# RESEARCH

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The use of osmoregulators and antioxidants to mitigate the adverse impacts of salinity stress in diploid and tetraploid potato genotypes (Solanum spp.)

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# Abstract

Background: Many arid and semi-arid areas endure from extensive salinization of agricultural land. Nevertheless, it must either develop salinity-tolerant varieties or use agronomic treatments to alleviate the symptoms of stress. Although the cultivated potato, Solanum tuberosum L., is relatively salt sensitive, salinity tolerance was demonstrated in several Solanum relatives. Knowledge of genetic variation for salinity tolerance across diverse species is required for breeding of salinity-tolerant cultivars. Higher osmotic pressures associated with salinity impede plant development and cause plant death; yet, the exogenous application of cellularly recognized molecules to withstand such stress might be a key method.

**Results:** In vitro studies were performed to determine how much genetic variability for salinity tolerance exists in S. tuberosum (tbr), a tetraploid species and S. chacoense (chc), a diploid species in which 13 genotypes were evaluated under 100, 200 or 300 mmol  $L^{-1}$  NaCl and the average tested parameters were compared with the control (no stress). A further experiment was conducted to investigate the effect of exogenous application of osmoregulators and antioxidants, namely, glycine betaine (GB), proline (P) and salicylic acid (SA) at 400, 200 and 100 mg  $L^{-1}$ , respectively, which applied solely to counteract the harmful effect of stress on potato plants. The results showed that when plants exposed to salinity, root characteristics, plantlet water content % (PWC), chlorophyll and K<sup>+</sup> content, and callus formation all substantially reduced; however, Cl<sup>-</sup> and Na<sup>+</sup> levels, as well as catalase and peroxidase activity, were elevated. In general, chc showed more tolerance compared to tbr with genetic diversity within and among species. Under stress, chc clones, 'A-6,' C-8' and 'D-2' and tbr cultivars, 'Diamond' and 'Russet Burbank' were more tolerant and yielded the greatest salinity tolerance index. Under stress but with applied GB, SA and P, the adverse consequences of stress were relieved. GB was found to be a good treatment for enhancing all the examined traits.

**Conclusion:** The results indicated that there is a significant genetic variation in salt tolerance between (*tbr*) cultivars and (chc) clones. GB followed by SA and P could completely or partly reverse the adverse impact of salinity stress on potato plants.

Keywords: Salinity tolerance, Solanum tuberosum, S. chacoense, Tetraploid and diploid potato, Osmoregulators and antioxidants, Morphological and physiological characteristics

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# Introduction

Potato, along with wheat and rice, is one of the world's most important food crops. Cultivated tetraploid potatoes (*Solanum tuberosum* L.) originated in the Andes of South America, where they were mostly farmed at elevations ranging from 2000 to 4000 m in an environment characterized by short daylength, high light intensity, cold temperatures, and relatively high humidity [1]. Potato was then transported to the world, where many environmental factors affected tuber production. On the other hand, a diploid potato (*S. chacoense* Bitt.) is recognized to be the most common, aggressive and adaptable wild potato in South America. Plant breeders are interested in wide genetic variety, and *S. chacoense* has piqued their interest due to its claimed resistance to more than 20 pests and diseases [2].

Tetraploid potato cultivars are moderately sensitive to moderately tolerant to salinity, depending on the criteria used for classification [3–5]. This sensitivity is related to the growth stage of the plant, being more sensitive to salinity in the early growth periods [6, 7]. Salinity stress has been found to delay emergence, delay root and shoot development, and reduce shoot weight [8] in addition, reducing plant growth, and tuber yield and size [9–11]. In vitro salinity tolerance was tested on European and North American potato varieties. Six vegetative growth parameters, i.e., shoot and root lengths, fresh and dry weights were assessed at harvest and compensated for cultivar vigor variations [12]. Differences in sensitivity have been observed in many cultivated varieties [6, 11-14], although no extensive evaluation of cultivated varieties in comparison with wild species has yet been reported. Therefore, it is very important to screen the cultivated and wild species for their salt tolerance to recommend varieties that can be cultivated in high saline soil conditions or to use salt-tolerant genotypes in breeding programs.

The detrimental influence of salinity stress, for example, osmotic stress, ionic stress, and oxidative stress, may be analyzed in three different directions; oxidative stress is believed to be most harmful [15-17]. In plant cells which generate membrane lipids, proteins and nucleic acid damage, oxidative stress leads to the formation of harmful reactive oxygen species (ROS) [18]. According to the findings by [19], combining Trichoderma and biochar was the most efficient in reducing the harmful effects of salt on spinach plants by enhancing antioxidant up-regulation and decreasing membrane permeability and ROS. Furthermore, endophytic Bradyrhizobium and Enterobacter inoculation caused a large increase in morphological and physiological metrics; nevertheless, these treatments caused significant decreases in ROS, electrolyte leakage %, and malondialdehyde content in stressed soybean plants [20]. In saline soils, higher osmotic pressures prevent plant development and lead to plant death [21]. Many researchers were working intensively to explore biological processes to salt stress to cope with salt injury. One strategy was the exogenous use of cellularly recognized molecules to withstand stressors, such as betaines, proline and antioxidants [22]. Glycine betaine (GB) and proline (P) are two main organic osmolytes which build up on different plant species for environmental stress, including drought, cold and salt [23]. P is an amino acid and is a compatible solute which plays a key function in osmoregulation and osmo-tolerance [24]. It shields membranes and proteins from the disruptive effects of abiotic stress dehydration. Moreover, it is capable of scavenging free radicals during stresses [23]. GB is an amino acid derivative that is recognized for its protection effects in larger plants against salinity stresses, not only through the maintenance of osmotic adjustment but through stabilizing many functional units, such as the oxygen-devolving PS-II complex. Therefore, a number of plants can defend themselves from abiotic stress by increased glycine betaine production and build-up [23, 25]. Salicylic acid (SA), as an antioxidant, is a phytohormone, considered to be a universal signaling molecule protecting plants from several cases of biological or abiotic stress [26]. SA has been found to be involved in regulatory activities in a number of plant physiological processes, such as ion absorption and permeability of cells and their photosynthetic rate [27, 28]. In addition, SA foliar spray or seed tuber soaking has a considerable impact on the improvement of potato yield and quality [29].

Therefore, the objectives of this study were to: (1) determine the extent of genetic variability for salinity tolerance among different cultivated tetraploid potato cultivars and diploid wild potato accessions. (2) identify if osmoregulators and antioxidants, i.e., GB, P, and SA may be exogenously applied to alleviate salt stress injury in the examined potato genotypes. Meanwhile, plant morphological and physiological characteristics, enzymes activity and callus induction for thirteen genotypes were studied in the absence and presence of NaCl without or with osmoregulators and antioxidants to assess the genotypes' varied degrees of salt tolerance.

# **Materials and methods**

# **Plant materials**

Salt responses and osmoregulators or antioxidants effects were investigated in tetraploid and diploid of thirteen cultivated and wild potato genotypes. The genotypes used in this study included seven tetraploid potato cultivars of *Solanum tuberosum* ('Agata', 'Atlantic', 'Anya', 'Diamond', 'Gala', 'Kennebec' and 'Russet Burbank') and six clones of four accessions of the diploid wild species *S. chacoense* ('A-6', 'B-7', 'C-8', 'C-9', 'D-2' and 'D-3'). Tuber seeds of tetraploid cultivars were obtained from Agricultural research center (ARC), Egypt and true seeds of diploid accessions were obtained from obtained from the NRSP-6 United States Potato Gene bank in Sturgeon Bay, WI. The genotypes were chosen because they exhibited significant genetic diversity, making them suitable plant resources for testing salt tolerance.

# **Plant micropropagation**

For the rapid propagation of the above-mentioned potato genotypes, a tissue culture method was implemented. In order to harvest the explants of the tetraploid (2n=4x=48) S. tuberosum (tbr) cultivars, the sprouted potato tubers were planted in a greenhouse in 15 cm Pro-Mix pots with basic soil composition at 25°/15°C (day/ night) temperatures. In a sequential process, the tips of the shoot were separated and finely cleaned. The tips were then surface sterilized in a solution of 0.5% (v:v) sodium hypochlorite for 5 min and thoroughly rinsed with sterile distilled water [30]. The diploid (2n = 2x = 24)S. chacoense (chc) six clones used in the present study consisted of four accessions (A = PI 275136, B = PI 320288, C=PI 537025 and D=PI 566738). Accession A was collected in 1959 in Jujuy, Argentina; accession B was collected in 1966 in Cordoba, Argentina; accession C was collected in 1989 in Chuquisaca, Bolivia; accession and D was collected in 1993 in Asuncion, Paraguay (www.arsgrin.gov/npgs/acc/acc\_queries.html). True seeds were planted in flats of ProMix in the greenhouse at 25°/15°C (day/night) and two weeks later they were transplanted into 15 cm pots. Shoot tips from the 10 most vigorous plants per accession were micro-propagated to produce the above listed clones [31]. The explants were surface sterilized with 70% ethanol for 1 min followed by sodium hypochlorite (2%) for 10 min and thoroughly washed with sterile distilled water three times and introduced into tissue culture. Successively, surface sterilized shoot tips were cultured on Agar-solidified (8 g  $L^{-1}$ ) and sucrose  $(30 \text{ g L}^{-1})$  Murashige and Skoog (MS) basic medium [32]. The pH was modified to  $5.7 \pm 0.1$  before the inclusion of agar and the corresponding autoclaving to 121 °C and 15 psi for 20 min. Tissue excision, implantation and transition is performed under sterile conditions. Cultivated shoot tips were incubated for four weeks in the incubation room at 16 h photoperiod,  $25 \pm 2$  °C and white cool fluorescent bulbs supplying approximately 90  $\mu$ mol/m<sup>-2</sup>/  $s^{-1}$  of PPFD. Subcultures were performed using the tip of the shoots.

# Plantlet regeneration under salinity with/without osmoregulators and antioxidants treatments

Individual stem nodes were transferred to tubes containing 8 mL of MS growth medium, 30 g  $L^{-1}$  sucrose and 8 g  $L^{-1}$  agar supplemented with NaCl concentrations of 0, 100, 200 or 300 mmol  $L^{-1}$  representing, no stress, low stress, moderately stress and high stress levels. pH was adjusted at 5.7 and explants were allowed to grow for 4 weeks to test their tolerance or sensitivity to salinity. For the exogenous application of osmoregulators and antioxidants experiment, MS growth medium was supplemented with NaCl concentrations of 300 mmol L<sup>-1</sup> (high stress level) and glycine betaine (GB) at 400 mg  $L^{-1}$  or proline (P) at 200 mg  $L^{-1}$  or salicylic acid (SA) at 100 mg L<sup>-1</sup>. pH was adjusted to 5.7 and plantlets were allowed to grow for 4 weeks to test the effect of osmoregulators and antioxidants under high stress level. Ten plantlets per genotype were evaluated for each treatment and the experiments were repeated three times. All subcultures were maintained under 16 h photoperiod,  $19\pm2$  °C and white cool fluorescent bulbs providing approximately 90  $\mu$ mol/m<sup>-2</sup>/s<sup>-1</sup> PPFD.

# Root traits analysis

Root's development changes due to salt stress with and without osmoregulators and antioxidants in culture were fully documented. After 4 weeks, plantlets were collected from the tubes: root parameters, i.e., roots fresh weight, surface area root, total root length, and number of roots/ plantlets were analyzed using WinRhizo Basic 2009 tool (Regent Instruments Canada, Inc.).

# Plant water content and ions analysis

In addition, the plantlets water content % (PWC) was measured according to [33]. Ions of sodium (Na<sup>+</sup>), chloride (Cl<sup>-</sup>) and potassium (K<sup>+</sup>) content was determined based on method of [34]. The analysis of Na<sup>+</sup> and K<sup>+</sup> was done using 200 mg of plantlets tissues which were transferred to 100 cm<sup>3</sup> digestion flasks containing 50 ml 0.1 N HNO<sub>3</sub>. After 30 min of shaking, the extracts were filtered (Whatman qualitative filter paper, Grade 1) and ions were determined by atomic absorption spectrophotometry, whereas Cl<sup>-</sup> was determined by potentiometric titration with 0.01 N AgNO<sub>3</sub>

# Leaf's chlorophyll and enzymes activity

The chlorophyll content of potato leaves was measured using a SPAD-502 chlorophyll meter [35]. The enzyme activity (U min<sup>-1</sup> g<sup>-1</sup> FW) of fresh leaves of potato plantlets were measured spectrophotometrically using the techniques of [36] for catalase (CAT) and [37] for peroxidase (POD).

# Callus induction under salinity with/without osmoregulators and antioxidants treatments

Callus cultures were initiated from leaves of the regenerated plantlets of the tested potato tetraploid cultivars and diploid accessions. The leaves were cultured on MS medium supplemented with myo-inositol (100 mg  $L^{-1}$ ), sucrose (30 g  $L^{-1}$ ) and solidified with Agar (8 g  $L^{-1}$ ). Plant growth regulators were 0.4 mg  $L^{-1}$  NAA and 2 mg  $L^{-1}$  kinetin [38]. Therefore, three different NaCl concentrations (100, 200 or 300 mmol  $L^{-1}$ ) were compared with the control (MS medium without NaCl) to test the genotypes' capacities for callus induction under salinity stress. For osmoregulators and antioxidants experiment, MS growth medium was supplemented with NaCl concentrations of 300 mmol L<sup>-1</sup> (high stress level) and GB at 400 mg  $L^{-1}$  or P at 200 mg  $L^{-1}$  or SA at 100 mg  $L^{-1}$ . Before autoclaving at 121 °C for 20 min, the pH of the medium was adjusted to 5.70. The cultures were kept in the dark for the first two weeks before being moved to light conditions for another 2 weeks under 16 h photoperiod, 19±2 °C and white cool fluorescent bulbs providing approximately 90  $\mu$ mol/m<sup>-2</sup>/s<sup>-1</sup> PPFD. Each explant was evaluated after four weeks for callus fresh weight, percentage of explants forming callus, and percentage of explants forming roots. The experiment was repeated three times with ten calli per genotype evaluated for each treatment in both experiments.

#### Salinity tolerance analysis

Response to salinity stress and impact of osmoregulators and antioxidants to alleviate the adverse effect was assessed using stress tolerance index (STI). STI for each genotype under salinity stress level and osmoregulators or antioxidants treatments was estimated according to the method of [39]. STI was calculated as the ratio of the trait performance at high stress level (300 mol L<sup>-1</sup> NaCl) with and without GB, P and SA to the trait performance at no stress (MS medium without NaCl) as described in the following formula:

$$STI = Ts/Tp$$

where:

*Ts* is the trait of genotype under stress with and without osmoregulators and antioxidants treatments.

*Tp* the trait under no stress.

# Statistical analysis

The experimental design was a  $13 \times 4$  (genotypes x salinity treatments) in a factorial experiment with three replications (n=10) in a split block design for in vitro based screening and all obtained data were subjected to the analysis of variance using the combined data from

Genotypes	Roots fresh	weight (g)	Surface are	a of root (cm <sup>2</sup> )	Total root le	ength (mm)	No. of roots	/plantlet
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Tetraploid cultivars of S	S. tuberosum (tbr)							
Agata	0.850	0.563	1.140	0.741	111.25	104.35	8.65	6.32
Atlantic	0.941	0.345	0.790	0.576	66.32	50.15	6.33	5.38
Anya	0.984	0.250	0.547	0.170	69.61	32.29	6.55	2.25
Diamond	1.290	1.025	1.110	0.898	94.32	83.71	8.79	8.06
Gala	0.910	0.308	0.980	0.187	98.17	22.05	8.16	1.71
Kennebec	0.904	0.507	0.721	0.502	57.65	53.12	6.24	5.79
Russet Burbank	1.049	0.799	1.110	0.787	89.63	72.85	9.88	8.35
Diploid clones of S. cha	acoense (chc)							
A-6	1.036	1.212	1.433	1.160	99.74	93.79	10.10	10.07
B-7	0.905	0.610	0.841	0.470	80.14	35.50	8.67	7.22
C-8	0.999	1.292	1.490	1.432	88.32	83.77	9.57	9.14
C-9	0.874	0.482	0.547	0.470	69.34	30.53	7.84	6.04
D-2	1.205	0.926	1.580	1.242	113.32	109.27	10.51	10.71
D-3	0.895	0.962	0.990	0.633	75.68	75.60	8.10	6.59
LSD (0.05)	0.06179		0.2304		1.0122		0.2413	

**Table 1** Roots fresh weight, surface area of root, total root length and number of roots/plantlet of 13 genotypes of potato divided into 7 tetraploid cultivars of *Solanum tuberosum (tbr)*, and 6 diploid clones of *S. chacoense (chc)* grown in vitro under stress conditions

Explants were grown on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol  $L^{-1}$ ) and the average tested parameters over the combined three levels of stress were compared with the control (no stress)

the mean stress levels; 100, 200 and 300 mol L<sup>-1</sup> NaCl. For osmoregulators and antioxidants experiment, the design was factorial experiment (13 potato genotypes x NaCl concentration "high level, 300 mmol L<sup>-1</sup> NaCl" + 3 osmoregulators and antioxidants treatments) with three replications (n = 10) in a split block design. Data obtained from this study were subjected to analysis using SAS, version 9.3 (Cary, NC). Differences among potato genotypes were tested by an analysis of variance (ANOVA), and mean significant differences were tested by the Least Significant Difference (LSD) test at the 0.05 level of significance.

# Results

The current study's findings show the effects of salinity, osmoregulators and antioxidants, and their interactions on root growth, plant water content % (PWC), ions content, leaf chlorophyll content, catalase (CAT) and peroxidase (POD) enzyme activity and callus induction capacity in potato tetraploid cultivars of *Solanum tuberosum (tbr)* and diploid clones of *S. chacoense (chc)*. In the first experiment, genotypes were grown in vitro on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol L<sup>-1</sup>) and the average tested parameters throughout the combined three levels of stress were compared with the control (no stress, MS medium without NaCl), whereas in the second experiment, to teste the

effect of exogenous application of osmoregulators and antioxidants on alleviating the adverse impact of salinity, glycine betaine (GB), proline (P) and salicylic acid (SA) were solely added to the MS growth medium which was supplemented with high stress level of NaCl (300 mmol  $L^{-1}$ ).

# **Root traits**

The effects of salinity stress on the roots fresh weight, mean surface area of root, total root length and no. of roots/plantlet of the tested potato genotypes grown in vitro under salinity stress are shown in Table 1. Roots traits decreased as the salinity stress level increased with reductions generally greater in tetraploid cultivars of tbr than in diploid wild species clones of chc (Additional file 1: Data S1). The obtained data revealed considerable variations in roots fresh weight at stress levels, an expected result since changes in root characteristics are one of the primary responses of plants to salinity stress [38]; however, there were clear differences in root growth at the higher NaCl concentrations among the cultivars. In general, many of *tbr* cultivars showed more roots fresh weight at the no stress level than any of the other clones of chc. With the combined stress levels, cultivars 'Anya' and 'Gala' developed the lighter roots (0.25 and 0.308 g, respectively); however, other cultivars for example, 'Diamond' cv., and 'Russet Burbank' cv. had higher average roots fresh weight (1.025 and 0.799 g, respectively) when subjected to the stress levels (100, 200 and 300 mmol L<sup>-1</sup> NaCl) as clear in Table 1. On the other hand, the fresh weight of the roots rose with increasing salt concentrations in two of the *chc* clones. According to statistical analysis, two *chc* accessions (clones 'C-8' and 'A-6') exhibited considerably greater roots fresh weight (1.292 and 1.212 g, respectively) than all other accessions when grown in NaCl-enriched medium and were closely followed by clones 'D-3' and 'D-2' (0.962 and 0.926 g, respectively). At the same time, *chc* clones 'C-9' and 'B-7' showed the lightest roots fresh weight (0.482 and 0.610 g, respectively).

The root surface area of potato cultivars tbr and the related *Solanum* wild species *chc* was examined (Table 1). Overall comparisons of the 13 examined genotypes indicated significant differences among cultivated potato and wild species in surface area of root at all tested stress levels and the control (Additional file 1: Data S1). The mean root surface area for most of the examined genotypes differed significantly under stress levels. S. tuberosum 'Diamond' cv. followed by 'Russet Burbank' cv. and 'Agata' cv. had higher root surface area (0.898, 0.787 and  $0.741 \text{ cm}^2$ ) compared to the other potato *tbr* cultivars. Under salt stress, chc species had substantially smaller root surface area, although it was still greater than tbr cultivars. The root surface area of chc clone 'C-8' was the greatest, although it remained lower than the control (no stress). Overall, the 13 genotypes' roots fresh weight and mean root surface area varied from 0.05 to 1.29 g and 0.11 to 1.49 cm<sup>2</sup>, with a 25.8 and 13.5-fold difference, respectively.

Furthermore, other root characteristics such as total root length, and no. of roots were examined as clear in Table 1. Some of the *tbr* cultivars had significantly longer and more roots than the chc accessions at control (no stress) treatment; however, the wild potato accessions chc formed longer roots with salinity stress than the tbr potato cultivars. Under control conditions, total root length ranged from 57.65 to 111.25 mm and from 69.34 to 113.32 mm whereas, no. of roots ranged from 6.24 to 9.88 and from 7.84 to 10.51 in tbr cultivars and chc clones, respectively. While under stress conditions, total root length ranged from 22.05 to 104.35 mm and from 30.53 to 109.27 mm whereas, no. of roots ranged from 1.71 to 8.35 and from 6.04 to 10.71 in tbr cultivars and chc clones, respectively. Cultivars 'Agata' and 'Diamond' produced the longest roots in *tbr* when clones 'D-2' and 'A-6' were superior in chc. Statistical analysis indicated that chc (clone 'D-2') had significantly more roots under stress conditions than other genotypes examined in this experiment.

In terms of the impact of osmoregulators and antioxidants treatments on potato root traits, a substantial beneficial effect of these exogenous applications was identified in both tetraploids' cultivars and diploids accessions under high stress level (300 mmol  $L^{-1}$  NaCl) which examined in the second experiment. In general, exogenous treatments with osmoregulators and antioxidants improved root growth with the superiority of GB treatment followed by SA and P treatments compared to control (no stress) and high stress level without osmoregulators or antioxidants (Fig. 1). The mean of increasing in root traits owed to the GB application under high stress above control (no stress) and it was calculated to be 1.07 and 1. 87 g for roots fresh weight, 0.85 and 1.134 cm<sup>2</sup> for surface area of root, 81.05 and 89.52 mm for total root length and 7.617 and 9.46 for no. of roots in tbr cultivars and chc accessions, respectively. Furthermore, the interaction between genotypes and osmoregulators or antioxidants was shown to be significant for examined potato root traits. When compared to potato plantlets that did not receive exogenous treatments, GB application resulted in the highest mean values of plantlets roots fresh weight in tbr cultivars, 'Diamond' and 'Russet Burbank' and in chc, clones 'C-8' and 'A-6' (Fig. 1A and A'), the highest mean values of surface area of root in tbr cultivars, 'Diamond' and 'Agata' and in chc, clones 'D-2', 'C-8' and 'A-6', respectively (Fig. 1B and B'), the highest mean values of plantlet total root length in tbr cultivars, 'Agata' and 'Diamond' and in chc, clones 'D-2' and 'A-6' respectively (Fig. 1C and C'), and the highest mean values of number of roots in tbr cultivars, 'Russet Burbank', 'Diamond' and 'Agata' and in chc, clones 'D-2', 'C-8' and 'A-6', respectively (Fig. 1D and D').

#### Plant water content

The effect of salinity stress on plantlet water content % (PWC) after 4 weeks of growth is shown in Table 2. PWC % was reduced significantly at all stress levels for tbr cultivars and chc clones (Additional file 1: Data S1), with tbr varieties revealing a much higher reduction than chc clones. Under control (no stress), PWC % varied from 91 to 94% in tbr and from 92 to 94% in chc; however, with stress conditions, PWC % ranged from 55 to 85% in tbr and from 72 to 90% in chc. chc clones 'A-6', 'C-8', and 'D-2' yielded the highest PWC percentages (90, 89, and 88%, respectively), whereas tbr cultivars 'Gala' and 'Anya' yielded the lowest percentages (55 and 58%, respectively). In comparison, exogenous application of GB, P and SA had a significant influence on PWC % in the plantlets of all tested cultivars of tbr and accessions of chc under examined stress level. GB, SA and P, respectively, resulted in a substantial increase in PWC % however, GB was generally more effective than SA and P. Using GB led to an enhancement of PWC % in the presence of high salt stress (Fig. 2A and A'). PWC % was 93% on average under





**Table 2** Plantlet water content % (PWC), chloride (Cl<sup>-</sup>), sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) content (mmol kg<sup>-1</sup> FW) of 13 genotypes of potato divided into 7 tetraploid cultivars of *Solanum tuberosum (tbr)*, and 6 diploid clones of *S. chacoense (chc)* grown in vitro under stress conditions

Genotypes	PWC %		Cl <sup>-</sup> (mmol l	⟨g <sup>−1</sup> FW)	Na <sup>+</sup> (mmol	kg <sup>−1</sup> FW)	K <sup>+</sup> (mmol k	g <sup>-1</sup> FW)
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Tetraploid cultivars of S	S. tuberosum (tbr)							
Agata	91.0	81.0	39.0	307.00	38.0	294.67	82.0	50.50
Atlantic	93.0	76.0	44.0	315.33	41.0	312.33	82.0	50.70
Anya	93.0	58.0	43.0	426.67	44.0	416.33	80.0	27.33
Diamond	93.0	85.0	34.0	215.00	33.0	211.67	81.0	56.67
Gala	93.0	55.0	43.0	440.03	45.0	423.53	80.0	26.40
Kennebec	94.0	78.0	35.0	316.67	30.0	299.00	85.0	52.03
Russet Burbank	94.0	77.0	55.0	242.00	52.0	240.00	82.0	54.47
Diploid clones of S. cha	acoense (chc)							
A-6	94.0	90.0	20.0	161.00	17.0	239.67	82.0	70.67
B-7	93.0	72.0	24.0	255.33	21.0	309.00	67.0	30.00
C-8	93.0	89.0	18.0	175.67	15.0	136.33	81.0	78.33
C-9	94.0	73.0	35.0	249.33	28.0	292.33	76.0	58.67
D-2	93.0	88.0	25.0	232.67	22.0	214.33	80.0	73.67
D-3	92.0	76.0	22.0	255.00	20.0	272.67	78.0	65.67
LSD (0.05)	3.401		9.241		10.341		2.333	

Explants were grown on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol  $L^{-1}$ ) and the average tested parameters over the combined three levels of stress were compared with the control (no stress)

no stress conditions, compared to 60 and 76% in *tbr* and *chc* genotypes under high stress without exogenous applications. Exogenous treatments increased the PWC % in *tbr* cultivars to 72, 78, and 81% and in *chc* accessions to 77, 85, and 91% for P, SA, and GB, respectively. When compared to other genotypes, plantlets of 'A-6' clone and 'Diamond' cv. had the highest PWC (94 and 90%) under stress level + GB treatment.

# Chloride (Cl<sup>-</sup>), sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) content analysis

For ions content, the Cl<sup>-</sup> and Na<sup>+</sup> concentrations (mmol kg<sup>-1</sup> FW) in plantlets increased significantly with increasing stress levels in both species of *Solanum*, *tbr* and *chc*, as compared to control (Table 2). At the stress levels, large Cl<sup>-</sup> and Na<sup>+</sup> accumulation was detected in plantlets of tetraploid species *tbr* with a trend toward greater accumulation in the 'Gala' cv. (440.03 and 423.53 mmol kg<sup>-1</sup> FW, respectively) compared to the other *tbr* cultivars. On the other hand, plantlets of

'Diamond' cv. accumulated the lowest amounts of Cland Na<sup>+</sup> (215 and 211.67 mmol kg<sup>-1</sup> FW, respectively). At control conditions, the ions content varied from 34 to 55 mmol kg<sup>-1</sup> FW for Cl<sup>-</sup> and from 30 to 52 mmol kg<sup>-1</sup> FW for Na<sup>+</sup>, while under stress conditions, the ions content ranged from 215 to 440.03 mmol kg<sup>-1</sup> FW for Cl<sup>-</sup> and from 211.67 to 423.53 mmol kg<sup>-1</sup> FW for Na<sup>+</sup>. In chc clones, accumulation of saline ions (Cl<sup>-</sup> and Na<sup>+</sup>) was greater in 'B-7', 'D-3' and 'C-9' than other clones, whereas the lowest content was observed on clone 'A-6' for Cl<sup>-</sup> ions and on clone 'C-8' for Na<sup>+</sup> ions. Under control (no stress), Cl<sup>-</sup> and Na<sup>+</sup> content ranged from 18 to 35 mmol kg<sup>-1</sup> FW and from 15 to 28 mmol kg<sup>-1</sup> FW, respectively whereas, under stress treatment, ions ranged from 161.0 to 255.33 and 136.33 to 309.0 mmol  $kg^{-1}$ FW, respectively. In contrary, treatments with increasing stress levels resulted in a significant decrease in K<sup>+</sup> content in the plantlets of all examined genotypes of thr and *chc*, although the K<sup>+</sup> reduction was greater for *tbr* than for chc under stress level (Table 2). In all cultivars

(See figure on next page.)

**Fig. 2** Plantlet water content % **A** and **A'**, chloride **B** and **B'**, sodium **C** and **C'** and potassium **D** and **D'** content of in vitro-cultured stem node of seven tetraploid potato cultivars *Solanum tuberosum* ('Agata', 'Atlantic', 'Anya', 'Diamond', 'Gala', 'Kennebec' and 'Russet Burbank') and six clones of four accessions of diploid wild species *Solanum chacoense* ('A-6', 'B-7', 'C-8', 'C-9', 'D-2' and 'D-3'). Explants were grown under conditions of high salt stress level (300 mmol L<sup>-1</sup> NaCl) with and without glycine betaine (at 400 mg L<sup>-1</sup>), proline (at 200 mg L<sup>-1</sup>) or salicylic acid (at 100 mg L<sup>-1</sup>) treatments. Values are means  $\pm$  SE, (*n* = 10) and the experiment was repeated three times





of the *tbr* species, a significant reduction in K<sup>+</sup> was observed, although 'Diamond' and 'Russet Burbank' cultivars showed statistically significant higher values at the levels of salinity stress (56.67 and 54.47 mmol kg<sup>-1</sup> FW, respectively). K<sup>+</sup> was reduced significantly by salt stress in the plantlets of *chc* started from the treatment using the lowest concentration of NaCl (Additional file 1: Data S1) however, clones 'C-8' and 'D-2' showed significantly low reduction across salinity levels (78.33 and 73.67 mmol kg<sup>-1</sup> FW, respectively).

Furthermore, as demonstrated in Fig. 2, the influence of osmoregulators and antioxidants on ions content, Cl<sup>-</sup>, Na<sup>+</sup>, and K<sup>+</sup> was significant in the tetraploid and diploid potato genotypes studied. GB, SA and P were shown to be the optimal treatments for a significant decrease in the plantlets' Cl<sup>-</sup> (Fig. 2B and B'), Na<sup>+</sup> (Fig. 2C and C') and a substantial rise in the plantlets' K<sup>+</sup> (Fig. 2D and D') of potato *tbr* cultivars and *chc* accessions produced under sever salt stress. The average reductions in Cl<sup>-</sup> and Na<sup>+</sup> ions was 229.1, 297.35 and 242.31, 287.7 mmol kg<sup>-1</sup> FW in tbr and chc, respectively. Meanwhile, the average increase in K<sup>+</sup> mmol kg<sup>-1</sup> FW of high stress (300 mmol  $L^{-1}$  NaCl)+GB compared to high stress level without exogenous treatments was 41.48 and 14.93 mmol kg<sup>-1</sup> FW in tbr and chc, respectively. In general, 'Diamond' cv. remained the most salt-tolerant cultivar among the tbr cultivars. chc 'C-8' was identified as the clone with superior  $K^+$  when subjected to high stress + GB.

The variation in Na<sup>+</sup>/ K<sup>+</sup> ratio was determined using sodium and potassium concentrations in the plantlets, as shown in Fig. 3. The greater Na<sup>+</sup>/K<sup>+</sup> ratio was observed on cultivars of the *tbr*; nevertheless, 'Diamond' cv. had the lowest ratio under the highest level of salinity (Fig. 3A). In diploid species; *chc* clones exhibited the lowest Na<sup>+</sup>/K<sup>+</sup> ratio compared with those from *tbr* at the highest salt level. *chc* 'C-8' was identified as the clone with superior tolerance to salt as Na<sup>+</sup>/K<sup>+</sup> ratio was the lowest among all genotypes examined. Regarding the use of osmoregulators or antioxidants, GB, SA and P enhanced the tolerance of all genotypes by decreasing the Na<sup>+</sup>/ K<sup>+</sup> ratio. With no significant differences, GB and SA were more effective than P (Fig. 3A and A').

# Chlorophyll contents and enzymes activity

The effect of rising salt stress level on potato plants was found to have a significant influence on the leaves' content of chlorophyll and catalase (CAT) and peroxidase (POD) enzymes' activities (Table 3). Salinity stress levels dramatically decreased chlorophyll content in all genotypes, and the decline was considerably larger in *tbr* cultivars than *chc* clones. The higher effect was detected on the *tbr's* 'Gala' cv. (22.7); nevertheless, the 'Diamond' cv. retained a significant concentration of chlorophyll (41.1) at the stress levels. Statistical analysis revealed that *chc* clones 'C-8', 'A-6' and 'D-2' had significantly higher chlorophyll content (42.4, 42.1 and 41.8,

Genotypes	Chlorophyll (S	PAD)	Catalase (U mi	in <sup>-1</sup> g <sup>-1</sup> FW)	Peroxidase (U FW)	$min^{-1}g^{-1}$
	Control	Stress	Control	Stress	Control	Stress
Tetraploid cultivars of S. to	uberosum (tbr)					
Agata	48.5	33.4	1.32	3.8	1.29	3.5
Atlantic	49.6	35.0	1.39	3.9	1.32	3.5
Anya	46.5	23.7	1.34	4.0	1.24	4.1
Diamond	50.4	41.1	1.33	3.7	1.35	3.4
Gala	45.6	22.7	1.33	4.03	1.23	4.1
Kennebec	47.6	32.8	1.35	3.9	1.25	3.9
Russet Burbank	49.5	34.7	1.36	3.8	1.31	3.5
Diploid clones of S. chace	pense (chc)					
A-6	47.5	42.1	1.41	2.1	1.34	1.8
B-7	48.5	28.2	1.45	3.2	1.25	3.2
C-8	47.6	42.4	1.44	1.9	1.35	1.7
C-9	49.5	35.7	1.42	4.3	1.26	3.5
D-2	47.1	41.8	1.43	2.1	1.32	1.9
D-3	47.3	35.4	1.41	4.2	1.3	3.3
LSD (0.05)	2.451		0.305		0.201	

**Table 3** Chlorophyll (SPAD), catalase (U min<sup>-1</sup>  $g^{-1}$  FW) and peroxidase (U min<sup>-1</sup>  $g^{-1}$  FW) of 13 genotypes of potato divided into 7 tetraploid cultivars of *Solanum tuberosum (tbr)*, and 6 diploid clones of *S. chacoense (chc)* grown in vitro under stress conditions

Explants were grown on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol L-1) and the average tested parameters over the combined three levels of stress were compared with the control (no stress)

respectively) under salinity conditions than the other accessions evaluated, whereas clone 'B-7' was the most sensitive genotype with the lowest chlorophyll content (28.2). In contrast, salt levels considerably enhanced the activity of the CAT and POD enzymes in all genotypes of *tbr* and *chc*, which peaked when potato stem nodes were cultured under high stress level. When compared to the no-stress level (control), the highest salinity level resulted in an increase in CAT and POD activities estimated at 35.29 and 31.36%, respectively in *tbr* cultivars and 35.53 and 31.56%, respectively in *chc* genotypes. 'Anya' cv. and 'Gala' cv. exhibited the greatest enzyme activity in *tbr* cultivars, whereas 'Diamond' cv. had the lowest. On the other hand, 'C-9' exhibited the greatest enzyme activity in *chc* clones, whereas 'C-8' had the lowest.

In respect of the effect of GB, P and SA exogenous treatments, the results in Fig. 4 demonstrated a substantial increase in chlorophyll content and decrease in enzyme activity under high stress level. The overall effect of GB, P and SA on the content of chlorophyll as well as the activities of the CAT and POD enzymes in potato genotypes leaves was found to be significant in their mean values with superiority of GB treatment. When the mean values of chlorophyll and the activity of CAT and POD enzymes were compared in the leaves of plants that received GB treatment to the high stress, it was found that this treatment caused an increase of chlorophyll of 19.1 and 12.9%, respectively (Fig. 4A and A') and a decrease of the activity of CAT of 2.3 and 1.32% (Fig. 4B and B') and POD of 2.3 and 1.22% (Fig. 4C and C'), on average for both potato species *tbr* and *chc*, respectively. In general, 'Diamond' cv. and 'C-8' clone were the best genotypes compared to other *tbr* cultivars and *chc* clones when treated with GB.

# **Callus induction capacity**

The effects of salinity on callus fresh weight, % of explants forming callus and % of explants forming roots after four weeks are shown in Table 4. Callus fresh weight was effectively diminished at every salt level for the tbr cultivars. Clones of chc executed relatively better across all saline levels, whereas callus growth was induced in the chc clones 'D-2', 'A-6' and 'C-8'. Under different levels of salinity, all evaluated tetraploid and diploid species could form callus; however, the percentage decreased with increasing salt concentration in all potato genotypes. Only 'Diamond' cv. was consistent in its ability to form callus across all salt stress levels and provided the highest percentage of explants forming callus among the tbr cultivars (84.89%). In chc accessions, clone 'C-8' was superior and produced the highest percentage of explants forming callus (89.22%) compared to other chc clones and tbr cultivars.



Furthermore, under salinity stress, all diploid potato accession of *chc* formed the highest proportion of callus compared to *tbr* cultivars; however, the proportion of callus that developed roots was significantly affected by salinity stress in all genotypes tested. The significantly larger effect was observed on *tbr* cultivars, but

'Diamond' cv. retained a significant ability to form roots. The cultivars 'Gala' cv. and 'Anya' cv., on the other hand, were the most affected and had the lowest percentage of callus forming roots (1.7 and 2.0%, respectively). Diploid *chc* accessions exhibited high salt tolerance, with 67.44–88.78% of all explants forming roots. Under stress

Genotypes	Callus fresh w	eight (g)	% of explants	forming callus	% of explants	forming roots
	Control	Stress	Control	Stress	Control	Stress
Tetraploid cultivars of S. to	uberosum (tbr)					
Agata	1.30	0.72	86.00	42.00	99.0	22.11
Atlantic	4.15	1.88	87.33	54.56	89.0	30.89
Anya	1.34	0.38	86.33	31.78	99.0	2.00
Diamond	3.45	3.12	86.67	84.89	99.0	57.78
Gala	1.35	0.37	86.81	33.42	98.0	1.70
Kennebec	2.03	1.11	86.00	51.22	88.0	20.89
Russet Burbank	2.72	1.37	86.33	42.00	99.3	16.44
Diploid clones of S. chaco	pense (chc)					
A-6	1.74	2.03	86.33	85.11	88.33	83.67
B-7	1.09	0.70	88.00	77.44	88.33	83.67
C-8	1.54	1.86	87.33	89.22	91.00	88.78
C-9	1.11	1.06	84.67	42.33	84.67	67.44
D-2	2.56	2.28	84.67	65.33	90.00	78.89
D-3	1.61	1.50	86.33	87.89	89.33	82.22
LSD (0.05)	0.102		1.304		1.221	

**Table 4** Callus fresh weight, % of explants forming callus and % of explants forming roots of 13 genotypes of potato divided into 7 tetraploid cultivars of *Solanum tuberosum (tbr)*, and 6 diploid clones of *S. chacoense (chc)* grown in vitro under stress conditions

Explants were grown on media supplemented with three different concentrations of NaCl (100, 200 or 300 mmol  $L^{-1}$ ) and the average tested parameters over the combined three levels of stress were compared with the control (no stress)

conditions, *chc* clone 'C-8' surpassed all other genotypes, yielding the highest percentage of explants that formed roots.

Treatments with GB, P, and SA enhanced callus induction capacity significantly under high stress level, as shown in Fig. 5. Exogenous applications considerably improved callus fresh weight (Fig. 5A and A'), the percentage of explants forming callus (Fig. 5 B and B'), and the percentage of explants forming roots (Fig. 5C and C'), with the effect being greater in *tbr* cultivars. In general, under high salt stress without exogenous applications chc accessions had more callus fresh weight, % of explants forming callus, and % of explants forming roots than tbr cultivars. With exogenous applications, many of tbr cultivars showed the same or even greater callus induction capacity than chc clones, and GB was more effective than SA and P. When exogenously treated with osmoregulators and antioxidants especially GB, cultivars 'Atlantic' and 'Diamond' generated the heaviest callus fresh weight, while 'Diamond' cv. and 'C-8' clone had the highest proportion of explants producing callus and explants developing roots compared to other *tbr* and *chc* genotypes.

#### Salinity Tolerance Index (STI)

Salt tolerance, as expressed by the salinity tolerance index (STI) under high stress without and with exogenous applications of GB, P, and SA is shown in Table 5. Of most interest from a salt tolerance standpoint are genotypes

where the morpho-physiological parameters are no less under high salt conditions with exogenous applications of GB, P, and SA than under non-salt conditions. The salttolerance index (STI) developed by [39] defines tolerance at a certain salinity level in comparison to a control. In this experiment, each genotype had a control (no salt). To compute the STIs for the investigated parameters, the ratio for each genotype and salt treatment (300 mmol  $L^{-1}$ NaCl) without and with exogenous applications of GB, P, and SA to that genotype 's no salt treatment was determined. In overall, the STIs of the examined parameters revealed that chc accessions had higher salt tolerance than tbr potato cultivars. STI indicated that callus growth specially, the percentage of explants forming roots in *tbr* cultivars and surface area of root  $(cm^2)$  in *chc* clones were the most impacted characteristics to salt stress when compared to other STI of other traits. 'Diamond' cv. from tbr cultivars and 'C-8' from chc clones, on the other hand, were more tolerant and generated the highest STI, and the values rose considerably with exogenous treatments of GB, SA, and P, respectively. Furthermore, exogenous treatments significantly improved the STI of cultivars 'Anya' and 'Gala,' which exhibited large reductions and gave the lowest values as a response to salt stress. STI of the percentage of explants forming roots, for example, improved in 'Anya' cv. from 0.0 to 51%, 56 and 78% and in 'Gala' cv. from 0.0 to 52%, 67 and 81% by the treatments of P, SA and GB, respectively.



# Values are means $\pm$ SE, (n = 10) and the experiment was repeated three times

# Principal component analysis (PCA)

To determine the most desirable salt stress tolerance criteria, the correlation between genotypes and the studied traits under stress and exogenous treatments of GB, P and SA was performed using the principal component analysis (PCA) as shown in Table 6. A PCA was executed utilizing root characteristics, PWC %, ions content, chlorophyll content, enzymes activity, and callus traits of thirteen potato genotypes evaluated for genetic diversity under high stress conditions (300 mmol  $L^{-1}$  NaCl) and GB, P, and SA treatment. Root characteristics, such as roots fresh weight, number of roots/plantlets, surface area of root, and total root length, had the greatest variations of 1.255, 0.956,

Table 5 Salinity tolerance index (STI) of 13 genotypes of potato divided into 7 tetraploid cultivars of Solanum tuberosum (tbr), and 6 diploid clones of S. chacoense (chc) grown
in vitro under conditions of high salt stress level (300 mmol L <sup>-1</sup> NaCl) with and without glycine betaine (at 400 mg L <sup>-1</sup> ), proline (at 200 mg L <sup>-1</sup> ) or salicylic acid (at 100 mg L <sup>-1</sup> )
treatments

ureaumenus													
Traits	S. tuberc	osum (tbr)						S. chacoe	ense (chc)				
	Agata	Atlantic	Anya	Diamond	Gala	Kennebec	Russet Burbank	A-6	B-7	8-' '	C-9	D-2	D-3
STI under high stress													
Roots fresh weight (g)	0.45	0.23	0.102	0.66	0.12	0.42	0.61	0.95	0.52	0.98	0.38	0.58	0.91
Surface area of root (cm <sup>2</sup> )	0.43	0.45	0.20	0.71	0.12	0.49	0.58	0.73	0.43	0.99	0.75	0.70	0.53
Total root length (mm)	0.89	0.61	0.32	0.80	0.15	0.74	0.75	0.86	0.32	0.86	0.36	0.89	0.93
No. of roots/plantlet	0.59	0.77	0.15	0.81	0.11	0.87	0.70	0.84	0.70	0.85	0.58	0.86	0.63
Plantlet water content%	0.77	0.70	0.43	0.83	0.39	0.70	0.70	0.94	0.66	0.94	0.69	0.91	0.76
Chloride (mmol kg <sup>-1</sup> FW)	9.13	8.32	11.37	8.18	11.53	10.86	5.45	10.50	12.50	12.78	8.51	11.52	13.64
Sodium (mmol kg <sup>-1</sup> FW)	9.21	9.07	10.84	8.03	10.70	12.23	5.75	17.00	18.43	11.93	13.46	12.09	18.90
Potassium (mmol kg <sup>-1</sup> FW)	0.37	0.37	0.28	0.47	0.26	0.38	0.43	0.70	0.34	0.95	0.54	0.84	0.73
Chlorophyll (SPAD)	0.52	0.57	0.33	0.76	0.33	0.51	0.59	0.83	0.44	0.84	0.61	0.84	0.66
Catalase (U min <sup>-1</sup> g <sup>-1</sup> FW)	3.11	2.95	3.28	3.01	3.31	3.26	2.94	1.77	2.83	1.46	3.45	1.68	3.62
Peroxidase (U min <sup>-1</sup> g <sup>-1</sup> FW)	3.08	3.11	3.55	2.89	3.66	3.44	3.13	1.567	2.880	1.333	3.175	1.667	3.154
Callus fresh weight (g)	0.27	0.24	00.0	0.79	00.0	0.34	0.20	1.09	0.46	0.91	0.63	0.77	06.0
Explants forming callus %	0.34	0.48	00:0	0.93	00.0	0.37	0.41	0.97	0.70	1.02	0.37	0.77	0.98
Explants forming roots%	00.00	0.00	00:0	0.49	00.0	0.01	0.00	0.91	0.91	0.93	0.72	0.65	0.82
STI under high stress + proline													
Roots fresh weight (g)	0.94	0.49	0.102	1.07	0.09	0.55	0.97	1.37	1.45	1.41	1.23	1.04	1.48
Surface area of root (cm $^2$ )	0.85	0.90	0.44	0.88	0.35	0.91	0.69	0.78	0.61	0.99	0.81	0.82	0.65
Total root length (mm)	0.91	0.73	0.50	0.85	0.38	0.85	0.79	0.91	0.88	0.91	0.80	0.96	0.96
No. of roots/plantlet	0.94	0.88	0.67	0.96	0.48	0.91	0.95	0.98	0.94	0.97	0.87	0.94	0.91
Plantlet water content%	0.89	0.81	09:0	0.95	0.54	0.80	0.81	0.83	0.80	06.0	0.74	0.87	0.82
Chloride (mmol kg <sup>-1</sup> FW)	3.85	3.66	4.06	3.50	3.96	4.31	2.27	7.52	5.85	9.15	3.95	5.21	6.43
Sodium (mmol kg <sup>-1</sup> FW)	4.09	4.21	4.03	4.88	4.07	5.80	3.18	8.22	6.46	8.57	4.53	5.46	6.39
Potassium (mmol kg <sup>-1</sup> FW)	0.43	0.36	0.32	0.45	0.31	0.28	0.47	0.67	0.52	0.73	0.43	0.39	0.44
Chlorophyll (SPAD)	0.73	0.78	0.65	0.88	0.65	0.63	0.78	0.87	0.73	0.89	0.80	0.86	0.84
Catalase (U min <sup>-1</sup> g <sup>-1</sup> FW)	1.82	1.22	1.87	1.05	1.80	1.85	1.32	1.49	2.14	1.53	1.90	1.47	1.99
Peroxidase (U min <sup>-1</sup> g <sup>-1</sup> FW)	1.86	2.05	2.18	1.41	2.28	1.76	1.30	1.42	2.00	1.33	1.59	1.29	1.54
Callus fresh weight (g)	0.62	0.82	0.26	0.85	0.29	0.84	0.71	1.11	0.81	0.98	0.82	0.82	0.94
Explants forming callus %	06.0	0.92	0.76	0.95	0.77	0.83	0.87	0.99	0.89	1.03	0.92	0.93	0.99
Explants forming roots%	0.46	0.45	0.51	0.71	0.52	0.47	0.70	0.96	0.92	0.96	0.83	0.75	0.86
STI under high stress + salicylic acic	σ												
Roots fresh weight (g)	1.31	0.49	0.142	1.28	0.13	0.96	1.25	1.57	1.63	1.56	1.42	1.12	1.70

Traits	S. tubero.	sum (tbr)						S. chaco	ense (chc)				
	Agata	Atlantic	Anya	Diamond	Gala	Kennebec	Russet Burbank	A-6	B-7	6-8 C-8	C-9	D-2	D-3
Surface area of root (cm <sup>2</sup> )	0.87	0.91	0.65	0.89	0.45	0.93	0.85	0.84	0.88	1.10	0.87	0.92	0.79
Total root length (mm)	0.95	0.84	0.64	0.96	0.49	0.91	0.90	0.96	0.98	0.96	0.96	1.01	1.02
No. of roots/plantlet	0.99	1.01	0.86	1.00	0.71	0.96	0.98	1.00	0.97	0.98	0.92	0.98	0.93
Plantlet water content%	0.93	0.82	0.77	0.95	0.73	0.86	0.82	0.95	0.86	0.96	0.87	0.92	0.89
Chloride (mmol kg <sup>-1</sup> FW)	2.82	2.75	3.13	2.32	3.03	3.17	1.55	2.03	2.14	1.98	1.78	2.22	2.24
Sodium (mmol kg <sup>-1</sup> FW)	3.48	3.67	3.80	3.76	3.81	5.47	2.53	2.32	1.77	2.37	1.16	1.29	1.74
Potassium (mmol kg <sup>-1</sup> FW)	0.74	0.72	0.56	0.83	0.56	0.48	0.86	0.91	0.81	0.87	0.81	0.76	0.79
Chlorophyll (SPAD)	0.80	0.84	0.75	0.92	0.76	0.77	0.87	0.96	0.83	0.98	0.86	0.92	06.0
Catalase (U min <sup>_1</sup> g <sup>_1</sup> FW)	1.59	1.08	1.57	1.05	1.58	1.63	1.18	1.77	2.00	1.46	2.18	1.47	2.06
Peroxidase (U min <sup>-1</sup> g <sup>-1</sup> FW)	1.32	1.44	2.34	1.56	2.39	1.44	1.60	1.34	1.68	1.26	1.59	1.21	1.46
Callus fresh weight (g)	0.76	0.94	0.41	0.93	0.50	0.96	0.88	1.14	1.01	1.08	0.99	0.86	1.03
Explants forming callus %	0.94	0.94	0.86	0.99	0.89	0.92	0.93	1.01	0.97	1.03	0.95	0.97	1.02
Explants forming roots%	0.54	0.56	0.62	0.80	0.67	0.59	0.73	0.98	0.96	0.97	0.92	0.83	06.0
STI under high stress + glycine bet.	aine												
Roots fresh weight (g)	1.43	1.02	0.36	1.54	0.50	1.02	1.55	1.93	2.01	2.02	1.95	1.52	2.07
Surface area of root (cm $^2$ )	0.96	0.96	0.84	0.99	0.55	0.96	0.91	0.97	0.94	1.17	0.91	0.96	06.0
Total root length (mm)	0.95	0.84	0.64	0.96	0.49	0.91	0.90	1.05	0.99	1.02	0.98	1.02	1.05
No. of roots/plantlet	1.03	1.06	0.93	1.02	0.77	1.01	1.01	1.11	1.01	1.00	0.94	1.16	0.95
Plantlet water content%	0.97	0.83	0.81	0.97	0.75	0.90	0.84	1.00	0.95	1.00	0.96	0.98	0.99
Chloride (mmol kg <sup>-1</sup> FW)	1.43	1.40	2.43	1.04	2.21	1.75	1.02	1.28	1.32	1.26	1.13	0.96	1.30
Sodium (mmol kg <sup>-1</sup> FW)	1.16	1.59	2.24	1.14	2.22	1.67	0.97	1.38	1.24	1.21	1.06	1.25	1.23
Potassium (mmol kg <sup>-1</sup> FW)	0.92	0.88	0.81	0.97	0.79	0.71	1.03	0.91	0.96	0.96	0.88	0.79	0.83
Chlorophyll (SPAD)	0.98	0.94	0.86	0.99	0.91	0.91	0.98	0.99	0.93	0.99	0.97	0.99	0.97
Catalase (U min <sup>-1</sup> g <sup>-1</sup> FW)	1.59	1.37	1.57	0.83	1.58	1.70	1.25	0.92	1.93	1.18	2.04	1.26	1.91
Peroxidase (U min <sup>-1</sup> g <sup>-1</sup> FW)	1.24	1.29	2.02	1.04	2.11	1.52	1.45	1.27	1.60	1.26	1.43	1.14	1.38
Callus fresh weight (g)	0.93	0.99	0.66	0.98	0.70	0.99	0.97	1.21	1.10	1.23	1.26	1.04	1.08
Explants forming callus %	0.98	0.99	0.93	1.01	0.95	0.97	0.96	1.03	1.01	1.04	1.01	1.01	1.03
Explants forming roots%	0.71	0.80	0.78	0.86	0.81	0.81	0.81	1.01	0.99	1.01	0.95	0.88	0.94

Table 5 (continued)

**Table 6** Principal component analysis (PCA) of all studied traits of 13 genotypes of potato divided into 7 tetraploid cultivars of *Solanum tuberosum (tbr)*, and 6 diploid clones of *S. chacoense (chc)* grown in vitro under conditions of high salt stress level (300 mmol  $L^{-1}$  NaCI) and glycine betaine, proline or salicylic acid treatments

Components	Variance	Proportion	Cumulative proportion
Roots fresh weight (g)	1.255	0.082	0.871
Surface area of root (cm <sup>2</sup> )	0.927	0.062	0.933
Total root length (mm)	0.847	0.060	0.941
Number of roots/plantlet	0.956	0.077	0.864
Plantlet water content (%)	0.441	0.029	0.962
Chloride (mmol kg <sup>-1</sup> FW)	0.208	0.014	0.976
Sodium (mmol kg <sup>-1</sup> FW)	0.148	0.010	0.986
Potassium (mmol kg <sup>-1</sup> FW)	0.811	0.051	0.992
Chlorophyll (SPAD)	0.043	0.003	0.994
Catalase (U min <sup>-1</sup> g <sup>-1</sup> FW)	0.037	0.002	0.997
Peroxidase (U min <sup><math>-1</math></sup> g <sup><math>-1</math></sup> FW)	0.019	0.001	0.998
Callus fresh weight (g)	0.031	0.001	0.874
Explants forming callus (%)	0.214	0.002	0.891
Explants forming roots (%)	0.921	0.063	0.911

0.927, and 0.847, respectively. From callus growth traits, % of explants forming roots had also high variance 0.921 followed by potassium content and PWC % with 0.811 and 0.441 variance. On the other hand, peroxidase activity was the least trait affected by the treatment with 0.019 variance.

# Discussion

Global potato productivity is rising, and more potatoes are being produced on saline soils or irrigated with saline water. Cultivated potato is frequently thought to be sensitive to salt however, a degree of tolerance has been found in related wild species and primitive cultivars of potato [12, 31]. At the same time, genetic diversity of wild species has previously been employed to enhance salinity tolerance in certain contemporary crop cultivars [38]. In this study, researchers attempted to test the tolerance of cultivated tetraploid potato and diploid wild species of Solanum to three distinct degrees of salinity stress (100, 200 or 300 mmol  $L^{-1}$  NaCl) in vitro. The diploid wild species (S. chacoense Bitt.) 'chc' was chosen for its predicted high salt tolerance at the total plant level, however tolerance varies among and/or within accessions and clones. In a previous research done by the corresponding author, the extent of genetic variation of *chc* was investigated using microsatellites and high level of heterozygosity for 15 SSR loci among and within ten accessions was found [40]. The tetraploid potato cultivars (S. tuberosum L.) 'tbr', on the other hand, were selected for their diversity in drought tolerance, making these cultivars suitable for salinity screening [30]. Plants under salt stress develop a range of metabolites known as compatible solutes because they do not interfere with plant metabolism, according to biochemical research [41]. Under the influence of salinity stress, plants engage in a variety of mechanisms that provide them with some levels of tolerance to such stress, such as boosting the osmotic pressure of their cells by increasing the concentration of some osmolytes such as glycine betaine and proline, while also increasing the activity of some antioxidants activities which have a pivotal role in scavenging reactive oxygen species, one of the most important manifestations of oxidative stress caused by saline stress [42-44]. Furthermore, the tetraploid and diploid genotypes were subjected to high salt level (300 mmol  $L^{-1}$  NaCl) with and without osmoregulators and antioxidants, i.e., glycine betaine (GB), proline (P) and salicylic acid (SA) to investigate the effect of exogenous application on salinity tolerance and stress alleviation.

In this study, stem nodes and calli were treated in vitro to various degrees of salt stress ranging from 100 to 300 mmol L<sup>-1</sup> NaCl, and growth was compared to normal conditions (no stress). Different researchers have employed NaCl for in vitro salinity screening in other plant species [19, 20, 45], however the amounts utilized were not as high as those used in this work. As the salt concentration rises, root growth suffers more than shoot growth [46, 47]. The first morphological features to be considerably altered by stress were root traits. Such findings contradict earlier research that showed that the initial effect of stress is a decrease in leaf number [48, 49]. The results, on the other hand, are consistent with earlier research on salt tolerance in potato and tomato plants [31, 38]. The salt effects on root development were more evident in *tbr* cultivars than in *chc* clones. Under salt stress, 'Diamond' cv. followed by 'Russet Burbank' cv. acquired better roots fresh weight and surface area of root when 'Agata' cv. had longer roots and 'Kennebec' cv. had more roots/plantlet however, still lower than control (no stress) treatment. On the other hand, roots fresh weight rose with increasing salt concentrations in chc clones. Clones 'C-8' and 'A-6' had substantially higher roots fresh weight and surface area of root when clones 'D-2' and 'D-3' had more and longer roots than tbr cultivars and all other chc clones. All of these findings indicate that there is significant diversity in salt stress response across and within species. These results are supported with previous findings [16, 17] that found that enhanced root growth is a developmental plant response to salinity stress.

In addition, salinity stress disrupted cell osmotic pressure, resulting in a significant reduction in plant water content [30]. In this investigation, the same results were found, with substantial differences between the tbr and chc genotypes. Plant water content % (PWC) was dramatically reduced during salt stress in tbr cultivars and chc clones, with chc significantly less affected than tbr. Under stress, PWC % ranged from 58 to 85% in tbr and 72 to 89% in chc. The greatest PWC percentages were obtained from chc clones 'A-6', 'C-8', and 'D-2', while the lowest percentages were obtained from tbr cultivars 'Gala' cv. and 'Anya' cv. At the same time, increasing salinity levels resulted to a significant drop in the amount of K<sup>+</sup>, with the reduction being considerably bigger in *tbr* than in *chc*. According to [50], the capacity to maintain potassium uptake in the context of large amounts of exogenous salt may be a desirable feature.

Furthermore, the *tbr* cultivars had greater Na<sup>+</sup> and Cl<sup>-</sup> accumulations than the *chc* accessions. The deposition of these ions increased considerably with increasing salinity stress in both species. The lowest content was observed on chc clone 'A-6' for Cl<sup>-</sup> ions and 'C-8' for Na<sup>+</sup> ions. This implies that ion buildup is the primary salt tolerance mechanism. According to the interpretation of this data, accessions of diploid wild species of chc were more salttolerant, not because they are better at restricting Cl<sup>-</sup> and Na<sup>+</sup> uptake at high NaCl levels than cultivars, but because they have a superior ability to tolerate high levels of Cl<sup>-</sup> and Na<sup>+</sup> in their tissues, as reported by [51]. It's also possible that excessive ion buildup is a necessary byproduct of survival. Other scientists have disclosed Cl- and Na+ accumulations in wild species [52-54], implying that Na<sup>+</sup> leaf concentrations could be used as a key attribute in evaluating germplasm for salt-tolerance breeding programs of cultivated tomato [55]. Salt stress could have an impact on ion transport in a cell-specific manner. Under salinity, a rise in cytoplasmic Na<sup>+</sup> and a decrease in K<sup>+</sup> trigger disruptions in membrane potential and osmotic pressure [56]. Under salt treatment, halophyte roots had a lower  $Na^+/K^+$  ratio than glycophyte roots [57]. Under salt stress, chc accessions (clones 'C-8', 'D-2', and 'A-6') had the lowest  $Na^+/K^+$  ratio, indicating that these were the genotypes with the highest tolerance to salt as determined by other criteria. Meanwhile, there was an overall correlation between growth in saline conditions and Na<sup>+</sup>/  $K^+$ , indicating that these genotypes might be beneficial to incorporate into stress tolerance breeding programs.

Previous research found that shoot fresh and dry weights dropped linearly with increasing salt level [12, 30], which might be due to a decrease in chlorophyll content in the leaves, as shown in the current study. Salinity stress levels dramatically decreased chlorophyll content in all genotypes, and the loss was considerably higher in tbr cultivars than chc clones. When chc clones 'C-8', 'A-6', and 'D-2' showed considerably greater chlorophyll content under salinity conditions than the other accessions, 'Diamond' cv. persisted to demonstrate significant chlorophyll content in tbr. Salt stress enhanced the activity of antioxidant enzymes including CAT and POD, which play a vital role in scavenging reactive oxygen species [42-44]. This is consistent with the findings of this study, which found a marked increase in the concentrations of CAT and POD enzymes with each increase in salinity in potato genotypes, as shown by [16, 43]. Yet, the increment in tbr cultivars was significantly greater than in chc clones. Diploid wild potato accessions chc have been shown to be more salt tolerant than cultivars of the tbr in callus culture. The callus fresh weight, % of explants producing callus, and % of explants developing roots in cultures after four weeks of stress treatment clearly demonstrated *chc's* better salt tolerance than that of *tbr*. The trend for diploid wild species of *Solanum spp.* to be more salt tolerant than tetraploid is comparable to that previously observed by [14, 15] however, our study employed genotypes from different species. All of the aforementioned data indicate that there is significant diversity in

response to salt stress among species and within species.

In this study, seven tbr varieties, namely 'Agata', 'Atlantic', 'Anya', 'Diamond', 'Gala', 'Kennebec', and 'Russet Burbank' were examined for salt stress (100, 200, and 300 mmol L<sup>-1</sup> of NaC1), thus 'Diamond' cv. and 'Russet Burbank' cv. were more tolerant and generated the highest salinity tolerance index. In another study undertaken by the authors, 21 tbr varieties were assessed, and clustering for drought response resulted in three separate groups based on variances in growth, physiology, and yield attributes: (i) tolerant group consisting of cultivars 'Russet Burbank', 'Diamond', 'Agata', 'Kennebec', 'Atlantic'; (ii) a moderately tolerant group consisting of cultivars 'Maritiema', 'Lady Balfour', 'Mizen', 'Marfona', 'Lady Rosetta', 'Marble', 'Desiree', 'Champion', 'Cara', 'Burren', and 'Almond', and (iii) a sensitive group consisting of cultivars 'Anya', 'Gala', 'Spunta', 'King Edward', and 'Gazella' [30]. In a previous study, a large population of cultivated potato varieties including some of the varieties evaluated in this study, were rated for salt tolerance using a multivariate analysis of the relative averages of six growth indicators tested at various salinity levels. Based on the sum of the relative ranking at 40, 80, and 120 mmol  $L^{-1}$  NaC1, the varieties were split into 8 groups. At all NaCl levels, the cvs. Amisk, BelRus, Bintje, Onaway, Sierra, and Tobique were in the top cluster groups and constituted the highest-rank [12]. In another study, six cvs. 'Diamant', 'Draga', 'Erntestolz', 'Kennebec', 'Marfona', and 'Spunta' were also evaluated for the same level of salt tolerance and ranked based on shoot length and fresh weight [58]; however, no differences were

found between the top-ranked 'Erntestolz' and the lowerranked 'Draga', 'Marfona', and 'Spunta' [12]. Likewise, [59] evaluated 6 varieties, i.e., 'Kennebec', 'Norchip', 'Red Pontiac', 'Russet Burbank', 'Russet Norkotah', and Superior, but another research found that the higher-ranked 'Russet Norkotah', 'Norchip', and 'Red Pontiac' were not as salt tolerant as the six top-ranked cvs. 'Amisk', 'BelRus', 'Bintje', 'Onaway', 'Sierra', and 'Tobique' [12]. The relative discrepancy in tolerance rankings among studies is most likely owing to the comparatively different degree of stress applied and the growth metrics examined.

Exogenous treatments of GB, P, and SA under stress conditions had a favorable and substantial influence on the various root development, PWC %, ions content, chlorophyll content, enzyme activity, and callus parameters of the thirteen potato genotypes assessed in this investigation. The implementation of the agronomic approach is linked to the perception of increasing the productivity of vegetable crops, prolonging production seasons and improving efficiency in a sustainable and clean direction and rationalizing the usage of natural resources [25, 26, 60]. This can also give an agronomic answer for stress reduction which may be exploited during search of genetic and physiologic remedies to this problem by crop breeders and biotechnologists. Exogenous treatments with osmoregulators and antioxidants improved the examined morphological, physiological and callus traits in tbr and chc. In general, GB treatment outperformed SA and P treatments when compared to controls (no stress) and high stress levels without osmoregulators or antioxidants. According to [61], GB optimized the ultrastructure of salt-stressed Oryza sativa seedlings. Under stressed conditions, the seedling's ultrastructure showed swelling of thylakoids, disintegration of grana, and disruption of mitochondria. However, when seedlings were treated with GB, these damages were significantly reduced. When GB was applied as a foliar spray to a stressed plant, it caused pigment stabilization as well as an increase in photosynthetic rate and growth [62]. GB is a nontoxic cellular osmolyte that intensifies the osmolarity of the cell throughout stress, and thus plays a significant role in relieving stress. GB also acts as a protective barrier through osmotic adjustment [63], protein stabilization [64], and protection of the photosynthetic apparatus from stress damage [62, 65]. GB accumulation is involved in a wide variety of plants from various taxonomic backgrounds.

On the other hand, SA and P treatments had a significant influence on the plantlets of all tested cultivars of *tbr* and accessions of *chc* under examined high stress level. All of the aforementioned is consistent with numerous studies that have been conducted in this regard [66, 67]. SA performs a variety of important physiological mechanisms in plants, including increasing nutrient uptake and the level of chlorophyll and carotenoid pigments, modifying the activities of some enzymes, and preserving the cell membrane's integrity [27, 28]. Such beneficial effects on plant growth and yield could be attributed to the role of SA in influencing plant hormone balances such as auxin, cytokinin, and abscisic acid under any conditions [68]. Plants frequently emit a stress response in an attempt to counteract the effects of the stressor, and levels of cellular compatible solutes such as P and sugars rise, conferring desiccation tolerance [69]. P buildup is one of the most frequently reported changes in plants caused by water deficit and salt stress, and it takes part in stress resistance mechanisms [70, 71]. P has been interconnected to the remedy of cellular osmotic stress, ammonia detoxification, protein and/or membrane stabilization, and the improvement of the stability of some cytoplasmic and mitochondrial enzymatic reactions [72]. However, in this experiment, P significantly increased the stress tolerance index of the tested genotypes while still trailing GB and SA. Because these compounds have different cellular modes of action, more research is needed to determine whether they can be combined to work synergistically. The findings suggest that there are exciting opportunities for reducing moderate to severe salt stress in the field.

# Conclusion

The findings of this study underline the adverse impacts of increased salt on the various performances of potato genotypes. The results suggest that there is a significant genetic variability for salt tolerance with and within Solanum tuberosum (tbr) cultivars, a tetraploid species, and S. chacoense (chc), a diploid species in which salt tolerance was observed in some of the genotypes studied. S. chacoense clones 'A-6', 'C-8' and 'D-2' were the clones showing the best salt tolerance overall. 'Diamond' cv. and 'Russet Burbank' cv. were the most tolerant cultivars among the tested S. tuberosum cultivars. These genotypes are good candidates for adoption in salt tolerance breeding schemes. Furthermore, the research results revealed that the detrimental consequences of salinity may be partially or completely negated by the exogenous application of GB, SA, and P. It was observed that GB may be a beneficial treatment for improving root development, PWC %, ions content, callus induction, chlorophyll content, and the measured enzyme activities of potato plants in salinestressed circumstances. Importantly, and in accordance with the STI and PCA values, roots fresh weight, number of roots, surface area of root, % of explants forming roots followed by total root length and potassium might be unambiguous indications of a potato plant's reaction to salt stress. Furthermore, because the treatments with GB, P and SA have diverse cellular mechanisms of action, additional research is needed to determine if they may be combined to operate synergistically. The analysis shows

that there is an intriguing possibility for lowering moderate to severe salt stress in the field; nevertheless, experimental studies are still required.

#### Abbreviations

*tbr. Solanum tuberosum; chc: Solanum chacoense;* GB: Glycine betaine; P: Proline; SA: Salicylic acid; STI: Salinity tolerance index; PWC: Plantlets water content; CAT: Catalase; POD: Peroxidase; PCA: Principal component analysis.

# **Supplementary Information**

The online version contains supplementary material available at https://doi. org/10.1186/s40538-022-00286-3.

Additional file 1: Data S1. Plant morphological and physiological characteristics, enzymes activity and callus induction of 13 genotypes of potato divided into 7 tetraploid cultivars of *Solanum tuberosum (tbr)*, and 6 diploid clones of *S. chacoense (chc)* grown in vitro under stress conditions. Explants were grown on media supplemented with four different concentrations of NaCl (0.0, 100, 200 or 300 mmol L<sup>-1</sup>).

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#### Authors' contributions

HEMZ: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, supervision, validation, visualization, writing—original draft, and writing—review and editing. KSAR: Data curation, formal analysis, investigation, methodology, visualization, writing—original draft, and writing—review and editing. Both authors read and approved the final manuscript.

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# Availability of data and materials

Not applicable.

#### Declarations

#### Ethics approval and consent to participate

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The authors agreed to the publication of the manuscript in CBTA journal.

#### **Competing interests**

The authors declare that they have no conflict of interest.

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