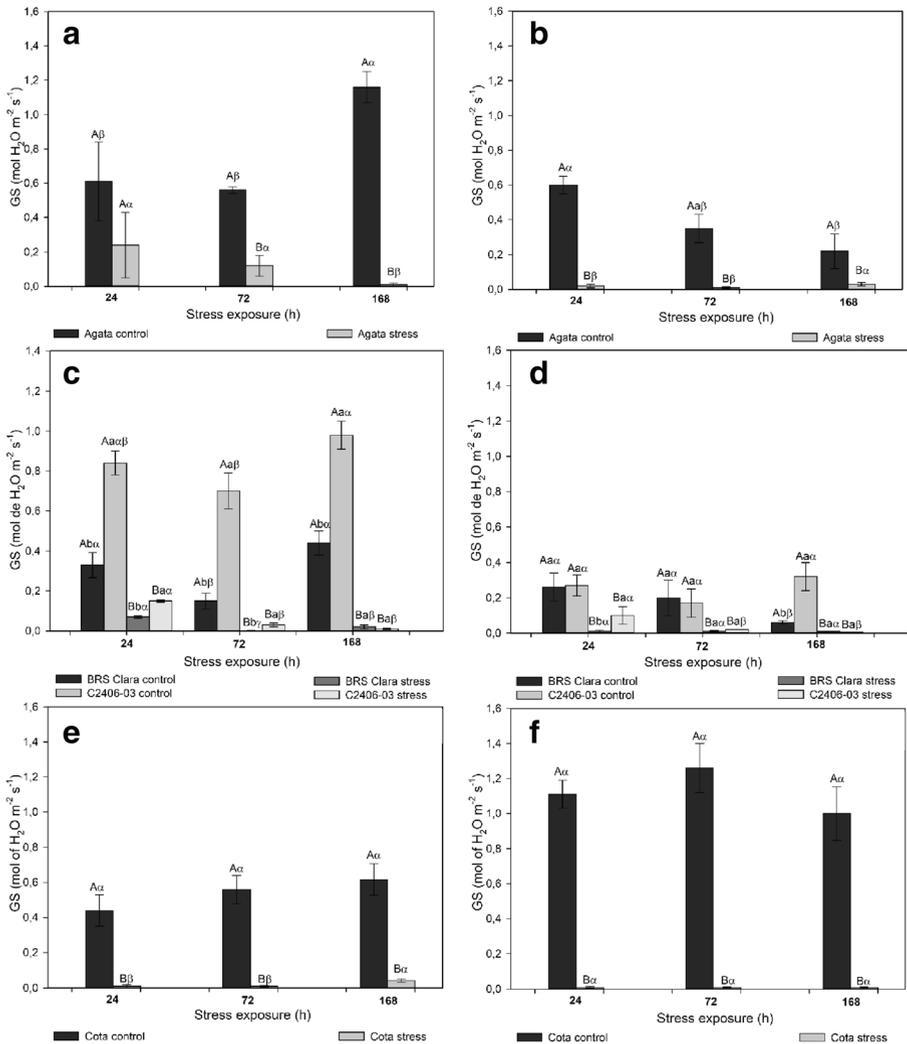


Fig. 3 Stomatal conductance (GS) in potato genotypes with early (**a** and **b**), intermediate (**c** and **d**) and late (**e** and **f**) tuberization, under water deficit by osmotic (**a**, **c** and **e**) and matric (**b**, **d**, and **f**) induction. Means followed by different letters, uppercase between osmotic conditions within the same genotype, lowercase between genotypes within the same osmotic condition and Greek between times of exposure to stress within the same genotype and treatment, indicate a significant difference according to the Tukey test ($P < 0.05$)

The thermographic analysis (heat) showed that the high heat accumulations were significant from the first hours of exposure to both stress inductions for all the plants tested (Fig. 5). Among the plants grown in hydroponics, Cota showed heat accumulation of more than 10 °C in the first 24 h of PEG exposure (Fig. 5 E). C2406-03 and BRS Clara cultivated in pots showed a gradual increase in the shoot temperature under water deficit by matric induction (Fig. 5D).

Plants grown in hydroponics also suffered significant losses in tuber production when exposed to PEG (Fig. 6A). The reductions in tuber numbers were reached in three

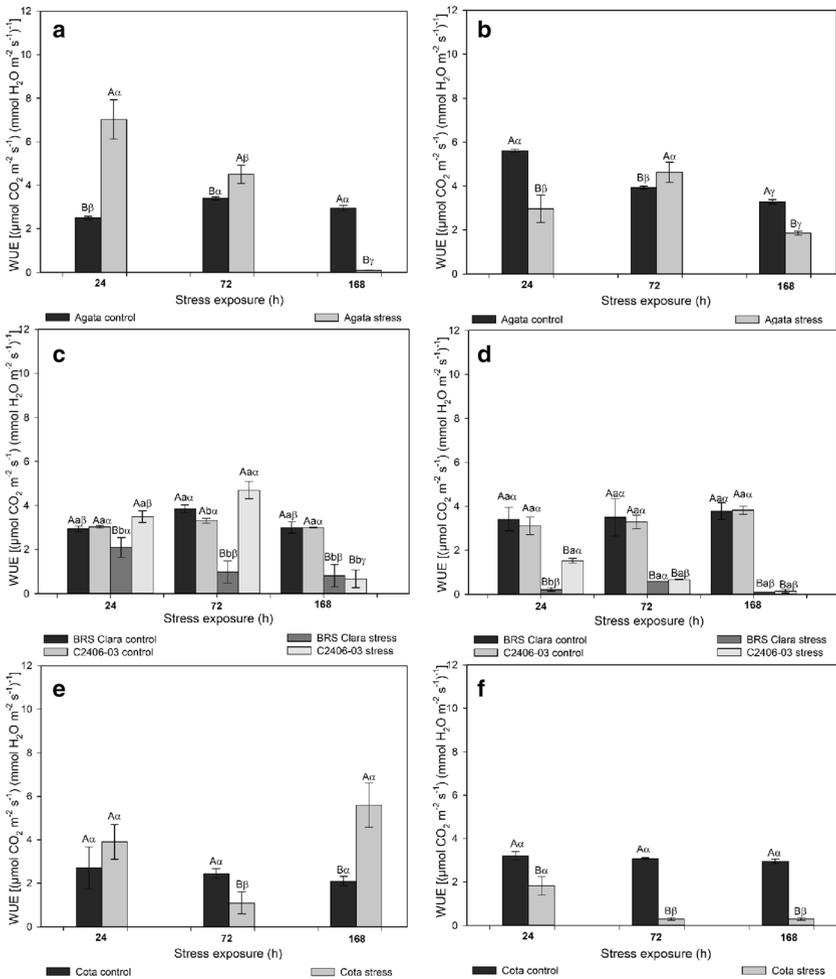


Fig. 4 Water Use Efficiency (WUE) in potato genotypes with early (a and b), intermediate (c and d) and late (e and f) tuberization, under water deficit by osmotic (a, c and e) and matric (b, d, and f) induction. Means followed by different letters, uppercase between osmotic conditions within the same genotype, lowercase between genotypes within the same osmotic condition and Greek between times of exposure to stress within the same genotype and treatment, indicate a significant difference according to the Tukey test ($P < 0.05$)

of the four tested genotypes. In addition to the reduction in the tuber number, there was a severe reduction in their weight when the plants were exposed to water deficit by osmotic induction (Fig. 6C). In BRS Clara and Cota exposed to PEG a significant decrease occurred in the percentage of tuber dry weight (Fig. 6E). On the other hand, plants grown in pots, although less productive than plants grown in hydroponics, did not show significant reductions in the number of tubers, even under water deficit by matric induction (Fig. 6B). Only Agata and C2406-03 showed a reduction in tuber fresh weight accumulation under water deficit by matric induction, compared to the other genotypes (Fig. 6D). None of the genotypes tested showed a reduction in tuber dry weight percentage when exposed to water deficit by matric induction (Fig. 6F).

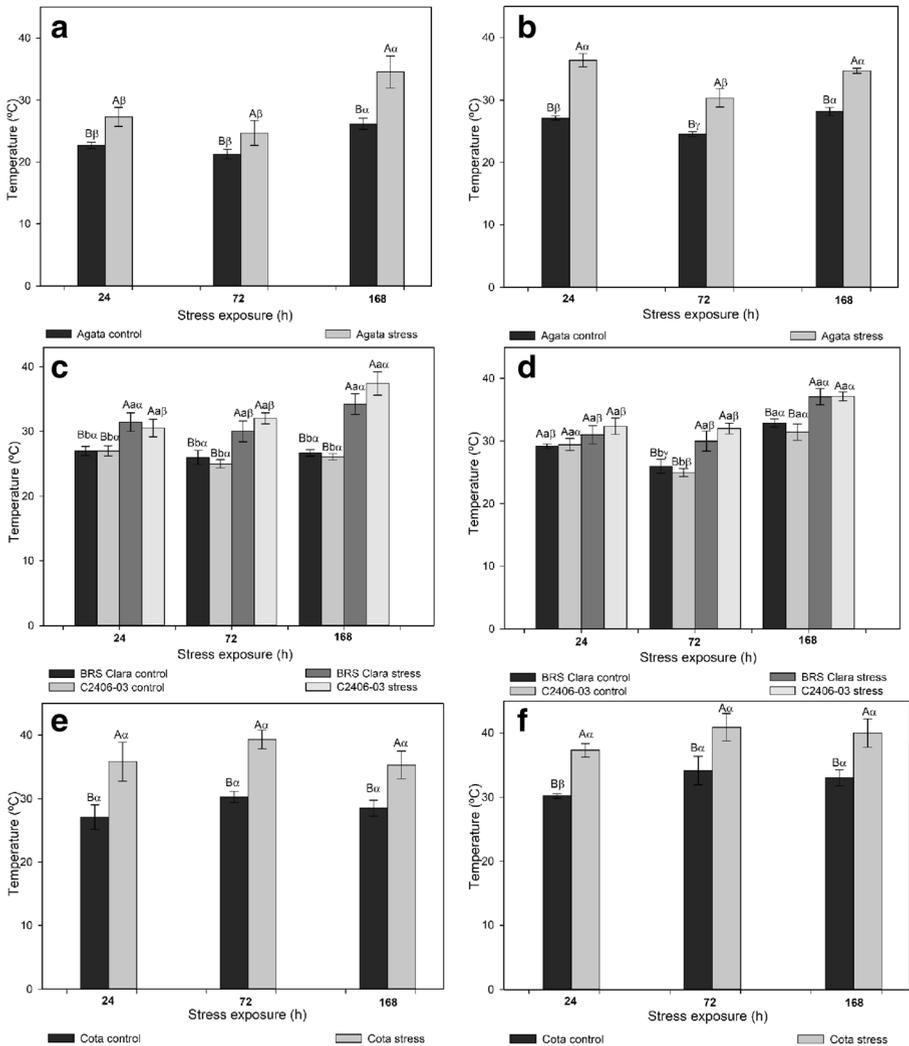


Fig. 5 Shoot temperature in potato genotypes with early (**a** and **b**), intermediate (**c** and **d**) and late (**e** and **f**) tuberization, under water deficit by osmotic (**a**, **c** and **e**) and matric (**b**, **d**, and **f**) induction. Means followed by different letters, uppercase between osmotic conditions within the same genotype, lowercase between genotypes within the same osmotic condition and Greek between times of exposure to stress within the same genotype and treatment, indicate a significant difference according to the Tukey test ($P \leq 0.05$)

Plants of Agata showed a significantly higher osmotic potential when exposed to PEG, which was even greater than the other genotypes (Fig. 7A). However, under cultivation conditions in pots, plants of Agata exposed to water deficit by matric induction, showed a reduction in osmotic potential (Fig. 7B). The other genotypes showed no significant difference between treatments. However, C2406-03 showed higher osmotic potential than the other genotypes when exposed to water deficit by matric induction (Fig. 7B).

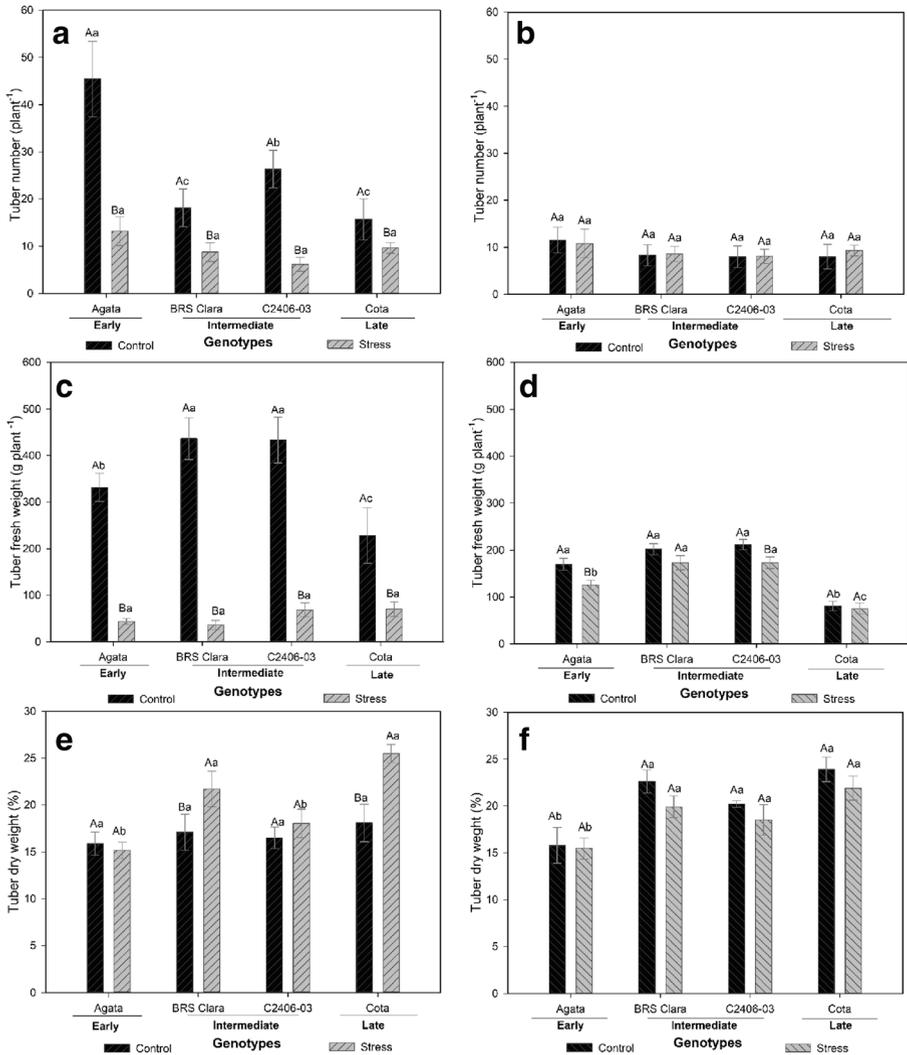


Fig. 6 Tuber number (a and b), tuber fresh weight (c and d), percentage of tuber dry weight (e and f) of potato genotypes with different tuberization cycles grown under water deficit by osmotic (a, c and e) or matric (b, d and f) induction. Means followed by different letters, uppercase between treatments in the same genotype and lowercase between genotypes in the same treatment, differ according to the Scott and Knott test, with a 5% probability of error

Regarding the accumulation of total soluble sugars (TSS) in the shoot, there was no significant difference between genotypes under optimal hydration conditions, for both cultivation conditions (Fig. 8). However, when exposed to water deficit by osmotic induction, all genotypes, except Cota, showed significantly higher TSS accumulation in comparison to their respective controls (Fig. 8A). Agata showed higher TSS accumulation in the shoot than other materials when exposed to PEG (Fig. 8A). When plants grown in pots were exposed to water deficit by matric induction, only Agata and BRS

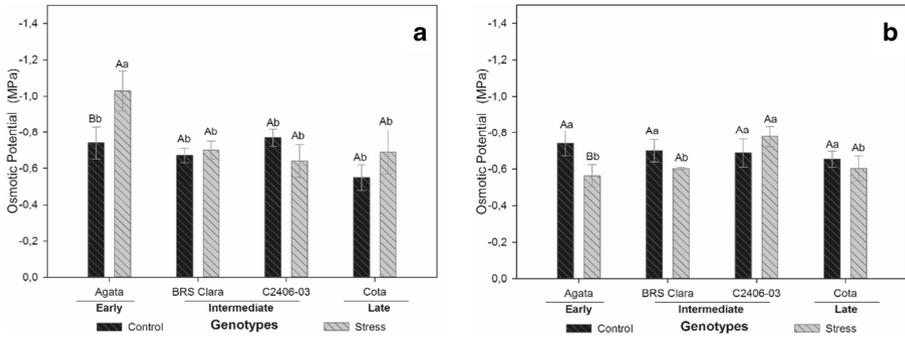


Fig. 7 Osmotic potential of potato genotypes with different tuberization cycles, grown under water deficit by osmotic (a) or matric (b) induction. Means followed by different letters, uppercase between treatments within the same genotype and lowercase between genotypes within the same treatment, differ according to the Scott and Knott test at 5% probability of error

Clara showed changes in shoot TSS levels (Fig. 8B). In Agata plants under water deficit by matric induction there was a reduction in shoot TSS accumulation, in comparison to its respective control plants. In contrast, in BRS Clara, there was a greater shoot TSS accumulation than their respective control (Fig. 8B). Agata plants

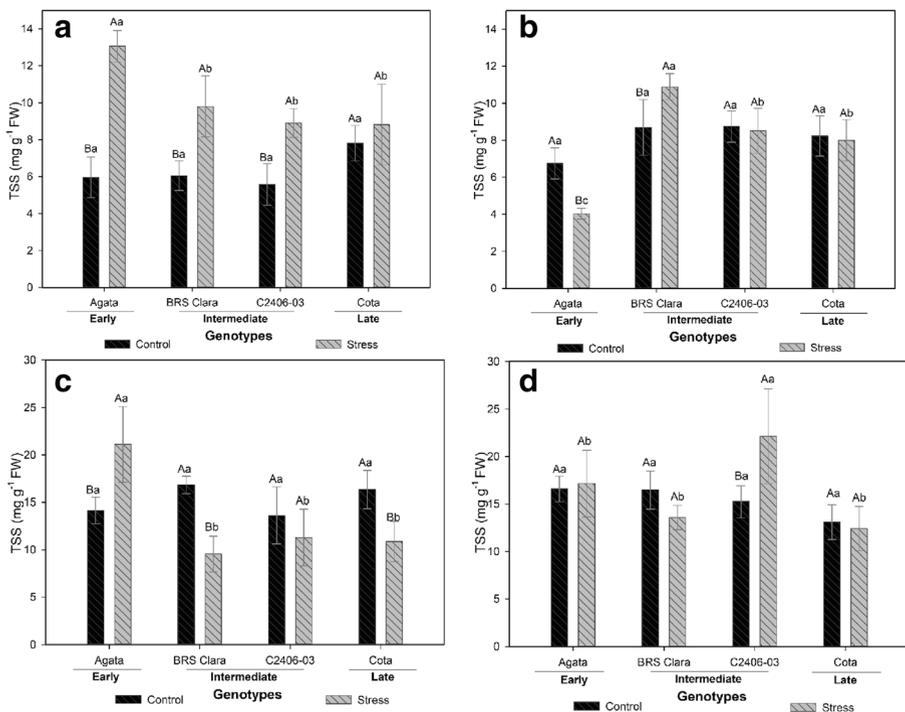


Fig. 8 Total soluble sugars content (TSS) accumulated in the shoot (a and b) and tubers (c and d) in potato genotypes with different tuberization cycles, grown under water deficit by osmotic (a and c) or matric (b and d) induction. Means followed by different letters, uppercase between treatments within the same genotype and lowercase between genotypes within the same treatment, differ according to the Scott and Knott test at 5% probability of error

grown in hydroponics also showed a singular behaviour concerning tuber TSS content (Fig. 8C). The highest TSS content occurred in Agata tubers exposed to PEG. However, in the other genotypes, the tuber TSS content was lower when exposed to water deficit by osmotic induction, except for C2406-03, where there was no significant difference for this trait (Fig. 8C). Tubers of most genotypes, when exposed to water deficit by matric induction, did not show significant changes in TSS accumulation compared to the respective controls. Only C2406-03 showed significantly higher TSS content, both in comparison to the control and to the other plants exposed to water deficit by matric induction (Fig. 8D).

Plants grown in hydroponics followed a sucrose shoot content (SUC) pattern like that observed in TSS content (Fig. 9). All cultivars showed an increase in shoot SUC contents when exposed to PEG, except Cota, which had not changed in response to the solution osmotic condition. Under optimal conditions of solution osmolality, there were no differences in shoot SUC content between the genotypes (Fig. 9A). Conversely, plants grown in pots did not suffer significant changes in the shoot SUC contents even under water deficit by matric induction (Fig. 9B).

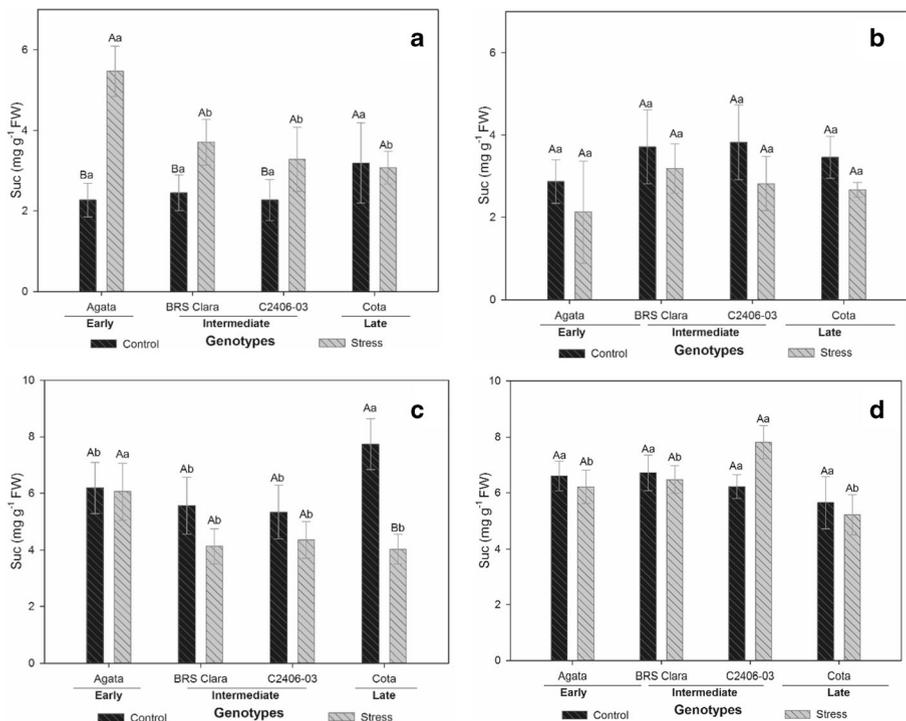


Fig. 9 Sucrose content (SUC) accumulated in the shoot (a and b) and tubers (c and d) in potato genotypes with different tuberization cycles, grown under water deficit by osmotic (a and c) or matric (b and d) induction. Means followed by different letters, uppercase between treatments within the same genotype and the lowercase between genotypes within the same treatment, differ according to the Scott and Knott test at 5% probability of error

Only Cota plants showed lower tuber SUC content when exposed to PEG (Fig. 9C). The other genotypes showed no significant difference in the tuber SUC content between treatments when grown in hydroponics. However, Agata plants showed a higher tuber SUC content under water deficit by osmotic induction compared to the other genotypes (Fig. 9C). Among the plants grown in pots, there was also no significant difference between treatments, but C2406-03 showed higher tuber SUC content, when compared to other plants exposed to water deficit by matric induction (Fig. 9D).

The plants demonstrated a constitutive shoot proline (Pro) content in both cultivation systems under optimal conditions (Fig. 10). When there was PEG exposure, Agata and BRS Clara had a significantly higher shoot Pro content compared to their respective controls (Fig. 10A). Between plants grown in pots, only Agata under water deficit showed no increase in the shoot Pro content (Fig. 10B). However, Agata plants grown in pots showed a higher tuber Pro content even under optimal irrigation conditions (Fig. 10D). Regarding the tubers of plants grown in hydroponics, only BRS Clara and Cota showed a significantly higher Pro content when exposed to PEG, compared to the respective controls (Fig. 10C).

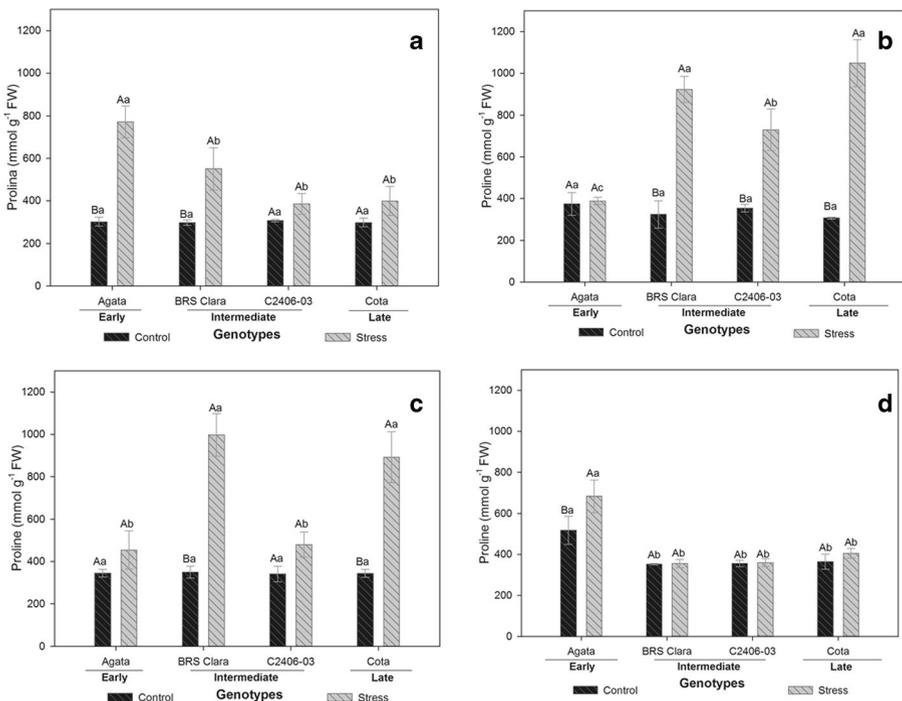


Fig. 10 Proline content accumulated in the shoot (a and b) and tubers (c and d) in potato genotypes with different tuberization cycles, grown under water deficit by osmotic (a and c) or matric (b and d) induction. Means followed by different letters, uppercase between treatments within the same genotype and lowercase between genotypes within the same treatment, differ according to the Scott and Knott test at 5% probability of error

Discussion

By converting the soil matric potential to MPa, it was possible to correlate the water availability in both systems. The soil matric potential used in the present study was like the potential used in experiments that evaluate the plant behaviour to water deficit (Hofer et al. 2017; Todaka et al. 2017; Tschaplinski et al. 2019). Thus, it is possible to affirm that the water availability in both cultivation conditions was similar even in the respective stress conditions.

However, it was observed that the plants showed a more negative osmotic potential than the nutrient solution, even with the PEG addition, but in general, they did not show significant changes in this parameter. It is possible that this potential presented by plants, compared to the solution potential, favours an osmotic force capable of carrying out some water absorption, even in the presence of PEG. In the work carried out by Yooyongwech et al. (2016), sweet potato plants under water deficit by matric induction showed less negative osmotic potential than the plants observed in the present study, even under optimal hydration conditions. This data may indicate that potato plants tend to tolerate osmotic induction stress better than sweet potato plants; however, sweet potato plants have better osmotic regulation when exposed to water deficit. The authors considered that sweet potato plants adjusted their osmolality when exposed to water deficit by matric induction. Therefore, it is possible that even in nutrient solution with an excessively negative osmotic potential, plants succeed to absorb water for some time. This is evident when looking at data on stomatal conductance and CO₂ fixation, where plants exposed to water deficit by osmotic induction took longer to completely stop the gas exchange. As for plants exposed to water deficit by matric induction, their gas exchange ceased within 24 h of exposure, even though they had an osmotic potential like that observed in plants grown in hydroponics. This is evident when observing the negative correlation between the osmotic potentials of both systems, showing that the behaviour of the plants is quite different between these cultivation conditions.

Carbohydrate and proline level determination was carried out as they are affected both in plants exposed to water deficit by osmotic (Kerepesi and Galiba 2000; Pál et al. 2018) and matric (Yooyongwech et al. 2016, 2017) induction. Plants under water deficit by osmotic or matric induction can adjust their osmolality by accumulating soluble sugars (Yooyongwech et al. 2016; Singh et al. 2018; Santos et al. 2019). According to Singh et al. (2015), soluble sugars and proline accumulation can act as osmoprotectants. Also, proline can improve antioxidant activity in plants under abiotic stress.

Although some genotypes tested show a significant increase in soluble sugars and sucrose, there is no significant modulation in their osmotic potential. Only Agata plants, when exposed to PEG, had their osmotic potential altered, becoming more negative. On the other hand, when exposed to water deficit by matric induction, the plants of this genotype had less negative osmotic potential. The interaction between metabolic content and the osmotic potential was only significant when the plants were grown in hydroponics. In pots, the osmotic potential only correlated with the shoot fresh weight, indicating that there may be another mechanism of osmotic adjustment.

The tubers of plants exposed to PEG showed an increase in TSS content, allowing an increase in the draining force of this tissue. The sugar content in tissues can modulate the source-sink ratio in plants (Durand et al. 2018). In this way, the tubers exert an increased sink force, leading the plant to prioritize this tissue, mainly in the exposure to PEG. With plants exposed to water deficit by matric induction, because there is not enough water in the substrate, it is possible that the plants have not increased TSS content and drain strength due to the consumption of these carbohydrates. This may be the reason why there was a reduction in the TSS content in the shoot, and consequently in osmotic potential, of Agata plants under water deficit by matric induction.

Some studies use proline levels as stress response indicators (Verbruggen and Hermans 2008; Verslues and Sharma 2010). In the present study, plants accumulated more proline when exposed to both water deficit inductions. However, Agata showed no increase in shoot proline content when exposed to water deficit by matric induction, or in the tubers when exposed to PEG. The shoot proline content of plants under both stress conditions has a negative correlation with gas exchange, indicating the importance of this amino acid in these conditions. However, in the plants grown in hydroponics, there was not a correlation between tuber proline content and gas exchange. Such correlations existed only in the plants grown in pots, which shows the tuber contribution to the water deficit response. It is possible that the highest levels of proline in plants exposed to water deficit by matric induction prevents oxidative damage to tissues, and thus maintains their productivity.

In general, plants exposed to water deficit by matric induction showed symptoms within a few hours of exposure (Banik et al. 2016; Gerhards et al. 2016). The same occurs in plants exposed to water deficit by osmotic induction, where it can be observed in studies such as that by Thalmann et al. (2016), in which *Arabidopsis thaliana* plants took only 2 h to show symptoms. PEG, on the other hand, has the characteristic of being biologically inert, so the stress induced by it is exclusive of osmotic nature (Ahmad et al. 2007; Wijewardana et al. 2018). Even so, PEG was able to induce a drastic decrease in transpiration in the plants, which can be seen in the water use efficiency (WUE), which is the ratio between net CO₂ fixation (A) and transpiration (Trmmol) (data not shown).

In this sense, it was observed that plants exposed to water deficit by osmotic or matric induction showed the same responses, with a WUE reduction in all tested plants. However, when exposed to PEG, Cota plants increased in WUE, due to the occurrence of CO₂ fixation, even under water deficit by osmotic induction. When observing this result, it would be possible to state that Cota can be tolerant to water deficit by osmotic induction and, therefore, tolerant of water deficit by matric induction. However, when observing this genotype under water deficit by matric induction, it was possible to notice that the plants behaved the same way as the others, with a significant reduction in WUE.

The thermographic data demonstrated that all plants underwent severe stress in both growing conditions. In general, this parameter can be used to diagnose water deficit (Obidiegwu et al. 2015). This is because plants exposed to water deficit show a significant increase in shoot temperature (Gerhards et al. 2016). This occurs because of a reduction in gas exchange, causing a reduction in transpiration and thus, there is no heat dissipation by the plant. Because heat accumulation is a

consequence of stoma closure, thermographic data had a negative correlation with gas exchange. Besides, the high canopy temperatures of the plants were negatively correlated with tuber production. On the other hand, the evaluation of canopy temperature shows that potato plants respond quickly to water deficit by osmotic or matric induction. This indicates that immediately upon exposure to stresses, plants are already suffering metabolic damage that may not be visualized.

The plants in the present work did not show significant loss in production when exposed to water deficit by matric induction for the same period that plants grown in hydroponics were exposed to PEG. This may be an indication that the time that these plants were under water deficit by matric induction was not enough to reduce production. On the other hand, the PEG exposure may have been more severe when compared to water deficit by matric induction, in the same period. Nevertheless, water deficit may not cause damage to the number of tubers produced, but damage to the size and quality of the tubers due to limited filling, even when irrigation conditions are resumed (Kammoun et al. 2018).

However, there are certain differences in the potato plant responses subjected to water deficit by osmotic or matric induction. Thus, it makes it difficult to interpret the PEG effect for the selection of potato genotypes tolerant to water deficit. PEG may promote damage to plants that does not occur when they are exposed to drought. This statement is more evident when we observe the correlation between the growth parameters of systems cultivation. This analysis does not show significant correlations between the same variables in the different systems, so possibly the tubers production depends on different factors in each culture system (Fig. 11). Besides, the behaviour of the plants after stress should be considered; that is, in the recovery time. It is possible that plants exposed to water deficit recover more quickly than plants exposed to osmotic stress.

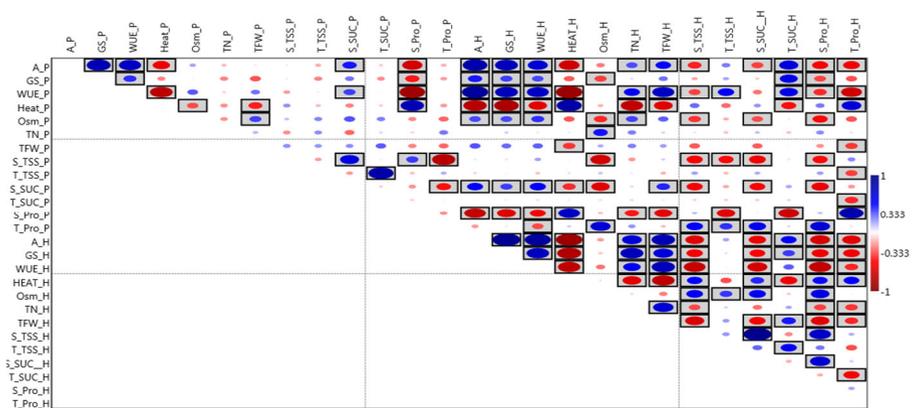


Fig. 11 Pearson’s correlation between the traits evaluated from plants and tubers of different potato genotypes submitted to water deficit by osmotic (H) and matric (P) induction. The grey boxes mark the significant correlations ($P < 0.05$); the blue ellipses indicate a positive correlation while the red ones indicate a negative correlation; the colour intensity and size of the ellipses refer to the strength of the interaction according to the correlation coefficient

Conclusions

Water deficit induction by osmotic induction can be recommended for phenotyping tolerance to this stress, due to the hydroponic system inducing greater tuber production per plant under optimal cultivation conditions. With this, it is possible to observe the responses of the plants, during the stress, and in the tuber production at the end of the cycle.

It is evident that there are strategies that can be advantageous for genotype selection using water deficit by osmotic induction, such as osmotic adjustment and an increase in the metabolite contents in the shoot, but which may not be beneficial when the plants are under matrix water deficit.

Funding This study was partially financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)–Finance Code 001.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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

References

- Ahmad MSA, Javed F, Ashraf M (2007) Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two indica rice (*Oryza sativa* L.) genotypes. *Plant Growth Regul* 53(1):53. <https://doi.org/10.1007/s10725-007-9204-0>
- Al Atalah BF, Mohsen WI, Kountar EKA, Albiski FI, Murshed RF (2019) Evaluating the tolerance of some wheat varieties towards water stress induced by sorbitol. *J Technol Applied Sci* 2:30–37. <https://doi.org/10.5281/zenodo.2582903>
- Aliche EB, Oortwijn M, Theeuwens TP, Bachem CW, Visser RG, van der Linden CG (2018) Drought response in field grown potatoes and the interactions between canopy growth and yield. *Agric Water Manag* 206:20–30. <https://doi.org/10.1016/j.agwat.2018.04.013>
- Azerêdo GA, de Paula RC, Valeri SV (2016) Germination of *Piptadenia moniliformis* Benth seeds under water stress. *Cienc Florest* 26(1):193–202. <https://doi.org/10.5902/1980509821112>
- Azevedo JA, da Silva EM (1999) Tensiometer: a practical device for irrigation control. Planaltina: Embrapa Cerrados, pp 1–37
- Banik P, Zeng W, Tai H, Bizimungu B, Tanino K (2016) Effects of drought acclimation on drought stress resistance in potato (*Solanum tuberosum* L.) genotypes. *Environ Exp Bot* 126:76–89. <https://doi.org/10.1016/j.envexpbot.2016.01.008>
- Bielecki RL, Turner NA (1966) Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. *Anal Biochem* 17(2):278–293. [https://doi.org/10.1016/0003-2697\(66\)90206-5](https://doi.org/10.1016/0003-2697(66)90206-5)
- Carvalho IR, de Souza VQ, Follmann DN, Nardino M, Schmidt D, Pellissari G, Baretta D (2016) Physiological performance of soybean by regulating water Manitol. *Agrarian* 9(31):34–43
- Durand M, Mainson D, Porcheron B, Maurousset L, Lemoine R, Pourtau N (2018) Carbon source–sink relationship in *Arabidopsis thaliana*: the role of sucrose transporters. *Plant* 247(3):587–611. <https://doi.org/10.1007/s00425-017-2807-4>
- Ferreira DF (2011) Sisvar: a computer statistical analysis system. *Ciênc Agrotec* 35(6):1039–1042. <https://doi.org/10.1590/S1413-70542011000600001>
- Gerhards M, Rock G, Schlerf M, Udelhoven T (2016) Water stress detection in potato plants using leaf temperature, emissivity, and reflectance. *Int J Appl Earth Obs* 53:27–39. <https://doi.org/10.1016/j.jag.2016.08.004>

- Graham D, Smydzuk J (1965) Use of anthrone in the quantitative determination of hexose phosphates. *Anal Biochem* 11(2):246–255. [https://doi.org/10.1016/0003-2697\(65\)90012-6](https://doi.org/10.1016/0003-2697(65)90012-6)
- Hammer Ø, Harper DA, Ryan PD (2018) PAST: paleontological statistics software package for education and data analysis. *Palaeontol Electron* 4(1):1–9
- Hofer D, Suter M, Buchmann N, Lüscher A (2017) Nitrogen status of functionally different forage species explains resistance to severe drought and post-drought overcompensation. *Agric Ecosyst Environ* 236: 312–322. <https://doi.org/10.1016/j.agee.2016.11.022>
- Kammoun M, Bouallous O, Ksouri MF, Gargouri-Bouzid R, Nouri-Ellouz O (2018) Agro-physiological and growth response to reduced water supply of somatic hybrid potato plants (*Solanum tuberosum* L.) cultivated under greenhouse conditions. *Agric Water Manag* 203:9–19. <https://doi.org/10.1016/j.agwat.2018.02.032>
- Kerepesi I, Galiba G (2000) Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. *Crop Sci* 40(2):482–487. <https://doi.org/10.2135/cropsci2000.402482x>
- Libardi PL (2004) Measurement of soil water potentials. In: Libardi PL (ed) *Soil water dynamics*. Piracicaba, SP, vol 61, pp 121–145
- Mantovani EC, Zambolim L, Souza DO, Sediyaama GC, Palaretti LF (2013) Yield and quality of potato tubers under different regimes of conventional sprinkler irrigation. *Hortic Bras* 31(4):528–533. <https://doi.org/10.1590/S0102-05362013000400004>
- Marques GN (2016) Substrate, combination of cultivars and strawberry seedlings produced in cultivation without soil. 150f. Thesis (Doctorate) - Post-graduate program in Family Agricultural Production Systems. Federal University of Pelotas, Pelotas
- Medeiros CAB, Ziemer AH, Daniels J, Pereira AS (2002) Production of potato pre-basic seeds in hydroponic systems. *Hortic Bras* 20(1):110–114. <https://doi.org/10.1590/S0102-05362002000100022>
- Meng S, Zhang C, Su L, Li Y, Zhao Z (2016) Nitrogen uptake and metabolism of *Populus simonii* in response to PEG-induced drought stress. *Environ Exp Bot* 123:78–87. <https://doi.org/10.1016/j.envexpbot.2015.11.005>
- Obidiegwu JE, Bryan GJ, Jones HG, Prashar A (2015) Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Front Plant Sci* 6:542–550. <https://doi.org/10.3389/fpls.2015.00542>
- Pál M, Tajti J, Szalai G, Peeva V, Végh B, Janda T (2018) Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. *Sci Rep* 8(1):1–12. <https://doi.org/10.1038/s41598-018-31297-6>
- Pelegriani LL, Borcioni E, Nogueira AC, Koehler HS, Quoirin MGG (2013) Effect of water stress simulated with NaCl, mannitol and peg (6000) on the germination *Erythrina falcate* Benth seeds. *Ciênc Florest* 23(2):511–519. <https://doi.org/10.5902/198050989295>
- Reisser Junior C, Castro CM, Medeiros CAB, Carvalho GC (2011) Methods for selection to drought tolerance in potatoes. *Acta Hort* 889:391–396. <https://doi.org/10.17660/ActaHortic.2011.889.48>
- Rena AB, Masciotti GZ (1976) Efeito do déficit hídrico sobre o metabolismo do nitrogênio e o crescimento de quatro cultivares de feijão (*Phaseolus vulgaris* L.). *Rev Ceres* 23(128):288–301
- Rohr A. (2016) Genetic parameters, responses associated with water deficit and population structure in potato germplasm (*Solanum tuberosum* L.). 128f. Thesis (Doctor in Agronomy) - Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas
- Santos CM, Endres L, da Silva ACS, Silva JV, de Souza Barbosa GV, Froehlich A, Teixeira MM (2019) Water relations and osmolite accumulation related to sugarcane yield under drought stress in a tropical climate. *Int J Plant Prod* 13(3):227–239. <https://doi.org/10.1007/s42106-019-00050-y>
- Scott AJ, Knott M (1974) Cluster analysis method for grouping means in the analysis of variance. *Biometrics* 30:507–512
- Silveira T (2018) Response to water deficit and characterization of the root system of potato genotypes. Embrapa Temperate Climate. 60f. Dissertation (Master in Agronomy) – Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas, 2018
- Singh M, Kumar J, Singh S, Singh VP, Prasad SM (2015) Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Rev Environ Sci Biotechnol* 14(3):407–426. <https://doi.org/10.1007/s11157-015-9372-8>
- Singh B, Mishra S, Bohra A, Joshi R, Siddique KH (2018) Crop phenomics for abiotic stress tolerance in crop plants. In: *Biochemical, physiological and molecular avenues for combating abiotic Stress tolerance in plants*, pp 277–296. <https://doi.org/10.1016/B978-0-12-813066-7.00015-2>
- Thalman M, Pazmino D, Seung D, Horrer D, Nigro A, Meier T, Kölling K, Pfeifhofer HW, Zeeman SC, Santelia D (2016) Regulation of leaf starch degradation by abscisic acid is important for osmotic stress tolerance in plants. *Plant Cell* 28(8):1860–1878. <https://doi.org/10.1105/tpc.16.00143>

- Tobe K, Li X, Omasa K (2000) Seed germination and radicle growth of a halophyte, *Kalidium caspicum* (Chenopodiaceae). *Ann Bot* 85(3):391–396. <https://doi.org/10.1006/anbo.1999.1077>
- Todaka D, Zhao Y, Yoshida T, Kudo M, Kidokoro S, Mizoi J, Toyooka K (2017) Temporal and spatial changes in gene expression, metabolite accumulation and phytohormone content in rice seedlings grown under drought stress conditions. *Plant J* 90(1):61–78. <https://doi.org/10.1111/tpj.13468>
- Tschaplinski TJ, Abraham PE, Jawdy SS, Gunter LE, Martin MZ, Engle NL, Yang X, Tuskan GA (2019) The nature of the progression of drought stress drives differential metabolomic responses in *Populus deltoides*. *Ann Bot* 124(4):617–626. <https://doi.org/10.1093/aob/mcz002>
- Van Handel E (1968) Direct microdetermination of sucrose. *Anal Biochem* 22(2):280–283. [https://doi.org/10.1016/0003-2697\(68\)90317-5](https://doi.org/10.1016/0003-2697(68)90317-5)
- Verbruggen N, Hermans C (2008) Proline accumulation in plants: a review. *Amino Acids* 35(4):753–759. <https://doi.org/10.1007/s00726-008-0061-6>
- Verlues PE, Sharma S (2010) Proline metabolism and its implications for plant-environment interaction. In: *The Arabidopsis Book/American Society of Plant Biologists*, vol 8, pp e0140–e0148. <https://doi.org/10.1199/tab.0140>
- Wijewardana C, Alsajri FA, Reddy KR (2018) Soybean seed germination response to in vitro osmotic stress. *Seed Sci* 39:143–154
- Yooyongwech S, Samphumphuang T, Tisarum R, Theerawitaya C, Cha-U S (2016) Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments via soluble sugar and free proline. *Sci Hortic* 198:107–117. <https://doi.org/10.1016/j.scienta.2015.11.002>
- Yooyongwech S, Samphumphung T, Tisaram R, Theerawitaya C, Suriyan CU (2017) Physiological, morphological changes and storage root yield of sweetpotato [*Ipomoea batatas* (L.) Lam.] under peg-induced water stress. *Not Bot Horti Agrobi* 45(1):164–171. <https://doi.org/10.15835/nbha45110651>
- Zhang C, Meng S, Li Y, Zhao Z (2014) Net NH₄⁺ and NO₃⁻ fluxes, and expression of NH₄⁺ and NO₃⁻ transporter genes in roots of *Populus simonii* after acclimation to moderate salinity. *Trees* 28(6):1813–1821. <https://doi.org/10.1007/s00468-014-1088-9>

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