Sodicity stress differently influences physiological traits and anti-oxidant enzymes in pear and peach cultivars

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Background. The growth and physiological responses to sodicity stress of pear and peach are poorly understood. Insights into how sodicity stress alters tree physiology remain vital to developing salt tolerant scion and rootstock cultivars.

Methods. The effects of sodicity stress (soil pH_s ~8.8) on tree growth and physiological traits on field grown trees of pear cultivars Punjab Beauty and Patharnakh, and peach cultivars Partap and Shan-e-Punjab were recorded using standard procedures. Sodicity-induced changes in oxidative stressors, proline, anti-oxidant enzymes and leaf ions were measured to draw inferences.

Results. Sodicity-induced reductions in vegetative growth were particularly marked in Patharnakh pear and Partap peach compared with other cultivars. Although sodicity stress triggered a significant increase in leaf malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), their levels relative to controls were much higher in peach than in pear; reflecting that peach suffered from greater oxidative stress. Interestingly, MDA and H_2O_2 levels did not seem to be deleterious enough to trigger proline-induced osmotic adjustment in pears. The activities of anti-oxidant enzymes strongly varied with the cultivar; specifically, the sodicity-induced increases in CAT and SOD activities were much higher in Punjab Beauty pear and Shan-e-Punjab peach. Principal Component Analysis revealed an explicit convergence between CAT and SOD activities in Punjab Beauty and Shan-e-Punjab cultivars in response to sodicity-induced oxidative stress. Correlation analysis revealed that leaf Na^+ strongly inhibited tree growth in peach than in pear. Leaf K^+ and proline were found to be the major osmolytes in sodicity-stressed pear and peach cultivars, respectively.

Conclusions. Our findings revealed a marked suppressive effect of sodicity stress on tree growth in peach than in pear. The sodicity-induced upticks in leaf malondialdehyde, hydrogen peroxide and Na^+ seemed to induce proline-mediated osmotic adjustment in peach but not in pear. The overall better sodicity tolerance in pear compared to peach was ascribed to increased activities of anti-oxidant enzymes catalase and superoxide dismutase enzymes together with restricted Na^+ uptake and better leaf K^+ levels.

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Abstract

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activities in Punjab Beauty and Shan-e-Punjab cultivars in response to sodicity-induced oxidative stress. Correlation analysis revealed that leaf Na^+ strongly inhibited tree growth in peach than in pear. Leaf K^+ and proline were found to be the major osmolytes in sodicity-stressed pear and peach cultivars, respectively.

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INTRODUCTION

Horticultural production will have to increasingly rely on salt-impaired lands in the coming decades (Singh et al., 2018a). Saline, sodic (alkali) and saline-sodic soils occupy ~60%, 26% and 14% of the global salt-affected area, respectively (Wicke et al., 2011). In contrast to the commonly accepted saturated soil paste pH (pH_s) threshold of 8.5, pH_s >8.2 seems to be more realistic for classifying the soils as sodic under Indian conditions: soil pH_s of 8.2 is often associated with an exchangeable sodium percentage (ESP) of 15; a limit above which the soils are generally considered to be sodic (Abrol et al., 1988). Generally, soil pH_s of 8.5 roughly corresponds to ESP of ~50, high enough to suppress the crop growth (Sharma et al., 2016; Abrol et al., 1988). Although alkali salts such as sodium carbonate (Na_2CO_3) are often more detrimental to plant growth than neutral salts (e.g. NaCl) (Yang et al., 2009), including fairly salt tolerant crops (Abbas et al., 2021), alkali stress research continues to receive little attention.

Sodic soils in the Trans-Gangetic Plains of India (study area) mostly have predominance of highly soluble $Na_2CO_3$and $NaHCO_3$ salts, and are thus prone to abrupt increases in the soil pH (Mandal, 2012).

In sodic soils, excessive Na^+ causes clay dispersion, surface crusting and deterioration in the soil physical properties (Qadir et al., 2007). Besides poor physical properties, high pH, osmotic and ionic stresses, and nutrient deficiencies are other limitations to plant growth in the sodic soils (Qadir & Schubert, 2002). Additionally, calcium carbonate ($CaCO_3$) concretions in the sub-soil also hamper plant establishment (Sharma et al., 2016). Considerable spatial variations in soil pH are also frequently seen in sodic soils; the more sodic parts of the field are often less congenial for crop growth (Samra et al., 1988). In fruit crops, sodicity stress adversely affects vegetative growth (Saxena & Gupta, 2006; Krishnamoorthy, 2009). Altered plant water relations (Li et al., 2020), decrease in photosynthetic pigments (Krishnamoorthy, 2009; Li et al., 2020), lipid peroxidation and oxidative stress (Ahmad et al., 2014), and ionic stress (e.g. Na^+) (Singh et al., 2018b) are the major limitations to plant growth. Sodicity-stressed plants accumulate osmolytes such as proline for osmotic adjustment (Krishnamoorthy, 2009; Ahmad et al., 2014; Singh et al., 2018a), and activate the antioxidant enzymes for scavenging the free radicals (Ahmad et al., 2014).

The physico-chemical properties of sodic soils improved following amendment application and salt leaching; however, such improvements are mostly transient and limited to the top soil (<15 cm) (Sharma & Singh, 2019) such that sub-soil constraints continue to persist (Kumar et al., 2019). Under such conditions, agronomic practices such as planting into amendment-treated auger-holes often give better results (Gill & Abrol, 1991; Saxena & Gupta, 2006). As a majority of fruit crops are mostly highly sensitive to salinity and sodicity stresses

(Singh et al. 2018a), development of salt tolerant scion and rootstock cultivars could help to sustaining fruit production in salt-affected soils (Mahmoud et al., 2020). Although salt tolerance is a complex polygenic trait, and is greatly influenced by the genetic and environmental cues (Flowers, 2004), there exists ample genotypic variation that needs to be explored for identifying the salt tolerant genotypes (Mahmoud et al., 2020; Singh et al. 2018a).

Although some studies have shown the adverse effects of salinity stress on pear (Myers et al., 1995; Oron et al., 2002; Musacchi et al., 2006) and peach (Boland et al., 1993; Karakas et al., 2000; Soliman et al., 2017) tree growth and physiology, their responses to sodicity stress remain elusive; barring some anecdotal evidence that high soil pH and the sub-soil constraints may suppress plant growth (Elkins et al., 2012; Abd-Elmegeed et al., 2013; Mestre et al., 2015; Tagliavini et al., 1995). Notably, not only the soil pH levels in these studies were rather low to draw reasonable inferences, the plausible physiological changes accounting for the reduced plant growth were also not investigated. Plant responses to salt often strikingly vary with the experimental conditions; for instance, depending on experimental conditions pears may either be highly sensitive (EC_e < 1.0 dS m^{-1}; Ebert, 1999) or moderately tolerant (~5.0 dS m^{-1}; Musacchi et al., 2006) to salinity. Interestingly, most of the aforementioned studies had used NaCl as the sole salinizing agent in relatively controlled short-term experiments; the results may be altogether different when a different salt is used to induce salt stress (Grieve et al., 2012). While an interplay among the oxidative stressors (e.g., malondialdehyde), osmolytes (e.g., proline), anti-oxidant enzymes and the leaf ions is known to greatly influence plant response to salinity stress in pears and peaches (Erturk et al., 2007; Wu& Zou, 2009; Dejampour et al., 2012; Yousefi et al., 2019), such responses remain uninvestigated under sodic conditions. Insights from other fruit crops [e.g., *Malus halliana* (Jia et al., 2019) and *Morus alba* (Hui-Hui et al., 2019)] also suggest

that plant physiological responses to salinity and sodicity stresses remarkably vary with each other: the effects of salinity cannot be used as a reliable proxy for those of sodicity.

To our knowledge, systematic studies have not yet been carried out to evaluate the effects of sodicity stress on tree growth and physiological relations of pears and peaches. This study intended to delineate sodicity-induced changes in tree growth, leaf oxidative stress markers, proline, enzymatic anti-oxidants and leaf ions in pear and peach cultivars, and how these changes influence the overall cultivar-specific tree responses to sodicity stress.

MATERIALS & METHODS

Location

The experiment was conducted between January, 2015 and April, 2019 at the experimental farm of Indian Council of Agricultural Research-Central Soil Salinity Research Institute, Karnal, India (29° 42′ 20.6″ N, 76° 57′ 19.80″ E, 243 m above mean sea level). The region has a semi-arid subtropical climate with hot summers and dry winters. The long-term average annual rainfall is ~700 mm. A sodic field was used to evaluate the pear and peach cultivars.

Experimental material

The pear cultivars Punjab Beauty (*Pyrus communis*) and Patharnakh (*P. pyrifolia*) grafted on Kainth (*Pyrus pashia*); and peach cultivars Partap and Shan-e-Punjab (*Prunus persica*) on Sharbati rootstock (*P. persica*) were evaluated. The bare root plants were planted on 17th January 2015 in auger-holes (diameter 20 cm, depth 120 cm) in a sodic field, keeping graft joint ~15 cm above the surface. The auger-holes were refilled with original soil and 5 kg of gypsum

($Ca_2SO_4.2H_2O$) before planting for better initial establishment (Sharma et al., 2014). The square system of planting was used, with between- and within-row spacings of 6 m each in both pear and peach. Trees were trained to the modified leader system, and the recommended management practices were followed for better tree growth. Irrigation water was applied through 1 m wide channels.

Treatments

Soil samples were collected from 24 random points of the field, from four depths (0-15, 15-30, 30-60 and 60-100 cm) using an auger. After air drying, the samples were ground and sieved (2.0 mm sieve) for determining the saturated soil paste pH (pH_s) and soil saturation extract electrical conductivity (EC_e) using digital pH and conductivity meters (Eutech, Singapore). Based on soil analysis, the experimental plants were grouped into control (mean soil pH_s= 8.22, EC_e= 0.71 dSm^{-1}) and sodic (mean pH_s= 8.80, EC_e= 0.94 dS m^{-1}) treatments for recording the observations. Both soil pH_s and EC_e increased with depth, and there were significant differences between control and sodicity treatments (pH_s F= 41.94, p <0.001; EC_e F= 27.52, p <0.001) (*Table S1*). The groundwater used in irrigation had the following composition: electrical conductivity- 0.65 dS m^{-1}, pH- 8.04, Na^+- 2.41 me L^{-1}, K^+- 0.15 me L^{-1}, Ca^{2+} + Mg^{2+}- 4.17 me L^{-1}, Cl^-- 0.98 me L^{-1} and HCO^-_3- 4.31 me L^{-1}.

Tree growth

Four trees of each pear and peach cultivar representing control and sodic treatments were randomly tagged for recording the observations. Tree height and trunk diameter were measured during the last week of April, 2019. Trunk diameter readings, recorded using a digital Vernier caliper (Mitutoyo, Japan) 15 cm above the graft union, were converted into trunk cross sectional

area (TCSA) by the formula: TCSA= $\pi(d/2)^2$; where d= mean of E-W and N-S trunk diameters. Canopy volume (CV) was computed by the formula: CV= $(w^2 \times h)/2$; where w= canopy diameter in east-west (E-W) and north-south (N-S) directions; and h= tree height.

Leaf physiological traits

The fully expanded leaves from middle of the shoots were collected from all the directions of each replicate tree (n= 4), pooled, packed in zip lock bags inside an ice-box, and immediately brought to the laboratory. Total leaf chlorophyll was estimated by overnight incubation of 200 mg chopped leaves in 80% acetone (Arnon, 1949) using a UV-VIS spectrophotometer (Electronics India). Lipid peroxidation, in terms of malondialdehyde (MDA) content, was estimated by the method of Heath & Packer, (1968). Hydrogen peroxide (H_2O_2) content was estimated using the procedure described in Sinha, (1972). Proline was extracted using 200 mg leaf tissue homogenized in 10 ml of 3% sulphosalicyclic acid (Bates et al., 1973).

Anti-oxidant enzymes

Fresh leaf sample (~250 mg) was homogenized in 0.1 M phosphate buffer (pH 7.5) containing 5% (w/v) polyvinyl polypyrrolidone, 1 mM EDTA, and 10 mM b-mercapto-ethanol for determining ascorbate peroxidase (APX, EC 1.11.1.11) and superoxide dismutase (SOD, EC 1.15.1.1) activities. APX activity was assayed as described in Nakano & Asada, (1981), and the enzyme activity was calculated using extinction coefficient of 2.8 mM^{-1} cm^{-1}. The SOD assay was performed following Beauchamp & Fridovich, (1971). The absorbance of the solution was measured at 560 nm with a UV-VIS spectrophotometer (UV 3200, Lab India Analytical). Catalase (CAT, EC 1.11.1.6) and peroxidase (POX, EC 1.11.1.7) were extracted in 0.01 M phosphate buffer (pH 7.5) with 3% (w/v) polyvinyl polypyrrolidone by homogenizing the fresh

158 leaf tissue (1.0 g). The homogenate was centrifuged at 4ºC for 15 min at 10,000 x g and clear
159 supernatant was used for the assay. CAT activity was determined as the disappearance of H_2O_2 at
160 240 nm (25ºC) for 1 min (Aebi, 1984). The POX was assayed by determining the rate of guaiacol
161 oxidation in the presence of H_2O_2 at 470 nm (Rao et al., 1998).

162 **Leaf Na^+ and K^+**

163 Leaf samples, dried to a constant weight at 60º C in a hot air oven (NSW, India), were
164 finely ground and 100 mg tissue sample was digested in the di-acid [HNO_3:$HClO_4$ (3:1)] mixture
165 for determining Na^+ and K^+ (mg g^{-1} DW) using a flame photometer (Systronics India).

166 **Statistical analysis**

167 The experiment was laid out in a randomized block design. The main and interaction
168 effects of treatment and cultivar on dependent variables were examined by a two-way Analysis
169 of Variance (ANOVA). The assumptions of equality of variances (Levene's test) and normality
170 (Q-Q plots) were checked prior to ANOVA, and some variables (tree height, proline and
171 peroxidase in case of peach data) were log-transformed to improve the ANOVA assumptions.
172 The effect size measure omega squared (ω^2) was computed to estimate the variance in the
173 response variable(s) accounted for by the explanatory variables. Tukey's test ($p < 0.05$) was used
174 for mean comparisons (JASP v. 0.15). Data are expressed as mean (n=4) ± standard error.
175 Principal Component Analysis (PCA) (Bartlett's test of sphericity, $p<0.001$) was applied to
176 reduce the dimensionality and to detect the key patterns in data (Jamovi v. 2.2). Pearson's
177 bivariate correlations between the measured traits were computed (Julkowska et al., 2019).

178

RESULTS

Analysis of Variance

The Analysis of Variance (ANOVA) results evinced strong repressive effects of sodicity stress on tree growth and leaf physiological traits in both pear and peach. Although sodicity-induced reductions in tree height (TH), trunk cross sectional area (TCSA) and canopy volume (CV) were highly significant (p <0.001) in both the crops, a perusal of the effect-size measure (ω^2) implied that TCSA was far less sensitive to sodicity stress than were both TH and CV; regardless of the crop (*Table 1*). Likewise, ω^2 values suggested a more adverse effect of sodicity stress on peach than on pear growth. The ω^2 values were low-to-moderate (<0.600) for most of the leaf physiological traits, but quite high (>0.700) for leaf proline, APX and Na^+ in pear. This suggested that explanatory variable (i.e., sodicity stress) accounted for a reasonably high variance in the latter. The pear cultivars differed markedly with other for all the traits except leaf proline, APX and Na^+/K^+ ratio (*Table 1*). The sodicity-triggered increases in leaf MDA and H_2O_2 levels were far greater in peach compared to pear in terms of ω^2 values. This, together with more or less similar values of ω^2 for the leaf proline, implied the greater sensitivity to oxidative stress of peaches compared to pears. Quite the contrary, the values of ω^2 evinced a moderate-to-strong increases in APX, POX and CAT activities in pear than in peach; while upregulation in SOD activity was quite similar in both the cases. This again indicated a better anti-oxidant system to cope with free radicals in pear than in peach (*Table 1*).

Tree growth

There were strong differences between the cultivars for sodicity-induced reductions in tree growth. For instance, the reductions in trunk cross sectional area (TCSA) and canopy

volume (CV) relative to controls were much lower in Punjab Beauty pear (16.49% and 44.50%, respectively) than in Patharnakh (41.89% and 69.28%, respectively) (*Table 2*). Sodicity stressed Partap peach trees displayed much higher reductions in tree height (TH, 36.24%), TCSA (74.28%) and CV (90.33%) than corresponding decreases of 29.23%, 11.94% and 58.58% in cv. Shan-e-Punjab (*Table 2*).

Leaf physiological traits

Sodicity stress caused appreciable reductions in total leaf chlorophyll (TC) in pear (Punjab Beauty- 26.95%, Patharnakh- 21.43%). Leaf malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline levels were invariably higher in sodic than in control treatment. Leaf MDA increased marginally (9.61%) in Punjab Beauty and moderately (19.92%) in Patharnakh (*Table 3*). Both the cultivars showed identical increases (~12.0%) in leaf H_2O_2. Leaf proline accumulation in sodic treatment was considerably higher in Punjab Beauty (33.86%) compared to Patharnakh (20.15%). Partap and Shan-e-Punjab peaches had 30.80 and 18.09% less TC, respectively, in sodic soils than respective controls (*Table 3*).

Leaf anti-oxidant enzymes

Sodicity-triggered increases in APX and CAT activities relative to controls were more pronounced in pear cv. Patharnakh (34.49 and 35.97%, respectively) than in Punjab Beauty (28.44 and 25.16%, respectively) (*Table 4*). Contrarily, POX and SOD activities were 2.6- and 1.4-folds higher, respectively, in sodicity-stressed Punjab Beauty leaves than in Patharnakh (*Table 4*). However, in absolute terms, only POX activity was higher in sodicity-stressed Patharnakh while both CAT and SOD activities much higher in Punjab Beauty. In case of peach, the sodicity-triggered upregulation in leaf APX and POX activities relative to controls were markedly higher in Shan-e-Punjab (29.41 and 22.99%, respectively) than in Partap (13.49 and

6.24%, respectively). Conversely, CAT and SOD activities were more noticeable in Partap (38.82 and 16.19%, respectively) compared to Shan-e-Punjab (8.37 and 14.79%, respectively). Nonetheless, Shan-e-Punjab significantly outperformed Partap for the absolute leaf CAT and SOD levels in the sodic soils (*Table 4*).

Leaf Na^+ and K^+

As expected, sodicity stress caused an increase in leaf Na^+ and a decrease in K^+, regardless of the cultivar. Pears Punjab Beauty and Patharnakh displayed considerably higher leaf Na^+ (43.91 and 74.57%, respectively) in sodic than in control soils. In contrast, leaf K^+ declined significantly in sodicity stressed Punjab Beauty (17.76%) and Patharnakh (28.69%) trees (*Table 5*). In sodic soils, peaches Partap and Shan-e-Punjab had 71.68 and 58.24% more leaf Na^+, respectively, and 31.97 and 24.21% less K^+, respectively (*Table 5*).

Principal component analysis

The PCA was quite efficient in reducing the dimensionality: the first two Principal Components (PCs) (Eigen value >1.0) alone explained 90.95% of the cumulative variance in data in pear (*Table S2*; Figure 1a), and 94.61% in peach (*Table S2*; Figure 1b). In case of pear, leaf Na^+, K^+ and Na^+/K^+ ratio alongside proline, APX and CV were the highly weighted variables on PC1; and TH, TCSA, HP, MDA and CAT on PC2 (*Table S2*). Likewise, for peach, TH, HP, proline and Na^+/K^+ ratio were the best represented variables on PC1, and TCSA, APX, POX, CAT and K^+ on PC2 (*Table S2*). Interestingly, PCA achieved a clear-cut discrimination for the cultivar- and treatment-specific effects in data: while PC1 unambiguously distinguished the control and sodicity treatments, PC2 clearly separated the tested cultivars from each other (Figure 1a, 1b). The PCA also unveiled some interesting patterns in data. For pear, tight

clustering of tree growth attributes, K and TC in the upper left quadrant implied a straightforward role of leaf K^+ in osmotic adjustment in the sodicity-stressed pears (Figure 1a). Likewise, there was an apparent cultivar-specific upregulation in CAT and SOD activities in response to oxidative (HP and MDA) and ionic (Na^+) stresses in Punjab Beauty trees; increased POX activity alone seemed to have alleviated salt stress in Patharnakh (Figure 1a). In case of peach, leaf K^+ did not have any obvious effect in alleviating the salt stress. Contrarily, osmoregulation through proline apparently increased in response to increasing HP, MDA and Na^+ levels. Of the enzymatic anti-oxidants, APX and POX were rather specific to Partap, and CAT and SOD to Shan-e-Punjab (Figure 1b).

Correlation analysis

In pear, growth traits (TH, TCSA and CV) and total leaf chlorophyll (TC) had strong positive correlations with leaf K^+ (r >0.800, p= 0.000). Expectedly, leaf Na^+/K^+ ratio had moderate-to-strong negative correlations with TH (r= -0.623, p= 0.010), TC (r= -0.602, p= 0.014) and CV (r= -0.788 p= 0.000) (*Table S3*, Figure 2a). A strong positive correlation between leaf MDA and H_2O_2 (r= 0.914, p= 0.000) reflected their synergistic adverse effects on pear tree growth and physiology. Leaf proline had moderate positive correlations with MDA (r= 0.578, p= 0.019) and HP (r= 0.519, p= 0.039). Similarly, strong positive relationships (r >0.800, p= 0.000) were found between antioxidants (CAT and SOD) and oxidative stress indicators (MDA and HP) (*Table S3*, Figure 2a). In peach, leaf K^+ had strong positive correlations with TC (r=0.887, p= 0.000), APX (r= 0.732, p= 0.001) and POX (r= 0.791, p= 0.000), strong negative correlations with CAT (r= -0.987, p= 0.000) and SOD (r= -0.839, p= 0.000), and a moderate negative correlation with HP (r= -0.655, p= 0.006) (*Table S4*, Figure 2b). In contrast to pear, elevated leaf Na^+ had strong inhibitory effects(r >-0.880, p= 0.000) on tree growth in peach. The marked

adverse effects of MDA and HP on peach tree growth were also apparent (r> -0.800 (*Table S4*, Figure 2b).

DISCUSSION

Sodicity stress caused by high soil pH and excessive Na^+ accumulation (Sharma et al., 2016) is a serious constraint on pear and peach productivity (Elkins et al., 2012; Mestre et al., 2015). The observation that sodic soil conditions impair pear and peach tree growth (*Thind et al., 2020*) is anecdotal in the sense that key physiological traits underpinning sodicity tolerance remain elusive. In this backdrop, our study aimed to unveil the effects of sodicity stress on tree growth and physiological attributes in commercially important pear cultivars Punjab Beauty and Patharnakh, and peach cultivars Partap and Shan-e-Punjab.

We found that peach cultivars were more adversely affected than pear cultivars. Moreover, sodicity stress had a far greater growth suppressing effect on Patharnakh pear and Partap peach than their counterparts. The experimental soils used in this study, though previously reclaimed using gypsum, still have pH in the sodic range (>8.5), particularly below 30 cm depth. Additionally, a clayey soil texture limits the water flux and nutrient availability; further hampering the plant growth (Kumar et al., 2019). This may partly explain the differential responses to sodicity stress by the crops and cultivar tested by us (Samra et al., 1988). Excess salts suppress leaf and stem growth, and biomass production in pears (Okubo et al., 2000; Matsumoto et al., 2006) and peaches (Massai et al., 1997, 2004); albeit in a genotype-specific manner such that some cultivars are more adversely affected than others (Krishnamoorthy, 2009). The depletion of leaf chlorophyll may be caused by the lime-induced chlorosis when pears (Ma et al., 2005) and peaches (Thomidis & Tsipouridis, 2005) are grown in high pH

calcareous soils. Interestingly, the sodic soils of the Trans-Gangetic Plains (study area) are often deficient in plant available Fe (Kaledhonkar et al., 2019), a key element in chlorophyll synthesis (Ma et al., 2005). Furthermore, high soil pH may inhibit the uptake of metal ions (Mg^{2+} and Fe^{2+}) needed for chlorophyll synthesis (Jia et al., 2019). The presence of bicarbonate ($NaHCO_3$, a major salt in the study area soils; Mandal, 2012) is known to suppress Fe availability to the peach (Molassiotis et al., 2005) and pear (Valipour et al., 2020) plants; and this may, in turn, hamper the leaf chlorophyll formation (Molassiotis et al., 2005). The apoplastic pH increases in the presence of bicarbonates which eventually limits the Fe transport to the root stele and restricts the Fe uptake (Molassiotis et al., 2005).

Sodicity stress triggered a significant increase in leaf MDA and H_2O_2 accumulation, the indicators of oxidative injury and cell membrane damage (Sorkheh et al., 2012; Shen et al., 2021), regardless of the crop and cultivar tested. Nonetheless, sodicity-induced increases in leaf MDA relative to controls were far greater in peach (~37.0-42.0%) than in pear (9.61-19.91%) as were the increases in leaf H_2O_2 (~22.0-27.0% and ~12.0%, respectively), suggesting that peaches in general were more adversely affected by the oxidative injury (Shahvali et al., 2020). Although pears and peaches have not been comparatively evaluated for salt-induced increases in leaf MDA and H_2O_2, studies have shown relatively higher sensitivity of *Prunus* spp. including peach (*Prunus persica*) to the salt-induced lipid peroxidation and oxidative injury (Erturk et al., 2007; Shen et al., 2021; Toro et al., 2021). Salt-induced osmotic and oxidative stresses more adversely affect osmotic-sensitive than osmotic-tolerant genotypes; the latter show relatively efficient reactive oxygen species (ROS) scavenging and better cell membrane stability when exposed to these stresses (Rajabpoor et al., 2014). High sensitivity of *Prunus* spp. to these stresses is also

evidenced by the inefficacy of some interventions (e.g., fungal symbiosis) otherwise known to alleviate salt-induced oxidative stress to a considerable extent (Shahvali et al., 2020).

Although direct evidence is lacking, insights from a related species (birch-leaved pear, *Pyrus betulaefolia*) suggest that salt-triggered increases in leaf MDA may not be deleterious enough to cause lipid peroxidation in pears (Wu & Zou, 2009). The overexpression of genes '*Pp14-3-3*' (from *P. pyrifolia*) and '*PbrNHX2*' (from *P. betulaefolia*) dramatically improved salt tolerance in transgenic tobacco and *P. ussuriensis* by upregulating the activity of antioxidant enzymes (Li et al., 2014; Dong et al., 2019). Specifically, increased APX activity may account for a better redox homeostasis in pear; '*14-3-3*' proteins interact with APX for scavenging the reactive oxygen species implicated in the oxidative damage (Li et al., 2014). This is in agreement with our results as sodicity-induced increases in APX activity were noticeably higher in pears (28.44-34.49%) than in peaches (13.49-29.41%). The cultivar differences for both MDA and H_2O_2 were highly significant ($p< 0.001$) for pear (Wu & Zou, 2009), and significant ($p< 0.05$) for peach (Toro et al., 2021); implying quite distinct and somewhat shared responses of pear and peach cultivars, respectively, to sodicity stress in terms of lipid peroxidation and cellular damage. Genotypic differences for these oxidative stress markers under salt and drought stresses are either pronounced or subtle in different *Pyrus* and *Prunus* species (Sorkheh et al., 2012; Rajabpoor et al., 2014; Zarafshar et al., 2014; Li et al., 2015; Toro et al., 2021).

Salt-stressed plants accumulate compatible osmolytes like proline that, in addition to osmoregulation, also minimize cell membrane damage by scavenging the ROS (Dejampour et al., 2011; Rahneshan et al., 2018; Yousefi et al., 2019). We found that the tested cultivars did not differ significantly for leaf proline in both control and sodic treatments (Wen et al., 2011). Furthermore, the increase in leaf proline in response to MDA and H_2O_2 accumulation was

moderate in pear (r= 0.578 and 0.519, respectively; Fig. 2a) but quite strong in peach (r= 0.870 and 0.930, respectively; Fig. 2b). Leaf proline levels may not always be sufficient enough to contribute to osmotic adjustment and antioxidant activity, as shown previously in different pear species (Larher et al., 2009; Wen et al., 2011) and other fruit crops (e.g., Singh et al., 2022). Despite being a typical compatible solute, proline may not essentially lessen the osmotic potential of pear leaves (Larher et al., 2009), and other organic osmolytes (e.g., glycine betaine) may be potentially involved in osmotic adjustment. The fact that MDA and H_2O_2 levels may not necessarily be toxic enough to induce an increase in proline activity (Wen et al., 2011) also supports our finding as leaf MDA and H_2O_2 were two-three folds higher in peach than in pear (Table 2). Plausibly, a lower than expected increase in proline activity (Regni et al., 2019) might reflect higher sodicity tolerance in pear than in peach (Ebert, 1999; Musacchi et al., 2006); increased leaf proline levels often reflect sensitivity rather than tolerance to the excess salt (Mademba-Sy et al., 2003).

The tested cultivars, regardless of the crop, displayed increased activities of leaf antioxidant enzymes (APX, CAT, POX and SOD) in response to sodicity stress. Enzymatic anti-oxidants efficiently protect salt-stressed plants from ROS (e.g., H_2O_2) induced oxidative stress, and are considered reliable markers for discriminating the salt-tolerant and salt-sensitive genotypes (Sorkheh et al., 2012; Yousefi et al., 2019; Aazami et al., 2021). The cultivar differences for anti-oxidant activities observed by us can be explained by the complex nature of anti-oxidant expression in plant cells (Racchi, 2013), cell organelle-specific activities of anti-oxidant enzymes (Niu & Liao, 2016), and the genotypic differences (Regni et al., 2019). The *P. pashia* rootstock was found to better protect the Flemish Beauty scions than clonal (Quince A and C) rootstocks against oxidative damage via enhanced CAT, POX and SOD activities

(Sharma & Sharma, 2008). Similarly, peach seedling and clonal rootstocks differed considerably for leaf antioxidant levels in the presence of $NaHCO_3$ (Molassiotis et al., 2005). The SOD constitutes the first line of defense in alleviating the ROS-triggered oxidative stress in plants; it dismutases the superoxide anion (O_2^-) to produce molecular oxygen (O_2) and H_2O_2. The CAT then decomposes H_2O_2 into O_2 and H_2O (Cavalcanti et al., 2004). Obviously, a balance between SOD and CAT activities, and not their relative levels *per se*, would be crucial to maintaining O_2^- and H_2O_2 levels in a steady-state (Azarabadi et al., 2017). The CAT and SOD activities were not only much higher (Table 3) but also had a clear synergistic effect (Fig. 1a,b) in the sodicity-stressed Punjab Beauty pear and Shan-e-Punjab peach; enabling them to better adapt to sodicity than other cultivars (Sorkheh et al., 2012). The decreased activity of CAT often comes at the expense of greater oxidative damage-characterized, for instance, by the increased accumulation of H_2O_2 (Molassiotis et al., 2005).

Sodicity-stressed pear and peach trees had significantly higher leaf Na^+ and lower K^+ than respective controls. In the sodic treatment, pear Punjab Beauty showed considerably lower increase in leaf Na^+ than Patharnakh; helping it maintain a higher leaf K^+. A more or less similar trend was also seen in peach. Restricted translocation of Na^+ to aerial plant parts (Matsumoto et al., 2006), achieved for example by Na^+ exclusion by the roots in common pears (Musacchi et al., 2006) prevents xylem loading and translocation of Na^+ to the leaves. Differential accumulation of leaf Na^+ and K^+ in response to salt stress has also been observed in both own-rooted and grafted peaches (Massai et al., 1997, 2004) and interspecific *Prunus* hybrids (Dejampour et al., 2011), with low Na^+ accumulators showing better salt tolerance (Massai et al., 1997). Reduced accumulation of leaf Na^+, achieved either by root exclusion (Musacchi et al.,

2006) or partitioning into basal leaves (Massai et al., 2004) together with maintenance of adequate leaf K^+ (Massai et al., 2004) improves the salt tolerance.

In this study, the PCA was highly efficient in reducing the dimensionality, and in differentiating cultivar- and sodicity-specific effects in data. Specifically, PCA delineated the putative traits linked to sodicity stress tolerance in the pear and peach cultivars. Previously, PCA has been used to unveiling key responses to salt in other fruit crops (Sorkheh et al., 2012; Abid et al., 2020). Multivariate techniques such as PCA are usually more suitable for detecting the key patterns in data having complex (multicollinear) variables (Julkowska et al., 2019). Additionally, graphical visualization of PCA loadings provides an easier and intuitive means to understanding the shared and contrasting physiological responses to salt stress (Sorkheh et al., 2012; Singh et al., 2022). Based on correlation analysis, MDA, H_2O_2 and leaf Na^+ were found to have a greater repressive effect on tree growth in peaches than in pears. Furthermore, a strong correlation between leaf K^+ and growth traits and leaf chlorophyll in pear, but not in peach, was indicative of leaf K^+ mediated osmotic adjustment in pears.

CONCLUSIONS

Although sodicity stress suppressed tree growth regardless of the cultivar, strong genotypic differences were quite apparent: Punjab Beauty pear and Shan-e-Punjab peach exhibited better tolerance to sodicity stress. We found that sodicity-triggered increases in leaf malondialdehyde, hydrogen peroxide and Na^+ had a greater repressive effect on tree growth in peaches than in pears, and induced proline-mediated osmotic adjustment in the former. The higher activities of catalase and superoxide dismutase enzymes coupled with restricted Na^+

uptake and the maintenance of adequate leaf K^+ are the plausible explanations for overall better sodicity tolerance in pear.

ABBREVIATIONS

ANOVA: Analysis of Variance; APX: Ascorbate peroxidase; CAT: Catalase; CV: Canopy volume; DW: Dry weight basis; EC_e: Soil saturation extract electrical conductivity; H_2O_2: hydrogen peroxide; MDA: Malondialdehyde; me L^{-1}: Milli equivalent per liter; PCA: Principal Component Analysis; PCs: Principal Components; POX: Peroxidase; pH_s: Soil saturated paste pH; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TCSA: Trunk cross sectional area; TH: Tree height

Declaration of Competing Interest: The authors declare that they have no competing interests.

Author contributions: Conceived and designed experiments- AMS; performed the experiments- AMS and AK, analyzed the data- AMS; prepared the figures and tables- AMS, AK, RK; drafted the manuscript and revised it critically for important content- all authors.

Acknowledgments: We thank Rashtriya Krishi Vikas Yojana, Govt. of Haryana, India for financial support. Head, Department of Fruit Science, Punjab Agricultural University, Ludhiana, India is thanked for providing the pear and peach plants. Mr. Dheeraj Kumar is appreciated for his technical help.

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Figure 1

Figure 1a Principal Component Analysis biplot showing variable loadings and cultivar-treatment groups on first two principal components in pear.

Lines radiating from the centre reflect relative contribution and directionality. PBC- Punjab Beauty control, PBS- Punjab Beauty sodic, PathC- Patharnakh control, PathS- Patharnakh sodic, Abbreviations: TH- tree height, TCSA- trunk cross sectional area, CV- canopy volume, TC- total leaf chlorophyll, MDA- malondialdehyde,HP- hydrogen peroxide (H_2O_2), APX- ascorbate peroxidase, POX- peroxidase, CAT- catalase, SOD- superoxide dismutase, Na- leaf Na^+, K- leaf K^+, Na.K- leaf Na^+/K^+ ratio.

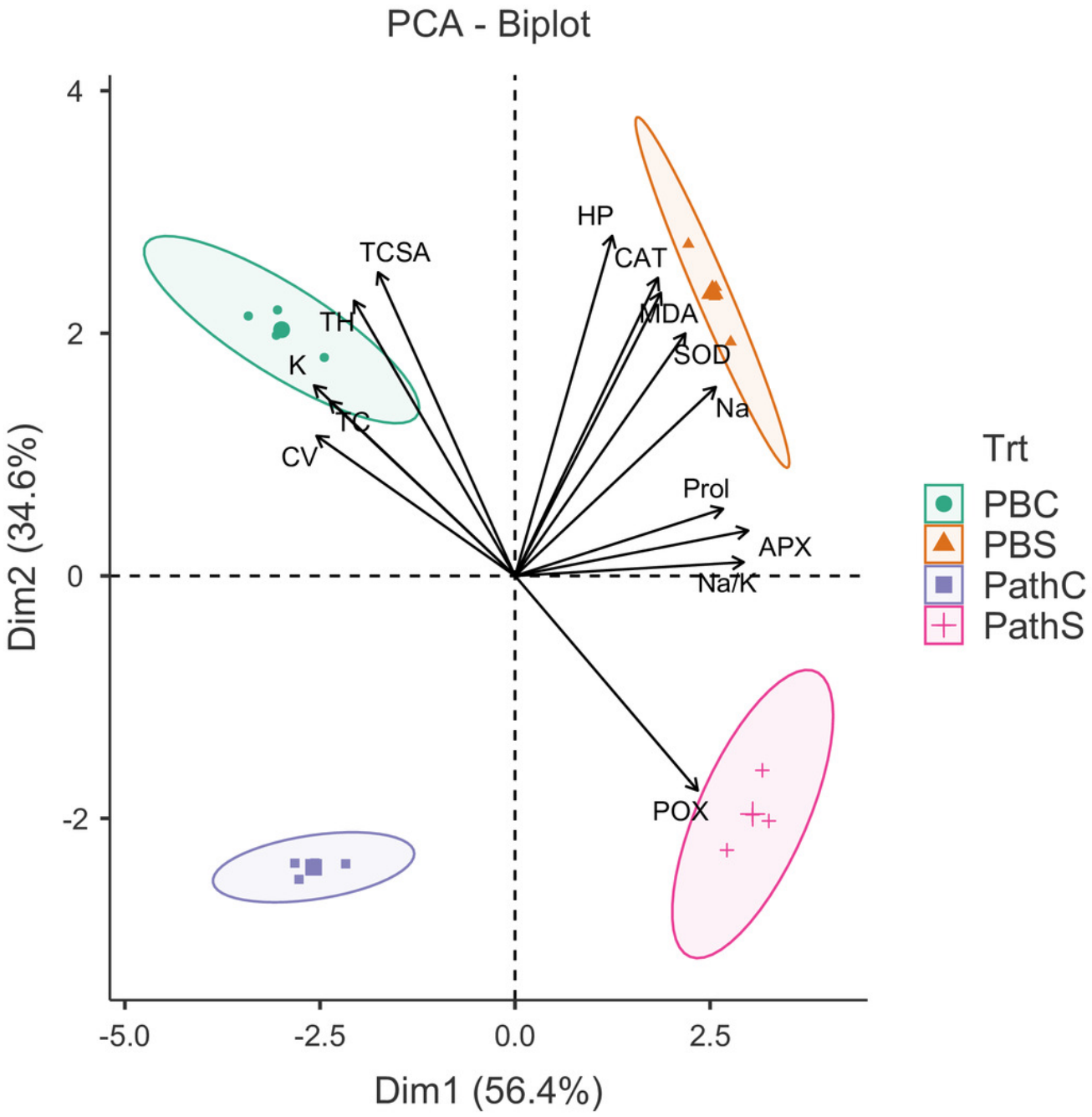

PCA - Biplot
Dim2 (34.6%)
Dim1 (56.4%)
4
2
0
-2
-5.0
-2.5
0.0
2.5
TCSA
TH
K
TC
CV
HP
CAT
MDA
SOD
Na
Prol
APX
Na/K
POX
Trt
PBC
PBS
PathC
PathS

Figure 2

Figure 1b Principal Component Analysis biplot showing variable loadings and cultivar-treatment groups on first two principal components in peach.

Lines radiating from the centre reflect relative contribution and directionality. PtpC- Partap control, PtpS- Partap sodic, SPC- Shan-e-Punjab control, SPS- Shan-e-Punjab sodic, Abbreviations: TH- tree height, TCSA- trunk cross sectional area, CV- canopy volume, TC- total leaf chlorophyll, MDA- malondialdehyde,HP- hydrogen peroxide (H_2O_2), APX- ascorbate peroxidase, POX- peroxidase, CAT- catalase, SOD- superoxide dismutase, Na- leaf Na^+, K- leaf K^+, Na.K- leaf Na^+/K^+ ratio.

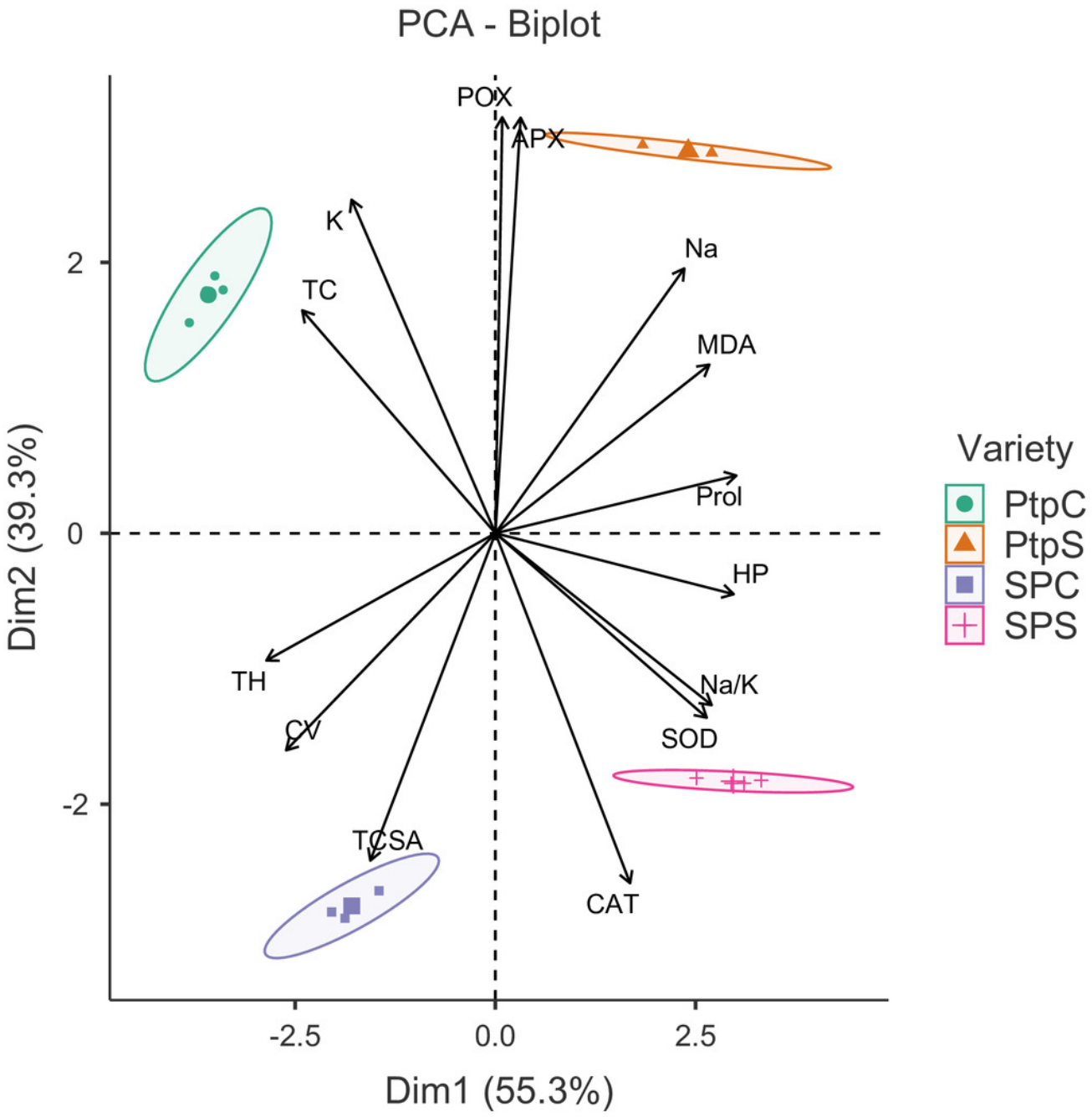

PCA - Biplot
POX
APX
K
Na
TC
MDA
Prol
HP
Na/K
SOD
TH
CV
TCSA
CAT
Dim2 (39.3%)
Dim1 (55.3%)
2
0
-2
-2.5
0.0
2.5
Variety
PtpC
PtpS
SPC
SPS

Figure 3

Figure 2a Correlation plot showing Pearson’s bivariate correlations between the measured traits in pear.

Ellipse size and color reflect the strength and direction (positive or negative) of the correlation. Individual cells marked with cross (X) denote non-significant correlations. For abbreviations, see Figure 1.

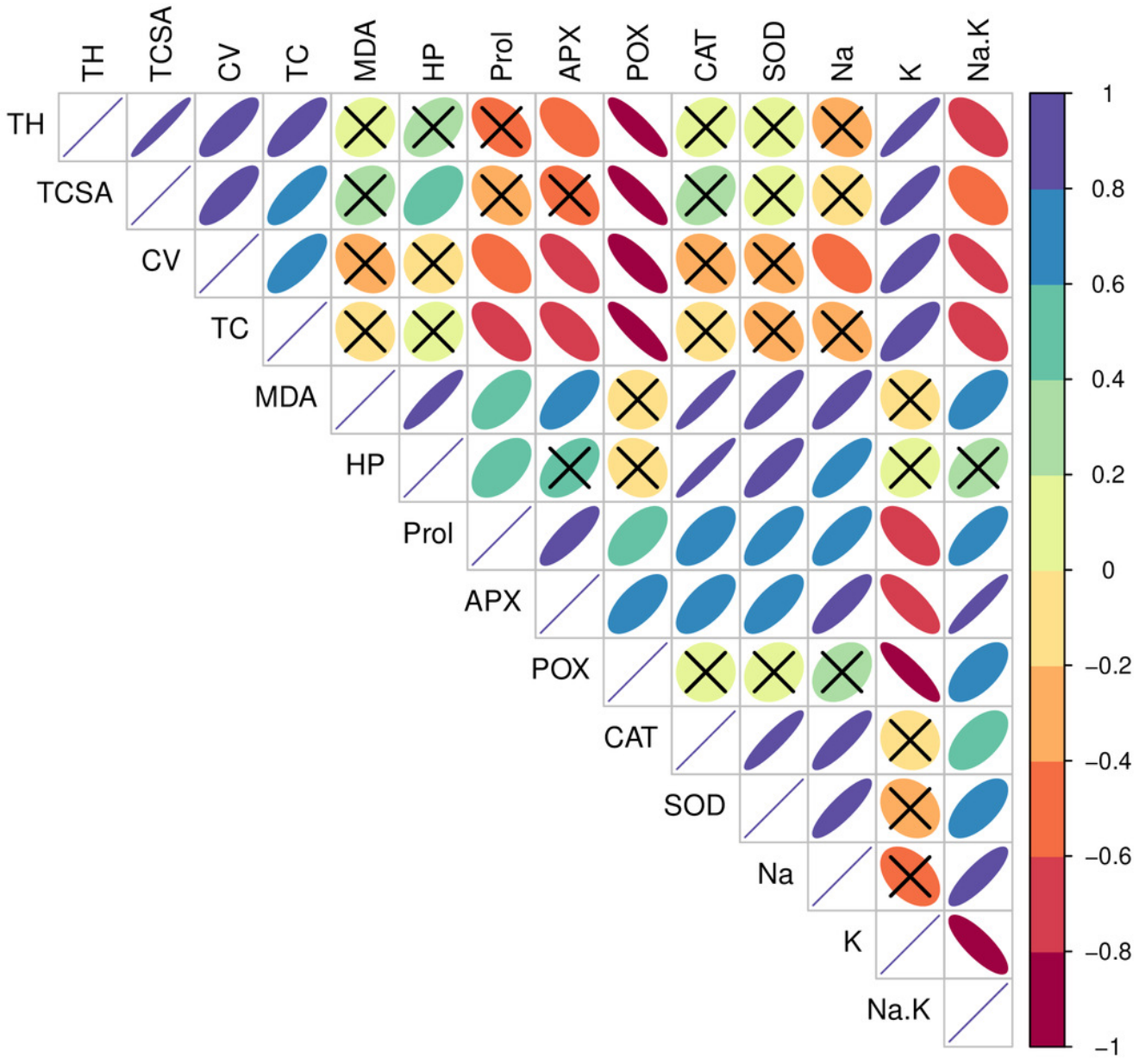
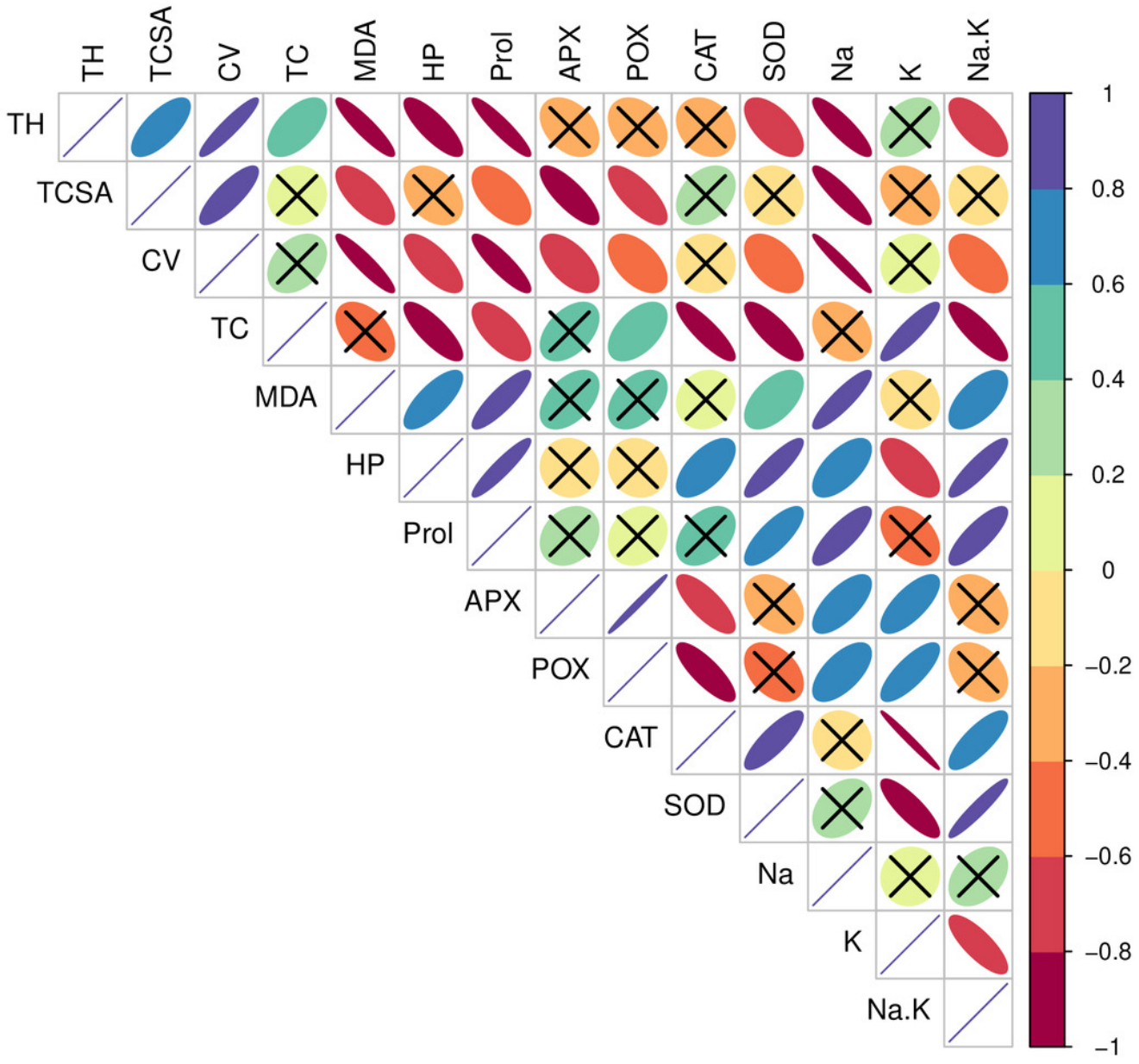

TH
TCSA
CV
TC
MDA
HP
Prol
APX
POX
CAT
SOD
Na
K
Na.K
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

Figure 4

Figure 2b. Correlation plot showing Pearson’s bivariate correlations between the measured traits in peach.

Ellipse size and color reflect the strength and direction (positive or negative) of the correlation. Individual cells marked with cross (X) denote non-significant correlations. For abbreviations, see Figure 1.


TH
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−0.8
−1

Table 1(on next page)

Table 1 Analysis of Variance (ANOVA) for different traits in pear and peach.

Table 1 Analysis of Variance (ANOVA) for different traits in pear and peach.

Trait	Source	F	p	ω^2	F	p	ω^2
		Pear			Peach		
Tree height (m)	Treatment (T)	83.55	<0.001	0.354	218.76	<0.001	0.902
	Cultivar (C)	134.00	<0.001	0.571	5.95	0.031	0.021
	T x C	2.35	0.151*	0.006	3.62	0.081*	0.011
Trunk cross sectional area (cm2)	Treatment (T)	50.98	<0.001	0.203	468.82	<0.001	0.379
	Cultivar (C)	179.72	<0.001	0.724	533.37	<0.001	0.431
	T x C	2.98	0.110*	0.008	219.17	<0.001	0.177
Canopy volume (m3)	Treatment (T)	38.93	<0.001	0.606	411.53	<0.001	0.846
	Cultivar (C)	10.20	0.008	0.147	53.81	<0.001	0.109
	T x C	0.50	0.495*	0.000	7.15	0.020	0.013
Total leaf chlrophyll (mg/g FW)	Treatment (T)	36.52	<0.001	0.509	46.78	<0.001	0.366
	Cultivar (C)	18.39	0.001	0.249	55.54	<0.001	0.436
	T x C	1.86	0.197*	0.012	9.69	0.009	0.070
Malondialdehyde (nmoles/g FW)	Treatment (T)	30.65	<0.001	0.376	81.82	<0.001	0.818
	Cultivar (C)	32.93	<0.001	0.405	3.96	0.070	0.030
	T x C	2.20	0.164*	0.015	0.03	0.861*	0.000
Hydrogen peroxide (mmoles/g FW)	Treatment (T)	84.27	<0.001	0.219	101.26	<0.001	0.772
	Cultivar (C)	281.57	<0.001	0.739	15.27	0.002	0.110
	T x C	0.95	0.349*	0.000	0.32	0.580*	0.000
Proline (mg/g FW)	Treatment (T)	40.49	<0.001	0.703	85.00	<0.001	0.853
	Cultivar (C)	0.42	0.530*	0.000	0.44	0.518*	0.000
	T x C	2.28	0.157*	0.023	0.06	0.814*	0.000
Ascorbate peroxidase (units/g FW)	Treatment (T)	147.59	<0.001	0.906	28.11	<0.001	0.092
	Cultivar (C)	0.42	0.527*	0.000	252.51	<0.001	0.856
	T x C	0.85	0.374*	0.000	0.21	0.652*	0.000
Peroxidase (units/g FW)	Treatment (T)	167.45	<0.001	0.500	130.39	<0.001	0.072
	Cultivar (C)	129.46	<0.001	0.386	1618.96	<0.001	0.898
	T x C	23.24	<0.001	0.067	39.18	<0.001	0.021
Catalase (units/g FW)	Treatment (T)	161.13	<0.001	0.421	197.25	<0.001	0.116
	Cultivar (C)	205.79	<0.001	0.539	1456.33	<0.001	0.860
	T x C	1.040e -4	0.992*	0.000	24.82	<0.001	0.014
Superoxde dismutase (units/g FW)	Treatment (T)	88.32	<0.001	0.542	107.21	<0.001	0.554
	Cultivar (C)	55.54	<0.001	0.338	71.48	<0.001	0.368
	T x C	4.35	0.059*	0.021	0.01	0.911*	0.000
Leaf Na^+ (mg/g DW)	Treatment (T)	159.87	<0.001	0.745	423.82	<0.001	0.730
	Cultivar (C)	38.69	<0.001	0.177	121.42	<0.001	0.208
	T x C	1.80	0.205*	0.004	21.05	<0.001	0.035
Leaf K^+ (mg/g DW)	Treatment (T)	109.07	<0.001	0.590	252.43	<0.001	0.144
	Cultivar (C)	58.23	<0.001	0.312	1407.86	<0.001	0.806
	T x C	2.96	0.111*	0.011	71.96	<0.001	0.041
Leaf Na^+/K^+ ratio	Treatment (T)	201.17	<0.001	0.871	651.86	<0.001	0.620
	Cultivar (C)	0.38	0.549*	0.000	360.43	<0.001	0.342
	T x C	15.25	0.002	0.062	25.19	<0.001	0.023

*Non-significant effect ($p > 0.05$).

Table 2(on next page)

Table 2 Mean comparisons for tree growth parameters in pear and peach cultivars.

Control and sodic treatments denote significantly different ($p < 0.001$) pH_s levels of ~ 8.2 and 8.8, respectively. Each data value represents mean (n=4) ± SD.

Table 2 Mean comparisons for tree growth parameters in pear and peach cultivars.

Cultivar	Treatment	Tree height (m)	Trunk cross sectional area (cm^2)	Canopy volume (m^3)
Pear				
Punjab Beauty	Control	3.86±0.20a	36.76±2.63a	1.91±0.21a
	Sodic	3.23±0.12b	30.70±2.64b	1.06±0.19bc
Patharnakh	Control	3.03±0.20b	23.68±1.87c	1.53±0.53ab
	Sodic	2.14±0.13c	13.76±1.62d	0.47±0.08d
Peach				
Partap	Control	4.25±0.31a	231.17±9.61b	28.96±2.90a
	Sodic	2.71±0.11b	59.45±2.62c	2.80±0.18b
Shan-e-Punjab	Control	4.31±0.14a	270.22±12.0a	34.28±3.09a
	Sodic	3.05±0.18b	237.96±10.57b	14.20±1.68b

Control and sodic treatments denote significantly different ($p < 0.001$) pH_s levels of ~ 8.2 and 8.8, respectively. Each data value represents mean (n=4) ± SD.

Table 3(on next page)

Table 3 Mean comparisons for leaf physiological traits in pear and peach cultivars.

Control and sodic treatments denote significantly different ($p < 0.001$) pH_s levels of ~ 8.2 and 8.8, respectively. Each data value represents mean (n=4) ± SD. TC- total leaf chlorophyll, MDA- malondialdehyde, H_2O_2- hydrogen peroxide.

Table 3 Mean comparisons for leaf physiological traits in pear and peach cultivars.

Cultivar	Treatment	TC (mg g^{-1} FW)	MDA (nmoles g^{-1} FW)	H_2O_2 (mmoles g^{-1} FW)	Proline (mg g^{-1} FW)
Pear					
Punjab Beauty	Control	1.41±0.10a	8.95±0.25b	130.41±1.99b	3.78±0.43b
	Sodic	1.03±0.10bc	9.81±0.35a	146.27±3.23a	5.06±0.25a
Patharnakh	Control	1.12±0.05a	7.43±0.22c	105.74±4.32d	3.92±0.28b
	Sodic	0.88±0.14c	8.91±0.69b	118.55±2.43c	4.71±0.31a
Peach					
Partap	Control	1.56±0.11a	7.74±0.36b	138.12±4.28c	4.06±0.11b
	Sodic	1.08±0.15b	10.58±0.94a	175.57±8.77a	5.31±0.18a
Shan-e-Punjab	Control	1.05±0.05bc	7.04±0.11b	153.89±9.19b	4.16±0.19b
	Sodic	0.86±0.05c	10.0±0.78a	187.33±4.32a	5.39±0.50a

Control and sodic treatments denote significantly different ($p < 0.001$) pH_s levels of ~ 8.2 and 8.8, respectively. Each data value represents mean (n=4) ± SD. TC- total leaf chlorophyll, MDA- malondialdehyde, H_2O_2- hydrogen peroxide.

Table 4(on next page)

Table 4 Mean comparisons for leaf anti-oxidant enzymes (units g^{-1} FW) in pear and peach cultivars.

Control and sodic treatments denote significantly different ($p < 0.001$) pH_s levels of ~ 8.2 and 8.8, respectively. Each data value represents mean (n=4) ± SD.

Table 4 Mean comparisons for leaf anti-oxidant enzymes (units g^{-1} FW) in pear and peach cultivars.

Cultivar	Treatment	Ascorbate peroxidase	Peroxidase	Catalase	Superoxide dismutase
Pear					
Punjab Beauty	Control	14.10±0.53b	183.68±5.80c	6.20±0.31b	64.09±1.88b
	Sodic	18.11±0.75a	224.07±4.92b	7.76±0.24a	80.62±4.40a
Patharnakh	Control	13.54±0.68b	220.47±3.47b	4.42±0.31c	56.23±1.29c
	Sodic	18.21±0.86a	238.84±3.74a	6.01±0.25b	66.84±2.08b
Peach					
Partap	Control	22.15±1.38b	307.41±5.85b	3.93±0.17d	46.88±1.31c
	Sodic	25.14±1.46a	326.60±5.42a	5.22±0.43c	54.47±1.55b
Shan-e-Punjab	Control	12.07±1.01d	178.22±6.93d	6.93±0.37b	53.00±1.57b
	Sodic	15.62±1.01c	219.20±5.92c	7.51±0.41a	60.84±1.54a

Control and sodic treatments denote significantly different ($p < 0.001$) pH_s levels of ~ 8.2 and 8.8, respectively. Each data value represents mean (n=4) ± SD.

Table 5(on next page)

Table 5 Mean comparisons for leaf Na^+ and K^+ and Na^+: K^+ ratio in pear and peach cultivars.

Control and sodic treatments denote significantly different ($p < 0.001$) pH_s levels of ~ 8.2 and 8.8, respectively. Each data value represents mean (n=4) ± SD.

Table 5 Mean comparisons for leaf Na^+ and K^+ and Na^+: K^+ ratio in pear and peach cultivars.

Cultivar	Treatment	Na^+ (mg/g DW)	K^+ (mg/g DW)	Na^+: K^+ ratio
Pear				
Punjab Beauty	Control	0.82±0.07c	9.74±0.52a	0.08±0.01b
	Sodic	1.18±0.09a	8.01±0.31b	0.15±0.01a
Patharnakh	Control	0.59±0.05d	8.54±0.63b	0.07±0.01b
	Sodic	1.03±0.07b	6.09±0.45c	0.17±0.02a
Peach				
Partap	Control	1.13±0.10c	14.42±0.59a	0.08±0.01d
	Sodic	1.94±0.10a	9.81±0.25b	0.20±0.01b
Shan-e-Punjab	Control	0.91±0.04d	5.70±0.37c	0.16±0.01c
	Sodic	1.44±0.09b	4.32±0.26d	0.33±0.02a

Control and sodic treatments denote significantly different ($p < 0.001$) pH_s levels of ~ 8.2 and 8.8, respectively. Each data value represents mean (n=4) ± SD.