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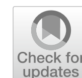

Salinity-induced changes in seed germination and the expression profile of antioxidant enzymes in peanut as early and late responses in emerging radicles

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Abstract

Reactive oxygen species (ROS) play a critical role in developmental and signal transduction processes during seed germination and early seedling establishment stages. Higher concentrations of ROS are known to have detrimental effects when the plant is under salt stress. In the present study, we aimed to test the early (1 h) and late (48 h) response of enzyme-driven ROS detoxification system in six peanut genotypes under salt stress at early seedling stage. Salt stress was imposed with three treatment concentrations of NaCl (50, 100 and 200 mM NaCl), all of which showed a reduction in seed germination and seedling vigour index. The 200 mM NaCl stress showed severe reduction of growth, while 100 mM NaCl stress resulted in rapid increase in $O_2^{\cdot-}$ and H_2O_2 contents. The $O_2^{\cdot-}$ content increased twofold in sensitive genotypes after 1 h of stress, whereas the tolerant genotypes showed ~ 60% rise. A prompt rise (> 50-fold) in SOD transcript was occurred within 1 h of salt stress in the tolerant genotypes (early response). But induction in SOD activity was observed only after 48 h of salt stress (late response). After 48 h of salt stress, the tolerant genotypes showed greater induction of POD activity, whereas in the sensitive genotypes CAT activity was more pronounced. We found POD and CAT played a greater role in H_2O_2 detoxification in tolerant and sensitive genotypes, respectively, during longer duration of the stress. This study summarizes the selective induction of different components of antioxidant enzyme system and their role in cellular fine tuning of ROS level in peanut under salt stress during seedling establishment stage.

Keywords Germination · Groundnut · Oxidative stress · Reactive oxygen species · Seedling emergence · Salt stress

Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
G_p	Germination percentage
G_r	Germination rate
GR	Glutathione reductase
MGT	Mean germination time
SVI	Seedling vigour index
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances

Introduction

Soil salinity limits plant growth and productivity in 7% of the total territorial area and in 20% of the irrigated arable land worldwide (Parihar et al. 2015). Peanut (*Arachis hypogaea* L.), a unique legume, is considered salt sensitive (Chakraborty et al. 2016a) and shows limitations in plant growth and biomass production beyond the critical level of soil salinity (Singh et al. 2008). The ability of the plants to absorb water decreases due to the osmotic effect of salt stress, causing poor seed germination and hindering initial growth and establishment (Singh et al. 2008). Plant responses vary not only with the intensity of the stress but

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also with the time and duration of exposure (Negrão et al. 2017). High substrate salinity and its detrimental effects vary with the developmental stage of the plant (Lafitte et al. 2004). Seed germination, seedling emergence, and early survival are most sensitive to soil salinity. Plant growth and survival after seedling establishment are less affected by low and moderate levels of salinity stress (Cuartero et al. 2006). Therefore, we need cultivars good enough to meet the challenges of saline environments during germination and seedling establishment to extend crop cultivation to coastal and saline areas.

The root systems of plants provide anchorage to ensure water and nutrient uptake. The roots also act as a sensory system by integrating changes in nutrient availability and water content. Under salt stress, plants tend to alter their root morphology and architecture to exploit the available resources most efficiently (Galvan-Ampudia et al. 2013; Gruber et al. 2013). To date, most of the studies on salt tolerance have focused on the aboveground parts (particularly leaves/mesophyll), which play critical roles in plant survival and productivity (Julkowska et al. 2014). However, under salinity stress, roots are the first contact point and must endure the detrimental effects of a high-external Na^+ environment (Negrão et al. 2017). A high level of salinity affects seed germination and plant growth via water deficiency (osmotic stress), ion toxicity and ionic imbalance or a combination of these factors (Läuchli and Grattan 2007). Thus, understanding the underlying mechanism associated with root growth is most critical for achieving salt tolerance. The short-term responses to salinity may be particularly relevant for an improved understanding of the biological significance of oxidative stress status in plants. Bailly et al. (2008) reported that transient production of reactive oxygen species (ROS) can exert a signalling and/or protective role, apart from causing oxidative damage if present in high concentrations.

Under high salinity damage is caused due to (1) ion toxicity, (2) decreased rhizospheric water potential and (3) increased formation of ROS (Levinsh 2006; Bose et al. 2014). The most prevalent ROS in plants include hydrogen peroxide, superoxide anions, hydroxyl radicals and singlet oxygen. These ROS interfere with normal cellular metabolism by oxidizing proteins, lipids, DNA and other cellular macromolecules under salt stress (Ahmad et al. 2016). Overaccumulation of ROS disturbs redox homeostasis by enhancing membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damage (Miller et al. 2010). However, the simultaneous production and scavenging of ROS (as a metabolic need or for signal transduction) occur in plants to maintain ROS within a favourable range (Miller et al. 2010). Hence, it is believed that a higher constitutive or salinity-induced antioxidant defence capacity would be better for salt tolerance (Abogadallah 2010; Chakraborty et al. 2016b). Plants differ in executing antioxidant defence strategies under different abiotic stresses. In fact, within the same species, genotypes may differ in their adaptive strategy for the selective induction of antioxidant enzyme networks. For instance, in peanut, one or few specific components of the whole defence system play a crucial role in salt tolerance (Chakraborty et al. 2016b) and may vary from one crop to another (Mittova 2004; Zhang et al. 2014).

Previous studies suggested that reactive oxygen species play a critical role in seed physiology and seedling establishment (Leymarie et al. 2011; Bailly and Kranner 2011). Bailly et al. (2008) reported that ROS play a critical role in plant developmental processes, including signal transduction during the seed germination process. In stressful environments, the production of these ROS is usually higher than that in non-stressful environments. In such conditions, plants face a challenge between production and detoxification of ROS for balancing the cellular ROS load. Under stress, ROS-induced damage should be minimized, while there should be a sufficient level of ROS necessary for normal seedling development (Müller et al. 2009). Such an intricate balancing mechanism has been studied relatively little in crop species such as peanut. Comprehensive information on the differential responses of an emerging radicle to salt stress in relation to the detoxification of ROS is particularly lacking in peanut. Thus, it is very important to study the early and late responses to salinity stress in relation to the detoxification of ROS and their subsequent effects on salt tolerance in peanut. The aims of the study were to understand (1) how early and late responses to salt stress differ in peanut roots and (2) how the responses, such as the activation of key components of enzyme-driven antioxidant defence, vary in tolerant and sensitive genotypes.

Materials and methods

Experimental conditions and plant materials

Two different sets of experiment were conducted in this study. In the first set, six peanut genotypes representing both bunch (Spanish type) and semi-spreading/spreading (Virginia type) habit groups and having differential salt sensitivity (see Supplementary Table S1 for genotype details and their salt stress response) were taken to determine critical level of salinity stress by assessing changes in various seed physiological traits. Fresh seeds of the six genotypes (Somnath, TPG 41, Girnar 1, CS 240, TMV 2 and NRCG 357) were surface sterilized with 0.1% $HgCl_2$ for 10 min, then thoroughly rinsed with distilled water and finally placed in the germination paper in petri dishes of 10 cm diameter. One petri dish consisting of 10 seeds was considered as single replication and each of the treatment × genotype combination was replicated 20 times

in this study. For control (0 mM NaCl) treatment, the seeds were grown in non-buffered basic salt media (BSM) solution (0.5 mM KCl + 0.2 mM NaCl + 0.1 mM $CaCl_2$, pH 5.7) (25 ± 2 °C), whereas for the salinity treatments (50, 100 and 200 mM NaCl) the appropriate amount of NaCl was mixed in the BSM solution from the beginning of the experiment. For germination and assessment of other seed physiological parameters, the seeds were grown in the dark for 10 days at room temperature (25 ± 2 °C).

In the second set of the experiment, the same experimental condition was laid down separately, except for the time and dose of salt stress imposition to determine the salinity induced early and late responses in oxidative stress build-up and selective induction of antioxidant enzyme activities. Here, the seeds were kept for germination in normal BSM solution for the first 4 days and then the emerging radicles were subjected to 100 mM of NaCl treatment (in BSM background solution). A separate set without NaCl treatment was kept as control. To study the salt induced early and late responses in the growing radicle, the samples were collected at 1 and 48 h after imposition of salt stress, respectively. The samples were immediately frozen in liquid nitrogen and subsequently stored at − 80 °C for oxidative stress-related studies and total RNA extraction. Here, we have chosen these two time points for early and late response to salt stress, because our earlier study (Chakraborty et al. 2016c) with 5-day old seedling showed rapid influx of Na^+ (within 20 min after exposure) inside the mature as well as meristematic root zone due to direct exposure to salt stress via immersion of roots in the saline solution. The salt-tolerant genotype started to efflux some amount of Na^+ back to the solution by active transport and the process got settled in about 48 h time.

Assessment of seed physiological parameters and seedling vigour

The number of seeds germinated each day was recorded until the 10th day, when the process of germination was stabilized. The seeds were scored as germinated if the tip of the radicle (~ 2 mm) was visibly rupturing the seed coat. At the end of 10th day, the length of radicle and plumule were measured and fresh biomass of both root and shoot were recorded. Then the samples were oven-dried at 60 °C for 72 h and the dry biomass was recorded. From the daily germination count, germination percentage (G_p), germination rate (G_r), and mean germination time (MGT) were calculated as per the following formulae (ISTA 2003):

$$\text{Germination percentage } (G_p) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100,$$

$$\text{Germination rate (GrR)} = \sum_{i=1}^{8} \frac{G_i}{D_i},$$

where 'G_i' is the total number seeds germinated on a particular day and 'D_i' is the corresponding day of 'G_i' (Bajji et al. 2002).

$$\text{Mean germination time (MGT)} = \frac{S_1D_1 + S_2D_2 + \cdots\cdots\cdots + S_nD_n}{S_1 + S_2 + \cdots\cdots\cdots + S_n},$$

where 'S' is the number of seeds germinated per day; 'D' is the day corresponding to 'S' and 'n' is the day of final count (Cantliffe 1991).

Seedling vigour index (SVI) was calculated as follows:

$$\text{SVI} = (\text{RL} + \text{SL}) \times \text{GP},$$

where RL = root length at 10th day; SL = shoot length at 10th day; GP = germination percentage (ISTA 2003).

Determination of level of oxidative stress

The build-up of oxidative stress in response to salinity treatment was determined by measuring the levels of various reactive oxygen species (ROS) in the growing root tissue. The superoxide radical content ($O_2^{\bullet-}$) was determined by measuring the reduction of nitroblue tetrazolium chloride (NBT) followed by absorption of the end-product at 540 nm as per the method described by Chaitanya and Naithani (1994) with minor modifications. A specified amount (~ 1 g) of root tissue was homogenized in pre-cooled phosphate buffer (0.2 M, pH 7.2) containing 1 mM diethyl dithiocarbamate, 3 mM KCN and 5 mM of H_2O_2 to inhibit Mn-SOD, Cu–Zn SOD and Fe-SOD for quantifying the maximum amount of $O_2^{\bullet-}$ produced (Sandalio et al. 1987). Hydrogen peroxide content from the root tissue was estimated by forming titanium–hydroperoxide complex (Rao et al. 1997). One gram of root tissue was ground to a fine powder with liquid nitrogen and mixed with 10 mL of chilled acetone in cold room. Subsequently, 4 mL of titanium reagent and 5 mL of ammonium solution was added to the filtered mixture to precipitate titanium–hydroperoxide complex. The blank was prepared by following the same procedure without using the root tissue. The precipitate was dissolved in 10 mL of 2 M H_2SO_4 and the absorbance was recorded at 415 nm against blank.

The extent of lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content produced in response to salt treatment (Heath and Packer 1968). The root sample (0.5 g) was homogenized in 10 mL 0.1% trichloro-acetic acid (TCA) and centrifuged at 15,000×*g* for 15 min. One millilitre of supernatant was mixed with 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA and heated at 95 °C for 30 min followed by cooling in an ice bath. After centrifugation at 10,000×*g* for 10 min, the absorbance of the supernatant was recorded at 532 nm and the interference of non-specific absorbance (measured at 600 nm) was subtracted from the final calculation.

Assay of antioxidant enzyme and total antioxidant activities

The extract for antioxidant enzymes (SOD, APX, GR, POD and CAT) was prepared by freezing 1 g of root tissue in liquid nitrogen followed by grinding in 10 mL of extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA in case of SOD, GR, POD, CAT and 1 mM ascorbic acid in case of APX) and filtered through 4 layers of cheese cloth (Chakraborty et al. 2015). The filtrate was then centrifuged for 20 min at 15,000×*g* and supernatant was used for enzyme assays.

The total SOD (EC 1.15.1.1) activity was estimated by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) by the enzyme (Dhindsa et al. 1981). A reaction mixture was prepared containing 13.33 mM methionine, 75 µM NBT, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium carbonate and 0.1 mL of enzyme extract and the final volume was made up to 2.9 mL by adding distilled water. The reaction was started by adding 0.1 mL of 2 mM riboflavin to the above reaction mixture and immediately keeping the tubes under two 15 W fluorescent lamps for 15 min. The absorbance was recorded at 560 nm, where a non-irradiated complete reaction mixture was used as a blank. A separate control (lacking the enzyme extract) was also prepared following the same procedure. One unit of enzyme activity was the amount of enzyme, which reduced the absorbance to 50% in comparison with the control tubes lacking enzyme. Catalase (EC 1.11.1.6) activity was assayed by measuring the loss of H_2O_2 (Aebi 1984) in a 3 mL reaction mixture consisting of 0.5 mL of 75 mM H_2O_2 and 1.5 mL of 0.1 M phosphate buffer (pH 7) and 50 µL of diluted enzyme extract and remaining amount of distilled water. The decrease in absorbance at 240 nm was observed for 1 min and enzyme activity was calculated in terms of the amount of H_2O_2 decomposed. Peroxidase (EC 1.11.1.7) activity was measured spectrophotometrically by recording the increase in absorbance at 470 nm, due to formation of tetra-guaiacol in a reaction mixture containing 50 mM phosphate buffer (pH 6.1), 16 mM guaiacol, 2 mM H_2O_2 and 0.1 mL of enzyme extract diluted to a final volume of 3 mL. The enzyme activity expressed as µmol tetra-guaiacol formed per min per mg protein was calculated using the extinction coefficient of its oxidation product, tetra-guaiacol $\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$ (Castillo et al. 1984).

Ascorbate peroxidase (EC 1.11.1.11) activity was assayed by recording the reduction in optical density of ascorbic acid at 290 nm (Nakano and Asada 1980). The 3 mL of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA and 0.1 mL of enzyme extract, where 0.1 mL of H_2O_2 was added to initiate the reaction. The APX activity was expressed as the decrease of ascorbic acid content (µmol) per min and per mg of protein. Glutathione reductase (EC 1.8.1.7) activity was assayed by measuring the reduction of GSSG per mg protein per min spectrophotometrically at 412 nm (Smith et al. 1988). The reaction mixture (3 mL) was prepared containing 66.67 mM potassium phosphate buffer (pH 7.5) and 0.33 mM EDTA (i.e., 1 mL of 0.2 mM buffer containing 1 mM EDTA), 0.5 mM DTNB (0.5 mL of 3 mM DTNB) in 0.01 M potassium phosphate buffer (pH 7.5), 66.67 µM NADPH (0.1 mL of 2 mM NADPH), and 0.1 mL enzyme extract was added with 0.1 mL of 20 mM GSSG to start the reaction before the time scan was initiated for 1 min.

The total antioxidant activity in terms of $ABTS^{\cdot+}$ radical-scavenging activity in peanut root tissue was estimated following the method described by Arnao et al. (2001). Freshly harvested root tissues (0.5 g) were homogenized in 25 mL of absolute methanol, kept at 4 °C for overnight and then centrifuged at 15,000 rpm for 20 min. The supernatants were transferred to a 100-mL volumetric flask. The pellets were re-dissolved in 25 mL of absolute methanol, centrifuged and pooled with the first extract. The process was repeated another time and total volume of extracts were made up to 100 mL with absolute methanol and used for analysis. The total antioxidant activity is expressed in µM Trolox equivalents (TE) g^{-1} of dry root tissue.

Gene expression studies

Total RNA from the root tissue was isolated using RNeasy Kit (Qiagen) following manufacturer's protocol with minor modifications. To eliminate genomic DNA contamination, the total RNA extract was subjected to DNase I treatment (using Qiagen kit as per manufacturer's protocol) before eluting the final product in nuclease free water. Further the integrity of the RNA was confirmed in the gel and absorbance of the isolated RNA was recorded by Nanodrop Spectrophotometer (ND 1000). Approximately 2 µg of total RNA from each biological replicate was used for cDNA synthesis using First-strand cDNA synthesis kit (Thermo SCIENTIFIC) and the synthesized cDNA was confirmed by PCR amplification using each of the cDNA samples (~ 100 ng) and primers of β-actin. To test gene specific primers (see Supplementary Table S2 for sequence details), similar PCR experiments were conducted for each set of primers. All the tested primers showed expected sizes of amplicon after 30 cycle reactions at 60 °C annealing temperature.

Changes in the transcript abundance of the studied genes were determined by Real-Time quantitative PCR using QuantiFast SYBR Green PCR reaction kit (Qiagen, USA). The reaction mixture contained ~ 100 ng of cDNA, 0.16 µM primers and 12.5 µL of QuantiFast SYBR Green PCR mix. The final volume of reaction mixture was maintained to 25 µL by adding sterile nuclease free water. Reactions were run in StepOnePlus™ Real-Time PCR System

(Applied Biosystem) with conditions as: 95 °C-5 min for 1 cycle; 95 °C-10 s and 60 °C-30 s for 40 cycles. A melt curve analysis was carried out at the end of the PCR cycles, to get the specificity of amplification. The fold changes in relative transcript abundance in treated roots were compared to control roots by comparative $2^{-\Delta\Delta Ct}$ method, where the *Ah-actin* gene was used as internal control to normalize the PCR reactions (Schmittgen and Livak 2008).

Statistical analyses

All the data recorded were the mean values ± standard error (mean) of 5–6 independent replications. The experiment was conducted in two-factor completely randomized design and the data were subjected to two-way ANOVA as per the experimental design. The ANOVA found significant differences for treatment × genotype interaction at 5% level of significance. A post hoc analysis for pair-wise comparison of treatment × genotype combinations was performed by Duncan's multiple range test (DMRT) using SPSS (version 16.0) software.

Results

Effect of salinity stress on seed germination

The first experiment was conducted to identify the level of NaCl stress, which can distinguish the tolerant and sensitive genotypes and subsequently could be used as the desired level of salt stress for the second experiment. The germination and early seedling growth under different levels of NaCl stress (0, 50, 100 and 200 mM) indicated that the seed physiological parameters, viz., Gp, Gr and MGT, were significantly affected beyond 100 mM NaCl stress (Table 1). The Gp at 200 mM NaCl stress was reduced to less than 20% in sensitive genotypes (TMV2 and NRCG357), while the tolerant genotypes (Somnath and TPG41) could maintain more than 40% Gp at similar levels of stress.

The germination rate (G_r) showed a significant reduction under different levels of NaCl stress (Table 1). The genotypes CS240 and NRCG357, with an inherently slow germination process, showed a further reduction in G_r in response to salt stress. Again, the tolerant genotypes (Somnath and TPG 41) showed a lesser (~ 50%) reduction in G_r, while both the sensitive genotypes (TMV 2 and NRCG 357) showed an ~ 80% reduction in Gr at 200 mM NaCl stress. The mean germination time (MGT) increased with increasing levels of salt stress (Table 1). The genotype Somnath germinated the quickest at the highest level of salt stress, with an MGT value of 3.29, while sensitive genotypes, viz., TMV2 and NRCG357, needed approximately 40% longer time to germinate at 200 mM salt stress.

Seedling growth and vigour index

The root growth was restricted significantly beyond the 100 mM NaCl treatment, with enough variability observed in root length (RL) in the studied genotypes. In tolerant genotypes such as Somnath and TPG41, the RL was reduced from 7.9 and 7.2 cm to 2.5 and 2.1 cm, respectively, at the highest level of stress (Table 2). However, in the sensitive genotype, viz., NRCG357, the RL was reduced to 0.7 cm only at 200 mM NaCl stress. Similarly, shoot growth was also reduced under salt stress in all the genotypes. A higher seedling vigour index was observed in the tolerant genotypes (> 100 in Somnath and TPG 41), while it was reduced to < 10 in the sensitive genotypes (TMV 2 and NRCG 357) at 200 mM NaCl stress (Table 2).

Initial biomass production

The total biomass after the 10th day of germination showed significant variability among the genotypes. The 200 mM salinity treatment was highly detrimental to peanut, as all

Table 1 Effect of different levels of salt (NaCl) stress (continued up to 8 days) on seed germination percentage, germination rate and mean germination time in peanut genotypes

	Germination (%)				Germination rate (seed day^{-1})				Mean germination time (days)			
	0 mM	50 mM	100 mM	200 mM	0 mM	50 mM	100 mM	200 mM	0 mM	50 mM	100 mM	200 mM
Somnath	96^{a}	92^{a}	88^{ab}	52^{fg}	0.88^{a}	0.82^{ab}	0.79^{b}	0.50^{fg}	3.00^{gh}	3.09^{g}	3.23^{fg}	3.29^{f}
TPG 41	83^{b}	72^{cd}	56^{ef}	44^{g}	0.84^{ab}	0.76^{bc}	0.45^{gh}	0.41^{hi}	2.81^{h}	2.79^{h}	3.64^{de}	3.93^{bc}
Girnar 1	72^{cd}	64^{de}	48^{fg}	35^{h}	0.70^{cd}	0.57^{ef}	0.37^{hi}	0.34^{ij}	3.16^{g}	3.47^{def}	3.75^{cd}	4.43^{a}
CS 240	80^{bc}	50^{fg}	35^{h}	30^{h}	0.63^{de}	0.51^{fg}	0.43^{g}	0.38^{hi}	3.47^{def}	3.40^{ef}	3.79^{cd}	4.09^{b}
TMV 2	88^{ab}	60^{d}	48^{fg}	20^{i}	0.89^{a}	0.59^{e}	0.27^{jk}	0.19^{kl}	2.77^{h}	3.56^{de}	4.45^{a}	4.50^{a}
NRCG 357	80^{bc}	48^{fg}	32^{h}	12^{j}	0.68^{cd}	0.44^{gh}	0.20^{kl}	0.13^{l}	3.26^{fg}	3.71^{cd}	4.00^{bc}	4.67^{a}

Mean values sharing the same letter for each treatment × genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

the genotypes showed more than a 95% reduction in both root and shoot biomass. However, in the 100 mM NaCl treatment, the tolerant and sensitive genotypes were sufficiently distinguishable (Tables 3, 4). On an average, the root FW declined by ~80% under 100 mM NaCl stress, but the reduction was <70% in the tolerant genotypes (Somnath and TPG 41) and >90% in NRCG357 and CS240 (Table 3). Similarly, the decline in root and shoot DW was more severe in CS240 and NRCG357 than in the other genotypes at 100 mM NaCl stress (Table 4).

Initial screening based on the seed physiological parameters revealed that different levels of salt stress during seed germination and early seedling establishment stage proved detrimental and had a significant negative impact on the germination process of peanut. Salt stress beyond 100 mM NaCl resulted in growth seizures in all genotypes, without a

Table 2 Effect of different levels of salt (NaCl) stress (continued up to 8 days) on root and shoot length and seedling vigour index in peanut genotypes

	Root length (cm)				Shoot length (cm)				Seedling vigour index			
	0 mM	50 mM	100 mM	200 mM	0 mM	50 mM	100 mM	200 mM	0 mM	50 mM	100 mM	200 mM
Somnath	7.9^{a}	6.3^{bc}	4.9^{de}	2.5^{ij}	4.7^{a}	2.3^{d}	1.5^{e}	0.8^{gh}	1402^{a}	789^{c}	561^{e}	148^{hi}
TPG 41	7.2^{a}	7.2^{b}	4.1^{efg}	2.1^{ij}	3.5^{c}	2.4^{d}	1.4^{ef}	0.5^{hi}	1070^{b}	690^{d}	307^{g}	131^{hij}
Girnar 1	5.4^{cd}	4.2^{ef}	3.4^{gh}	1.8^{jk}	4.1^{b}	3.5^{c}	1.1^{fg}	0.4^{ij}	681^{d}	493^{ef}	216^{gh}	80^{ijk}
CS 240	4.4^{ef}	3.5^{fg}	2.9^{hi}	1.1^{kl}	2.1^{d}	1.5^{e}	0.9^{g}	0.1^{j}	522^{e}	252^{g}	132^{hij}	33^{kj}
TMV 2	5.2^{cde}	3.8^{fgh}	2.4^{ij}	0.4^{m}	4.4^{ab}	3.1^{d}	1.4^{ef}	0.2^{ij}	847^{c}	421^{f}	181^{hi}	8^{k}
NRCG 357	7.0^{b}	2.9^{hi}	1.4^{kl}	0.7^{l}	3.1^{d}	3.7^{c}	0.9^{g}	0.1^{j}	808^{c}	317^{g}	72^{ijk}	8^{k}

Mean values sharing the same letter for each treatment × genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Table 3 Effect of different levels of salt (NaCl) stress (continued up to 10 days) on root, shoot and total biomass (fresh weight) in peanut genotypes

	Root FW (g)				Shoot FW (g)				Total biomass FW (g)			
	0 mM	50 mM	100 mM	200 mM	0 mM	50 mM	100 mM	200 mM	0 mM	50 mM	100 mM	200 mM
Somnath	4.47^{a}	2.24^{e}	1.39^{gh}	0.11^{j}	0.998^{a}	0.395^{de}	0.285^{fgh}	0.129^{jk}	5.471^{a}	2.635^{e}	1.679^{gh}	0.234^{j}
TPG 41	4.11^{a}	2.29^{e}	1.32^{h}	0.09^{j}	0.629^{b}	0.376^{def}	0.254^{ghi}	0.056^{klm}	4.738^{b}	2.665^{e}	1.571^{gh}	0.146^{j}
Girnar 1	2.67^{d}	1.95^{ef}	0.58^{i}	0.05^{j}	0.333^{efg}	0.164^{ij}	0.115^{jkl}	0.025^{lm}	3.001^{e}	2.110^{f}	0.694^{i}	0.075^{j}
CS 240	1.69^{fg}	1.22^{h}	0.34^{j}	0.02^{j}	0.202^{hij}	0.199^{hij}	0.059^{klm}	0.014^{m}	1.893^{fg}	1.416^{h}	0.196^{j}	0.034^{j}
TMV 2	3.60^{b}	1.70^{f}	0.74^{i}	0.01^{j}	0.438^{cd}	0.207^{hij}	0.186^{ij}	0.008^{m}	4.035^{c}	1.907^{fg}	0.926^{i}	0.018^{j}
NRCG 357	3.78^{b}	1.24^{h}	0.14^{j}	0.03^{j}	0.518^{c}	0.112^{jkl}	0.049^{klm}	0.021^{lm}	4.303^{c}	1.352^{h}	0.184^{j}	0.052^{j}

Mean values sharing the same letter for each treatment × genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Table 4 Effect of different levels of salt (NaCl) stress (continued up to 10 days) on root, shoot and total biomass (dry weight) in peanut genotypes

	Root DW (g)				Shoot DW (g)				Total biomass DW (g)			
	0 mM	50 mM	100 mM	200 mM	0 mM	50 mM	100 mM	200 mM	0 mM	50 mM	100 mM	200 mM
Somnath	0.727^{a}	0.348^{d}	0.244^{ef}	0.018^{i}	0.265^{a}	0.102^{c}	0.078^{de}	0.028^{ghi}	0.992^{a}	0.450^{d}	0.322^{e}	0.046^{h}
TPG 41	0.534^{b}	0.245^{ef}	0.185^{g}	0.010^{i}	0.127^{b}	0.065^{e}	0.048^{f}	0.010^{jk}	0.660^{b}	0.310^{e}	0.233^{f}	0.020^{h}
Girnar 1	0.263^{e}	0.201^{fg}	0.083^{h}	0.004^{i}	0.046^{f}	0.027^{ghi}	0.027^{ghi}	0.008^{jk}	0.310^{e}	0.228^{f}	0.110^{g}	0.013^{h}
CS 240	0.230^{efg}	0.118^{h}	0.055^{i}	0.003^{i}	0.033^{fgh}	0.029^{ghi}	0.024^{hi}	0.007^{jk}	0.263^{ef}	0.147^{g}	0.039^{h}	0.010^{h}
TMV 2	0.453^{c}	0.264^{e}	0.112^{h}	0.002^{i}	0.088^{cd}	0.050^{f}	0.041^{fg}	0.005^{k}	0.541^{c}	0.314^{e}	0.153^{g}	0.003^{h}
NRCG 357	0.424^{c}	0.192^{g}	0.010^{i}	0.001^{i}	0.085^{cd}	0.033^{fgh}	0.017^{ij}	0.008^{jk}	0.509^{cd}	0.225^{f}	0.027^{h}	0.009^{h}

Mean values sharing the same letter for each treatment × genotype combination, are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

distinguishable impact between tolerant and sensitive genotypes. Hence, 100 mM NaCl stress was found to be the most effective stress level for obtaining differential responses from tolerant and sensitive genotypes. Accordingly, a subsequent experiment to study the early and late responses of salt stress at the seedling establishment stage was carried out under 100 mM NaCl stress.

Oxidative stress and ROS production

The content of superoxide radical ($O_2^{\bullet -}$) showed an initial increase as an early response to salt stress. After 48 h, the $O_2^{\bullet -}$ content in the root tissue decreased to some extent from the initial increase (Fig. 1a, b). After 1 h of 100 mM NaCl treatment, all the genotypes showed a sharp increase in

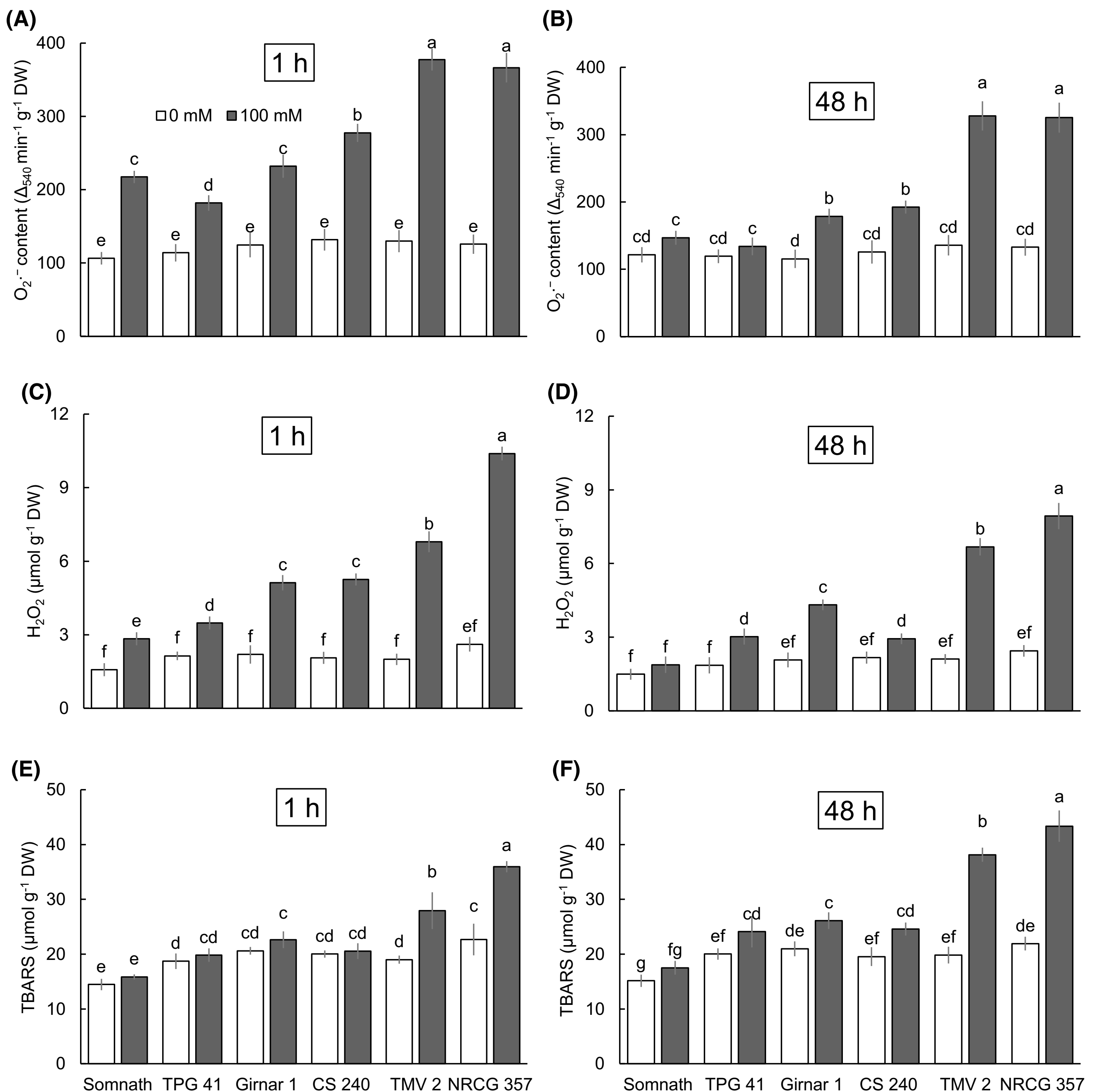

Fig. 1 Changes in superoxide radical content after 1 h (**a**) and 48 h (**b**); hydrogen peroxide content after 1 h (**c**) and 48 h (**d**); lipid peroxidation after 1 h (**e**) and 48 h (**f**) in peanut roots in response to 100 mM NaCl stress at seedling establishment stage. Mean values (±SE) sharing the same letter for each treatment×genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

$O_2^{\bullet-}$ content, with NRCG357 and TMV2 showing the highest increase (~ threefold rise in both the genotypes). The tolerant genotypes, viz., Somnath and TPG41, showed ~ 100 and 60% increases in $O_2^{\bullet-}$ content as an early response, suggesting the role of $O_2^{\bullet-}$ as a signalling molecule to trigger defence reactions. However, at 48 h after stress, the situation changed completely, and the increase in $O_2^{\bullet-}$ content showed a perfect resemblance to the nature of salt tolerance of the genotypes. The tolerant and moderately tolerant genotypes (Somnath, TPG 41, Girnar 1, CS 240) were able to reduce the $O_2^{\bullet-}$ content to a normal level or very close to it, while the sensitive genotypes TMV2 and NRCG357 continued to show a > 165% increase after 48 h of stress.

Similarly, the H_2O_2 content also showed an increase during the early response (1 h); however, its level was reduced at 48 h (late response) after salt stress (Fig. 1c, d). After 1 h of stress, the H_2O_2 content increased more in the sensitive genotypes (> 250% in NRCG 357 and TMV 2) than in the tolerant ones (< 100% in Somnath and TPG 41). A longer duration of stress (48 h) resulted in comparatively less H_2O_2 build-up, which may be due to the activation of the antioxidant defence network. A distinguishable response was observed between the sensitive and tolerant genotypes at 48 h of NaCl stress. The increase in H_2O_2 content was restricted to a mere 20 and 65%, respectively, in Somnath and TPG41, while it was ~ 190 and 230%, respectively, in NRCG357 and TMV2 at 48 h of salt stress. Lipid peroxidation, another indicator of oxidative damage under salt stress, showed a significant increase with increased duration of stress. A significant increase in lipid peroxidation was observed at 1 h of NaCl stress in the sensitive genotypes (Fig. 1e). Two sensitive genotypes (NRCG 357 and TMV 2) showed an ~ 50% increase in lipid peroxidation, whereas in the remaining genotypes, the increase was not significant after 1 h of stress. Similarly, at 48 h of stress, the damage (in terms of lipid peroxidation) was much less in the tolerant and moderately tolerant genotypes (showing a 25–30% increase in lipid peroxidation) than in the two sensitive genotypes (NRCG 357 and TMV 2), which showed a 90–100% increase in lipid peroxidation (Fig. 1f).

Antioxidant enzyme system and total antioxidant activity

The early response to salt stress did not alter the activities of the key antioxidant enzyme SOD (except in Somnath, TPG41 and Girnar 1); however, the late response showed significant induction of SOD activity in most of the genotypes (Fig. 2a, b). Compared with the sensitive genotypes, both the tolerant (Somnath and TPG 41) and moderately tolerant genotypes (Girnar 1 and CS 240) showed a much higher increase in SOD activities at 48 h after salt stress, while sensitive genotypes such as NRCG357 and TMV2 showed a modest increase of ~ 25% after longer exposure to NaCl stress (Fig. 2c). At the transcriptional level, the trend was completely opposite, where a sharp increase in SOD transcripts was observed at 1 h after imposition of NaCl stress (Fig. 2d). The tolerant genotypes (Somnath and TPG 41) and Girnar 1 (moderately tolerant) showed a more than 50-fold increase in SOD transcript level compared to that in the control conditions, indicating an immediate induction of the antioxidant defence network in these genotypes compared to the sensitive genotypes, viz., NRCG357 and TMV2, which showed a mere 1–5-fold increase in SOD transcripts. With increased duration of salt stress, the relative abundance of SOD transcripts stabilized, and little difference in relative transcript abundance was observed between the tolerant and sensitive genotypes at 48 h after imposition of salt stress, as Somnath and TPG41 showed 2- and 3.5-fold increases, respectively, while NRCG357 showed a > fourfold increase.

Similarly, the POD activity showed minimal induction upon short-term exposure of salt stress in most of the genotypes. Comparatively, much less induction in POD activity was observed after 1 h of stress imposition. Although the increase in POD activity was observed in most of the genotypes except TMV2, the highest increase was observed in TPG41 (~ 45%) and CS240 (~ 35%) at 1 h of stress (Fig. 3a). After 48 h of NaCl stress, the most tolerant genotypes (Somnath and TPG 41) showed the highest level of induction (105 and 85%, respectively) (Fig. 3b, c). The POD mRNA level increased sharply at 1 h after treatment in all the tolerant and moderately tolerant genotypes, while the level of induction was ~ fivefold in the sensitive genotypes. Unlike the SOD transcripts, whose level of expression decreased sharply from a very high initial induction level, the POD transcripts showed a stable induction level even after 48 h of NaCl treatment.

The APX and GR activities did not show any induction after 1 h of stress imposition (Figs. 4, 5); however, with increased exposure to stress (48 h), minimal induction in APX activity was observed in the tolerant genotypes only (Fig. 4b). The same results were confirmed by q-PCR data, which showed that the APX transcript levels were almost unaffected or were downregulated at 1 h of NaCl stress (except Somnath), while 48 h of stress resulted in little induction compared to that of other enzymes (SOD or POD) (Fig. 4d). Similarly, the GR activity showed no induction at 1 h of stress in most of the genotypes except CS240, which showed significant upregulation, while this activity was downregulated in Girnar 1 and NRCG357. However, after 48 h of stress imposition, Somnath and TPG41 showed significant upregulation in GR activity, while in the remaining genotypes, it was unaffected (Fig. 5). Unlike GR enzyme activity, GR transcript levels were upregulated in all genotypes with increased durations of stress (48 h). The CAT activity was relatively highly induced in the sensitive

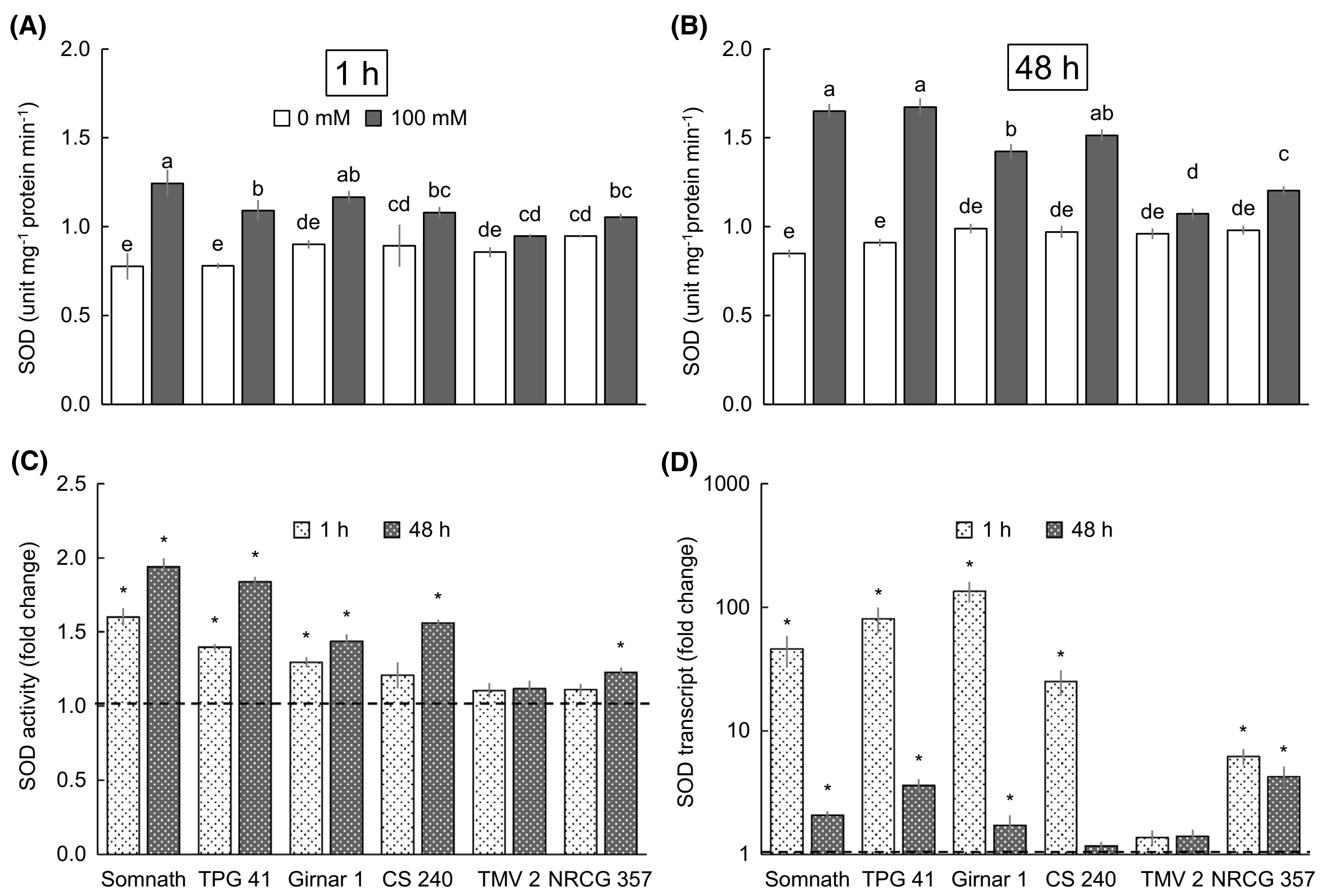

Fig. 2 Changes in superoxide dismutase (SOD) activity after 1 h (**a**) and 48 h (**b**) in peanut roots in response to 100 mM NaCl stress at seedling establishment stage. Mean values (± SE) sharing the same letter for each treatment × genotype combination are not significantly different ($P \le 0.05$) according to Duncan's multiple range test. Fold change in enzyme activity (**c**) and relative transcript abundance (**d**) were shown compared to respective controls. Mean values (± SE) having '*' and '§' denotes significant ($P \le 0.05$) upregulation and down-regulation compared to control, while the dotted horizontal lines represent the enzyme activity and transcript abundance in non-treated control roots (**c**, **d**)

genotypes (TMV 2 and NRCG 357) in response to both short and long durations of stress, which is completely opposite the pattern of induction for the other four antioxidant enzymes (Fig. 6). The induction level was ~ 50% in the sensitive genotypes but was 25–35% in the tolerant genotypes, but it did not show any increase in CS240 after 48 h of stress imposition. The CAT transcript abundance also showed the highest induction in NRCG357 and TMV2 (> 2- and > 4.5-fold increases in mRNA levels at 1 and 48 h after stress, respectively) (Fig. 6d).

The total antioxidant activity (TAA) measured in terms of Trolox equivalents in the root tissues of salinity-treated and non-treated seedlings showed significant differences after 1 h of stress imposition in all the genotypes (Fig. 7). The genotypes Somnath and CS240 showed maximum levels of TAA (91.8 and 90.5 µM TEA g^{-1} DW, respectively), whereas the level of TAA was lowest in the two sensitive genotypes, NRCG357 and TMV2. After 48 h of stress imposition, the TAA showed a significant increase in all the tolerant and moderately tolerant genotypes, while the increase in TAA was relatively much less in both NRCG357 (22.2%) and TMV2 (21.9%) in response to salt stress.

Discussion

Negative effects during the seed germination process under salinity stress can be due to both ionic and osmotic factors of NaCl stress (Machado Neto et al. 2004). Water is taken up by physical processes to activate the metabolic activities that lead to the breakage of dormancy and the mobilization of food reserves (Katembe 1998). Due to the increased osmotic potential of the growth media, seeds under saline conditions often fail to take up sufficient amount of water required for germination and initial plant growth, thereby delaying the process of germination (Werner and Finkelstein 1995). Most glycophytic crop species across different genera are reported to exhibit slow seed germination and subsequent seedling

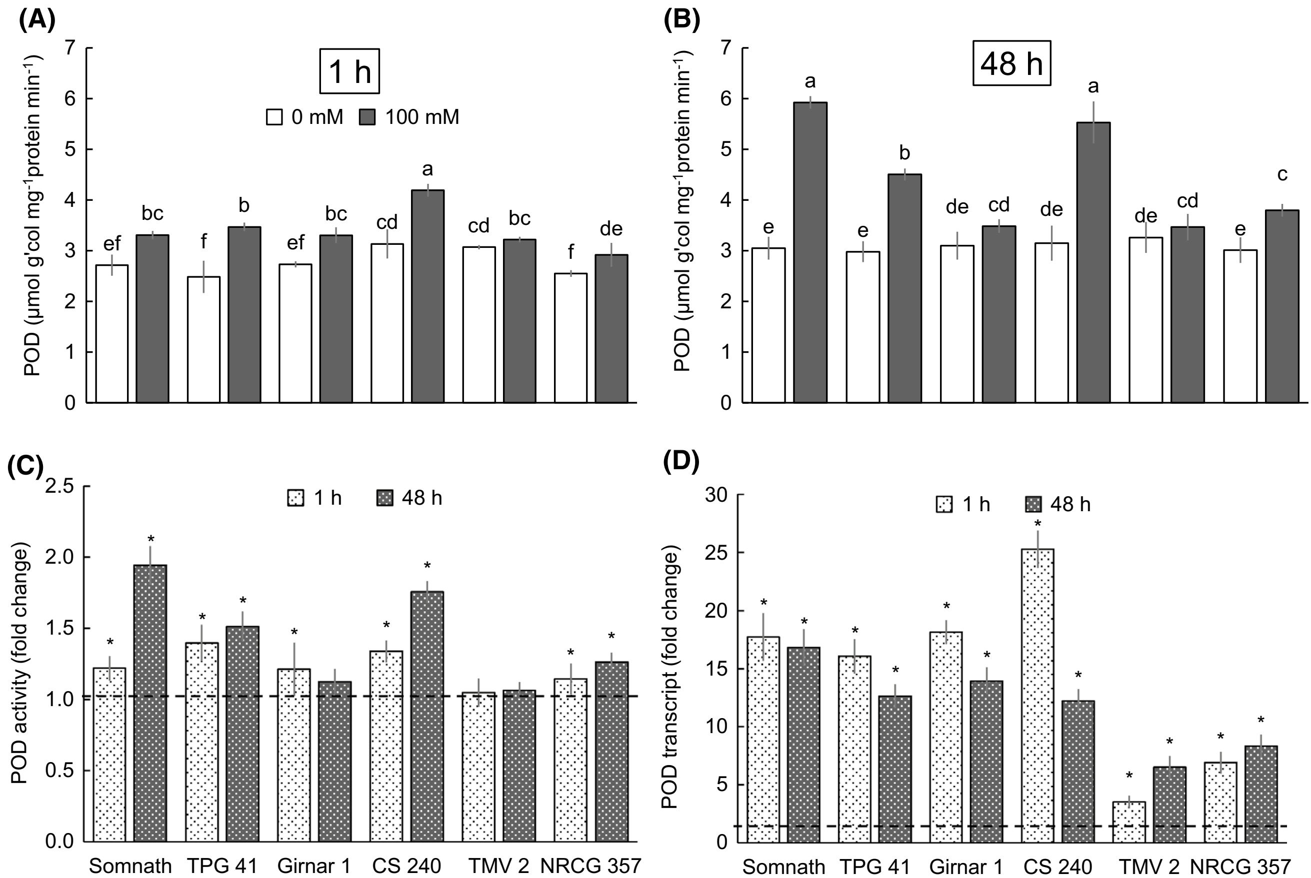

Fig. 3 Changes in peroxidase (POD) activity after 1 h (**a**) and 48 h (**b**) in peanut roots in response to 100 mM NaCl stress at seedling establishment stage. Mean values (±SE) sharing the same letter for each treatment × genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. Fold change in enzyme activity (**c**) and relative transcript abundance (**d**) were shown compared to respective controls. Mean values (±SE) having '*' and '§' denotes significant ($P \leq 0.05$) upregulation and down-regulation compared to control, while the dotted horizontal lines represent the enzyme activity and transcript abundance in non-treated control roots (**c**, **d**)

establishment under salinity stress (Almansouri et al. 2001; Luo et al. 2005; Singh et al. 2007).

Peanut genotypes showed considerable vulnerability to salt stress at the seed germination and early seedling establishment stages in this study. In our first set of experiments, different levels of NaCl stress were tested, where significant negative effects on seed germination and the early seedling establishment process were observed at 100 mM or higher NaCl concentrations. Aside from Somnath, the other five genotypes showed significant reductions in Gp, Gr and MGT, even at 50 mM NaCl stress. Similarly, the seedling vigour and initial biomass of the roots and shoots showed significant reductions with increasing levels of salt stress in most genotypes. Our initial screening revealed Somnath > TPG41 > Girnar 1 > CS240 > TMV2 > NRCG357 as the order of salt tolerance at the germination and seedling establishment stages. Comparing these results with those of our previous report (Chakraborty et al. 2016b), we found that a few genotypes, viz., Somnath and TPG41, which were tolerant to long-term (beyond the reproductive phase) salt exposure, also showed a significant level of tolerance at the seedling establishment stage. In contrast, genotypes such as CS240 showed relatively higher tolerance under long-term salinity stress (Chakraborty et al. 2016b) but failed to do so during the initial growth stages. This finding indicated that the mechanism of salt tolerance during early growth stages may not be similar as a whole with the mechanism operating at late growth stages. Several components of the stress tolerance network may vary in peanut during the early vegetative and reproductive stages. How different components of the oxidative stress tolerance network vary in early and late responses to salt stress is discussed in this study.

Both the osmotic and ionic components of salt stress cause metabolic imbalance and hence contribute towards the production of reactive oxygen species (ROS), leading to severe oxidative stress (Adem et al. 2014). The young and metabolically active tissues during the early stages of plant growth, viz., seedling establishment and early vegetative

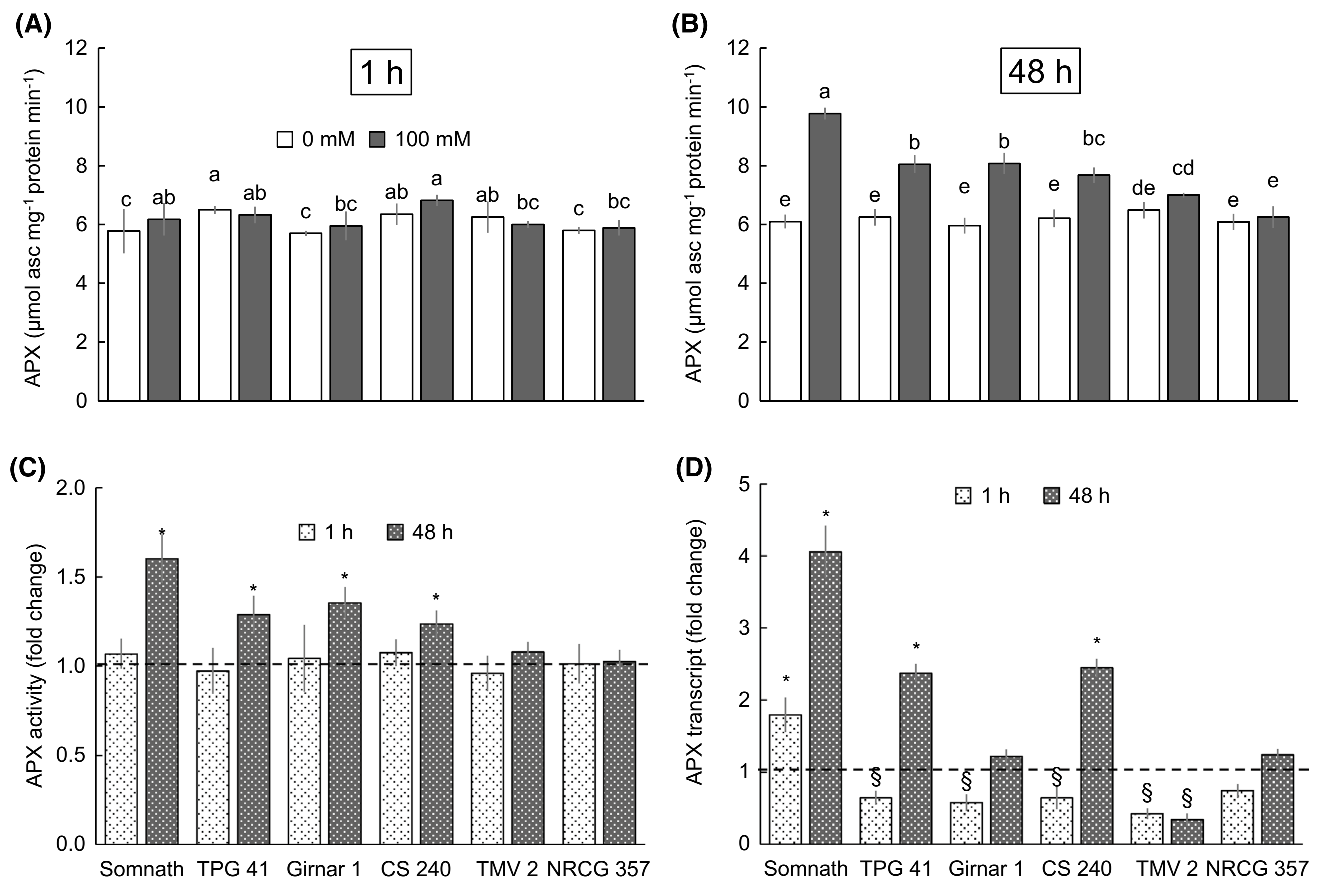

Fig. 4 Changes in ascorbate peroxidase (APX) activity after 1 h (**a**) and 48 h (**b**) in peanut roots in response to 100 mM NaCl stress at seedling establishment stage. Mean values (±SE) sharing the same letter for each treatment × genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. Fold change in enzyme activity (**c**) and relative transcript abundance (**d**) were shown compared to respective controls. Mean values (±SE) having '*' and '§' denotes significant ($P \leq 0.05$) upregulation and down-regulation compared to control, while the dotted horizontal lines represent the enzyme activity and transcript abundance in non-treated control roots (**c**, **d**)

stages, are sensitive to stress induced by ROS (Bose et al. 2014). It is well known that ROS can even be produced during the seed imbibition process as a metabolic requirement for the signal transduction network, which must be maintained at a metabolically favourable range for seedling growth and development (Bailly et al. 2008; Leymarie et al. 2011). The scenario becomes adverse in the presence of external stress factors, viz., salinity, which leads to disruption of the controlled production of ROS, resulting in cellular damage (Miller et al. 2010). It is essential for plants to overcome such metabolic imbalance by the action of well-coordinated ROS detoxification machinery, where the selective and judicious induction of each component of the network needs to be activated (Miller et al. 2010; Abogadallah 2010). In this study, we found very prompt responses in terms of changes in both superoxide radical and H_2O_2 contents. Both components increased sharply after just 1 h of salt stress imposition, whereas lipid peroxidation did not show significant changes at 1 h after stress, particularly in the tolerant genotypes. Such a sharp increase in $O_2^{\bullet-}$ content probably stimulated the enzyme-driven antioxidant defence network. This phenomenon was evident from the strong upregulation of SOD transcripts (50–100-fold in tolerant genotypes) in just 1 h of NaCl treatment. A quick upregulation of SOD and CAT activity under salinity stress was reported in germinating cucumber seeds (Fan et al. 2013). Stress-induced upregulation of CAT and POD was reported to be an essential part of antioxidant defence in sugar beet and rice (Bor et al. 2003; Demiral and Turkan 2005). Unlike our previous report, where little variation in SOD transcript induction was observed in tolerant and sensitive genotypes under prolonged salt stress at the reproductive stage, in this study, a distinctly higher SOD transcript induction (1 h after treatment) was observed in the tolerant genotypes as compared with the sensitive ones. The enzyme activity and/or transcript abundance of major H_2O_2-detoxifying enzymes, viz., POD and CAT, also showed induction at 1 h after stress, albeit certainly to a lesser degree than that of

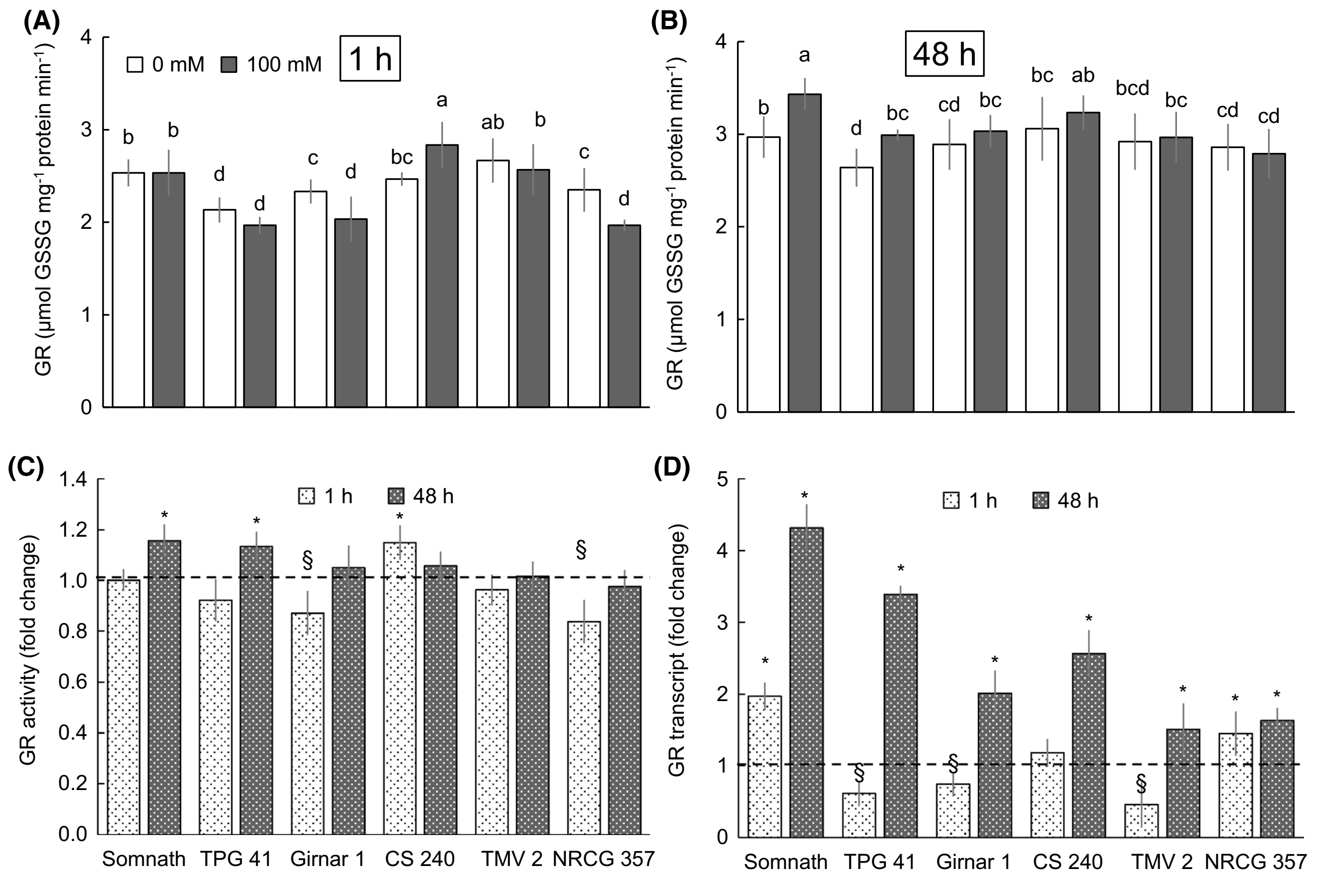

Fig. 5 Changes in glutathione reductase (GR) activity after 1 h (**a**) and 48 h (**b**) in peanut roots in response to 100 mM NaCl stress at seedling establishment stage. Mean values (±SE) sharing the same letter for each treatment × genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. Fold change in enzyme activity (**c**) and relative transcript abundance (**d**) were shown compared to respective controls. Mean values (±SE) having '*' and '§' denotes significant ($P \leq 0.05$) upregulation and down-regulation compared to control, while the dotted horizontal lines represent the enzyme activity and transcript abundance in non-treated control roots (**c**, **d**)

SOD. Relatively much lower induction was observed for both APX and GR transcripts, reaffirming the minimal role of this H_2O_2 detoxification pathway in peanut. Overall, the evidence points towards the initial induction (1 h after salt stress) of SOD as a major defence response in peanut roots under salt stress at the early seedling establishment stage.

Salt tolerance, particularly at the early stage, is closely associated with the plant's ability to detoxify major ROS produced because of salt stress (Mittova et al. 2002; Chakraborty et al. 2016c). With subsequent exposure to salinity, plants try to alter their stress-induced antioxidant defence response to counteract the ROS load by a number of routes (Mittler 2002; Bor et al. 2003). Electrolyte leakage and cellular injury associated with relatively high lipid peroxidation have been used to assess the extent of salt stress-induced damage in various crop species (de Azevedo Neto et al. 2006). In this study, there was an initial increase in $O_2^{\bullet-}$ and H_2O_2 contents at just 1 h after imposition of salt stress, which subsequently stabilized (at 48 h), particularly in the tolerant genotypes. However, in contrast, lipid peroxidation was found to increase after 48 h of stress imposition, especially in the sensitive genotypes. Such a high initial content of ROS might activate the defence reaction promptly enough to counterbalance the excess ROS load and return it to a favourable level by the action of SOD. However, over time, the activities of H_2O_2-detoxifying enzymes, particularly POD and CAT, played a more important role in managing the ROS load and became a key component of the stress tolerance network. The relatively more efficient system in tolerant genotypes probably managed the ROS load more effectively, resulting in less lipid peroxidation, a subsequent effect of ROS stress. A longer duration of salt stress resulted in the strong upregulation of activities and transcript abundance of POD and CAT. Comparatively greater upregulation of CAT activity was observed in the sensitive genotypes (NRCG357 and TMV2) than in the tolerant genotypes. Despite having the same substrate (H_2O_2), the activities of CAT and POD differ based on the

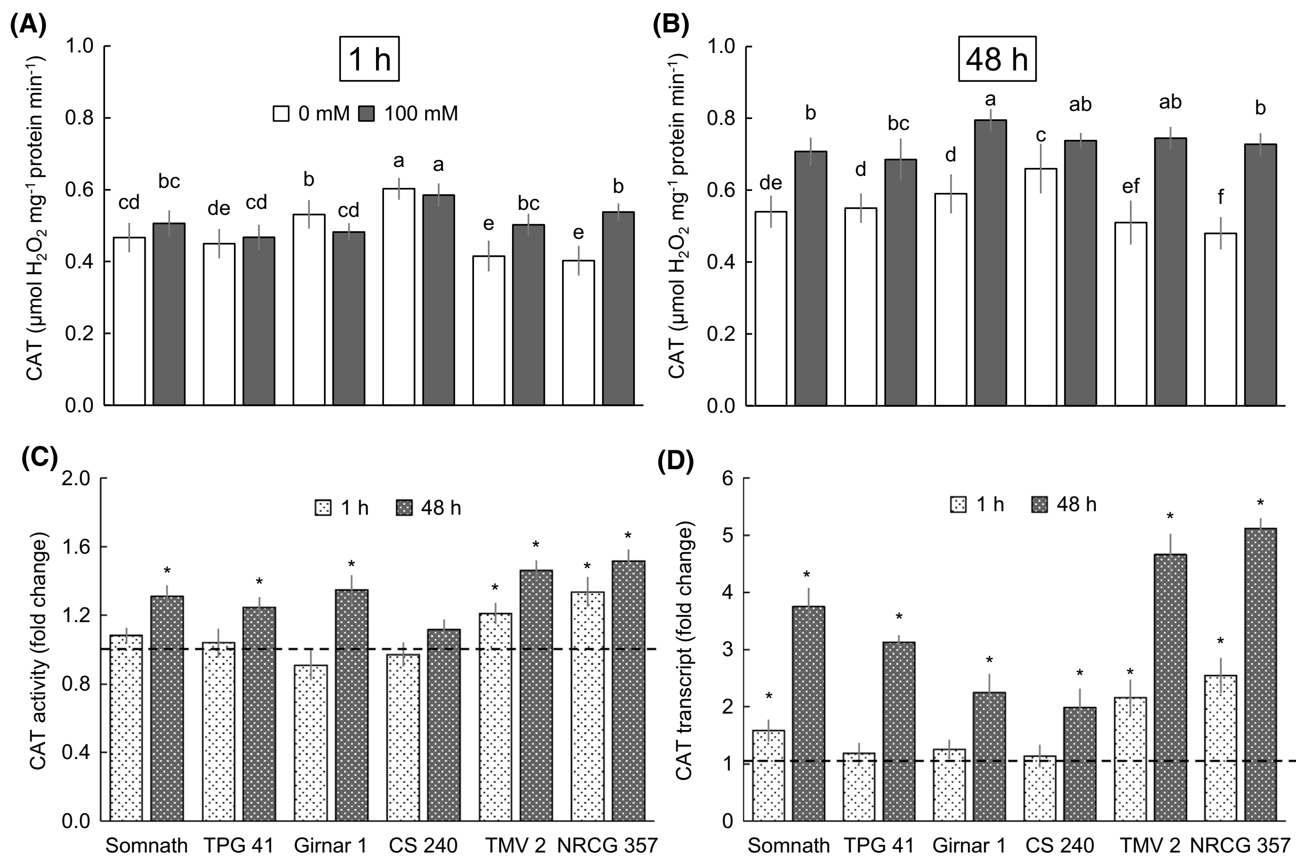

Fig. 6 Changes in catalase (CAT) activity after 1 h (**a**) and 48 h (**b**) in peanut roots in response to 100 mM NaCl stress at seedling establishment stage. Mean values (±SE) sharing the same letter for each treatment × genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. Fold change in enzyme activity (**c**) and relative transcript abundance (**d**) were shown compared to respective controls. Mean values (±SE) having '*' and '§' denotes significant ($P \leq 0.05$) upregulation and down-regulation compared to control, while the dotted horizontal lines represent the enzyme activity and transcript abundance in non-treated control roots (**c**, **d**)

substrate concentration. CAT detoxifies H_2O_2 at relatively high cellular concentrations, whereas POD acts at much lower substrate concentrations, maintaining a fine balance of cellular H_2O_2 levels (Mittler 2002; Abogadallah 2010). Greater CAT activity in salt-sensitive genotypes has been reported in peanut and other crop species (Abogadallah et al. 2010; Chakraborty et al. 2016b). Some induction of both APX and GR transcripts was observed at 48 h after NaCl stress, particularly in the most tolerant genotypes (Somnath and TPG41). This phenomenon might have complimented the more strongly induced POD activity for the fine tuning of the H_2O_2 load in these genotypes. Moreover, this study showed a greater pool of total antioxidants, as evidenced by the total antioxidant activity in the tissue of the tolerant genotypes at both 1 h and 48 h of stress imposition. In contrast, in the sensitive genotypes, although there was an initial increase in total antioxidant activity, after 48 h, it was reduced or remained unchanged. Relatively high constitutive and induced levels of total antioxidant activity in the tolerant genotypes helped them scavenge ROS generated during salt stress relatively more efficiently, as reported by Amor et al. (2006) for halophytes.

Conclusion

This study clearly demonstrated the changes in the activation of an enzyme-driven antioxidant defence network in peanut root as early (1 h) and late (48 h) responses to salt stress at the early seedling emergence stage. This indicated a mechanism for judiciously balancing the ROS level in germinating peanut roots. At early seedling stage, an initial shock of 100 mM NaCl resulted in burst of $O_2^{\bullet-}$ and H_2O_2 content in roots followed by strong upregulation of SOD transcript as an early response to salt stress in tolerant genotypes. This prompt induction of SOD transcript within 1 h of NaCl stress may be considered as a defence response

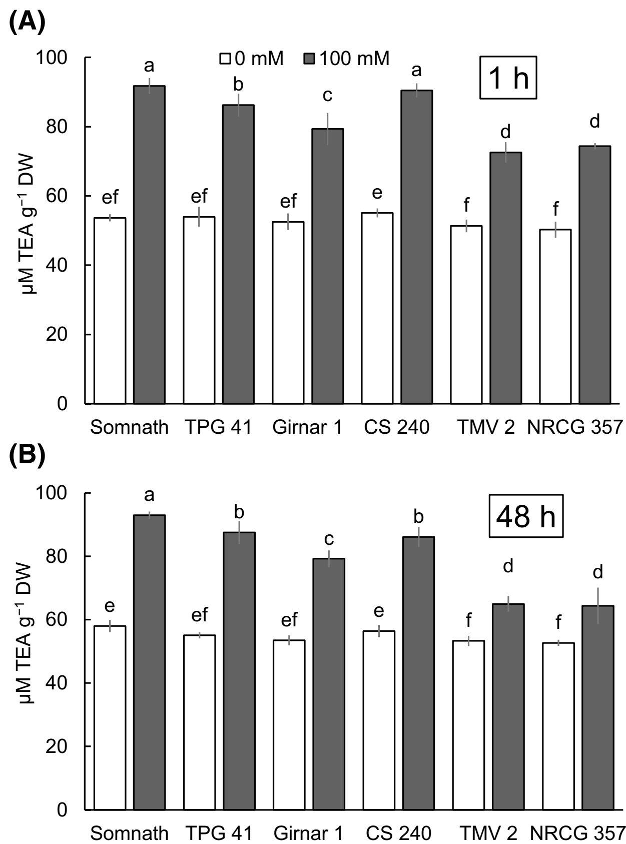

Fig. 7 Changes in the total antioxidant activity measured as Trolox equivalent in peanut roots at 1 h (**a**) and 48 h (**b**) after imposition of 100 mM NaCl stress at seedling establishment stage. Mean values (±SE) sharing the same letter for each treatment × genotype combination are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

to initiate the antioxidant enzyme-mediated detoxification network. The induction pattern was also corresponded with overall salt tolerance nature of the studied genotypes. Comparatively longer duration of stress (48 h) changed the selective induction pattern of antioxidant enzymes, where POD and CAT played a greater role in H_2O_2 detoxification and salt-tolerance in these genotypes. The immediate and adaptive response for selective induction of antioxidant enzyme system provides cues for cellular fine tuning of ROS load during seedling establishment process in peanut.

Author contribution statement KC conceptualized the whole study, KC and SKB designed the experiments, PVZ performed the seed physiological studies, NG carried out the biochemical work. KC, NG and DB analysed the data and done the statistical analysis. KC and SKB carried out the molecular work. KC, SKB, ALS and DB drafted the manuscript.

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