biologia plantarum
an international journal for experimental botany

BIOLOGIA PLANTARUM (2025) 69: 106-117 DOI: 10.32615/bp.2025.011

Comparative alterations in root cell wall constituents and cation-exchange capacity of two tomato cultivars under salinity induced by NaCl and $CaCl_2$

Victoria Oko OTIE[1,2], Anthony Egrinya ENEJI[1], Idorenyin Asukwo UDO[3],
Michael Okoi ITAM[4], Isong Abraham ISONG[1], Xiaohui FENG[5], Haruyuki FUJIMAKI[2],
and Ping AN[2,*]

[1] Department of Soil Science, Faculty of Agriculture, University of Calabar, P.M.B. 1115, Calabar, Nigeria
[2] Arid Land Research Centre, Tottori University, Hamasaka, Tottori 680-0001, Japan
[3] Department of Crop Science, Faculty of Agriculture, University of Calabar, P.M.B. 1115, Calabar, Nigeria
[4] Department of Plant and Environmental Sciences, Clemson University, Clemson, SC 29634, USA
[5] Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, 268 Huaizhong Road, Shijiazhuang, Hebei Province, 050023, P.R. China

[*]Corresponding author: E-mail: an.ping@tottori-u.ac.jp

Abstract

Background: Tomato plants exposed to salinity stress may experience dynamic changes in root growth and cell wall (CW) composition and structure.
Aims: Here, we determined the CW composition and cation-exchange capacity (CEC) of two tomato cultivars (*Daniela*, salt-tolerant and *Naomi*, salt-sensitive) as well as their growth and root characteristics.
Methods: Seedlings of the tomato cultivars were exposed to six NaCl plus $CaCl_2$ concentrations hydroponically, root growth and CW chemical composition were measured.
Results: The root growth of *Naomi* was adversely ($P \leq 0.05$) reduced at the elongation zone, but there was little change in the chemical composition of the CW under salinity. A marked reduction occurred in the CW-constituting polysaccharides of *Naomi* relative to *Daniela*, whether at the 8.00 dS m^{-1} NaCl treatment or its combination with $CaCl_2$. For both root zones, CW viscosity was better enhanced under NaCl and $CaCl_2$ combinations, but the contents of uronic acid across the CW constituents increased under sole treatment with $CaCl_2$ at the mature root zone of *Naomi*. The root CW CEC increased ($P \leq 0.05$) with increases in the ionic concentration of the external solution. Salt concentrations at 8.0 dS m^{-1} NaCl or 8.0 dS m^{-1} NaCl + $CaCl_2$ increased ($P \leq 0.05$) the CEC of the CW, especially for *Daniela*.
Conclusions: The overall results showed that $CaCl_2$ could enhance some tolerance in CW polysaccharides of tomato under salinity stress. The salt-tolerant *Daniela* with higher CW and ionic contents had superior stability in cell structure under salt stress.

Keywords: *Lycopersicon esculentum* L., salinity stress, salt tolerance, uronic acid.

Received 31 May 2025, *last revision* 11 September 2025, *accepted* 24 November 2025.
Abbreviations: AGDW - above ground dry weight; CEC - cation-exchange capacity; CDTA - cyclohexylenedinitrilotetraacetic; CW - cell wall; CWI - cell wall integrity; Hem - hemicellulose; Hem.1 - hemicellulose 1; Hem.2 - hemicellulose 2; PC1 - principal component 1; PC2 - principal component 2; PCA - principal component analysis; RDW - root dry weight; ROS - reactive oxygen species; UA - uronic acid; UA_Cellulose - uronic acid in cellulose; UA_Hem.1 - uronic acid in hemicellulose 1; UA_Hem.2 - uronic acid in hemicellulose 2; UA_Pectin - uronic acid in pectin.
Acknowledgements: We thank the Arid Land Research Center (ALRC) of Tottori University, Japan for partially funding this study through the corresponding author.
Data availability statement: Data generated or analyzed during this study are available from the corresponding author upon reasonable request.
Conflict of interest: The authors declare that they have no conflict of interest.

Introduction

Under high salinity, nutritional disorder and oxidative stress occur in plants as roots can no longer uptake water from the soil due to toxic concentrations of sodium ions (Na^+) as in the eukaryotic cells (Annunziata et al., 2017). Tomato (*Lycopersicon esculentum* L.) is an important vegetable, and moderately tolerant to salinity (Raza et al., 2017). Salt stress induces accumulation of reactive oxygen species (ROS) and retards root growth through the absorption of Na^+ (Tanveer et al., 2019). Although tomato is classified as a moderately sensitive crop and can tolerate electrical conductivity of the saturated soil extract up to 4.0 dS m^{-1} without a significant reduction in yield (Munns and Tester, 2008), any increase of 1 dS m^{-1} above its threshold value may compromise yield (Reina-Sánchez et al., 2005).

Diverse changes occur in the chemical composition of root cell walls (CWs) in response to salt stress. Cellulose is the most important component of the primary cell wall that forms cell skeletal structure. Hemicellulose (HeM) does cross-link with cellulose and the degree of desorption affects cell elongation, whereas pectin is found in the gap between cellulose and Hem and is involved in the porosity of the CW (Byrt et al., 2018). The addition of exogenous $CaCl_2$ to saline medium has been reported to enhance cell membrane integrity where the calcium ions bind to phospholipid molecules on cell membrane thus reducing specific ion toxicity (Guo et al., 2021). Recently, Liu et al. (2025) observed that exogenous $CaCl_2$ significantly alleviated oxidative damage of cell membranes by activating the production of oxidative enzymes, increased oxidoreductase and hydrolase activities in lignification, induced transduction of H_2O_2, ethylene, abscisic acid as well as up-regulated chlorophyll-binding proteins culminating to NaCl stress tolerance by alfalfa plants. Salinity effect on root cell wall and its ion-exchange capacity has been studied previously (Meychik et al., 2021). Cellulose and hemicelluloses do not contain any ion-exchange groups that are ionized at physiological pH. However, pectin within root CWs is the major binding site due to its high ion-exchange capacity (Loix et al., 2017). They are distinguished by their high cation-exchange capacity (CEC) due mainly to high (up to 70% by weight) content of (poly)galacturonic acid (Albersheim et al., 2010). A previous study reported that the higher the CEC value, the higher the plant's tolerance of salt conditions (Volkov, 2015). In soybean (*Glycine max*), salt treatment significantly decreased the percentage of pectin in salt-sensitive cultivar (An et al., 2014). This was indicative that the higher the abundance of pectin and cation-binding capacity in a salt-tolerant cultivar, the greater the Na^+ adsorption by the root CWs, and the lower the amount of Na^+ in the aboveground partitions (Zhao et al., 2019). Much less is known about the chemical nature, including the structural alterations in root cell wall polymers in response to salt stress in tomato plants.In this study, two tomato cultivars with contrasting salt tolerance, *Daniela* (salt tolerant) and *Naomi* (salt sensitive) were studied for changes in cell wall-constituting polysaccharides and CEC under NaCl and $CaCl_2$ stress.

Materials and methods

Site trial management: The experiment was conducted in the Glass House of the subdivision of Plant Eco-Physiology, Arid Land Research Center (ALRC), Tottori University, Tottori-Japan (134°14'E, 35°30'N and altitude 41 m).

Plant material and growth conditions: Seeds of two cultivars of tomato were procured from *HAZERA Seeds Co., Ltd.* The salt tolerance status of cultivars was confirmed by An et al. (2005). Based on their strength and weaknesses in salt tolerance, *Naomi* was sensitive, while *Daniela* was rated tolerant.

Seeds of both cultivars were surface sterilized with 0.5% sodium hypochlorite solution for 15 min, and then thoroughly rinsed with distilled water. The seeds were then germinated on moistened filter paper and transferred to a growth chamber (*MLR-350HT*, *Sanyo*, Osaka, Japan) at 25/21°C (day/night intervals), 75% relative humidity in the dark. Two weeks after sowing, the seedlings were transplanted into plastic containers (69 cm long × 54 cm wide × 30 cm deep) with 50 L of water floating with a 2.5 cm thick *Styrofoam*. A 2.5 cm diameter hole was made in the *Styrofoam*, and each plant was sown at 2.5 × 2.5 × 2.5 cm^2 sponges while floating on the surface of the water. Plants were fertilized with half strength nutrient solution (formulated by *Otsuka Chemical Co., Ltd.*) throughout the experimental period. The solution pH was set from 5.3 to 5.8 and adjusted daily with 1 M KOH solution. The hydroponic solution was changed weekly. Salinity treatment commenced two weeks after transplanting with the six salt concentrations of 0.00 dS m^{-1} NaCl (control), 0.00 dS m^{-1} NaCl + 0.20 dS m^{-1} $CaCl_2$, 4.00 dS m^{-1} NaCl + 0.20 dS m^{-1} $CaCl_2$, 8.00 dS m^{-1} NaCl, and 8.00 dS m^{-1} NaCl + 0.20 dS m^{-1} $CaCl_2$. Both cultivars were harvested 5 weeks after the onset of salt treatment. Plant height was measured at harvest. Shoot and root dry weights were measured after oven drying at 80°C for 72 h. The longest root of each cultivar was also measured. The roots were used to measure the CEC of the cell wall (CW).

Root sampling: Roots were sampled 5 weeks after salt treatment. The root tip was cut into 0 - 5 mm (extension area) and 5 - 10 mm (mature area) with a laboratory blade. The root partitions were frozen in liquid nitrogen for 10 min and stored at –30°C. Root samples were taken per treatment for CW analysis.

Measurement of chemical components in root cell wall: Root CW components were measured following the procedure of Zhong and Lauchli (1993). The root partitions were homogenized with cold 0.1 M Tris-HCl buffer (pH 7.4) and Tris buffer-saturated phenol using a *µT-12* bead crusher (*Taitec Corporation Ltd.*, Tokyo, Japan). The homogenate was centrifuged at 1 957 *g* for 10 min at 7°C. The supernatant was discarded, and cell wall pellets were subjected to further purifications by incubating and centrifuging in cold 0.1 M Tris-HCl, ethanol (80%, v/v), acetone (100%) and the mixture of methanol and chloroform (1:1, v/v), acetone and finally in ethanol. The CW extracts were treated with 0.2 mg mL^{-1} pronase in 0.05 mM phosphate buffer (pH 6.5) containing 5% ethanol for 16 h at 30°C. After centrifugation, the supernatant was disposed. Extraction of pectin fractions was carried out five times with cyclohexylenedinitrilotetraacetic acid (CDTA) at pH of 6.5 at 20°C. Cell walls extractions were done three times with hot CDTA at 100°C for 1 h each time. The CDTA extracts were denoted as the pectin-enriched fractions. Sequential extraction of hemicellulose 1 (Hem.1) and hemicellulose 2 (Hem.2) was done with 1 M and 4 M KOH solutions, respectively. The extractions in KOH solutions were repeated three times for 4, 16, and 4 h each, respectively. The alkaline-insoluble precipitate was designated as cellulose-enriched fractions which were dissolved in 0.5 mL of 72% (v/v) sulphuric acid for an hour, further diluted once with distilled water for the determination of total sugars. The concentrations of uronic acid and total sugars in each extract were measured using the M-hydroxy diphenyl colometric (Blumenkrantz and Asboe-Hansen, 1973) and phenol-sulphuric acid (Hodge and Hofreiter, 1962) methods, respectively.

Cell wall preparation: Cell walls were isolated from the roots following the methods of Meychik et al. (1999) and Meychik and Yermakov (2001). The excised roots were placed in a 250 ml glass ion-exchange column and washed in 1% alkali and acid solutions and with distilled water until complete eradication of Cl^- in the washing water, and then dried to a constant weight in the presence of $CaCl_2$ at 60°C. The quality of the CW isolated was assessed by staining the preparations with fluorescent dye DAPI (4',6-diamidino-2-phenylindole) (*Sigma-Aldrich Ltd.*, Darmstadt, Germany) and further examined microscopically to ascertain the absence of other intracellular structures in the preparations.

Cation-exchange capacity (CEC) of cell walls at different NaCl concentrations: The CEC of the root cell wall was measured according to Meychik et al. (2005). Root samples were washed sequentially with 10 mM sodium hydroxide, distilled water, and 10 mM hydrochloric acid, and then finally washed thoroughly with distilled water. They were then dried at 80°C to a constant weight. This was used as the root CW sample. Forty (40) milligrams of the root cell wall were transferred into 50 mL centrifuge tubes, and 10 mL of NaCl solution with varying concentrations (0.5, 2.0, 4.0, 8.0 and 12.0 dS m^{-1}) were added to each sample. After 24 h, the samples were discarded. The initial pH^{in} and final pH^{f} or equilibrium (after contact with root samples) pH values were measured in each tube with a pH-meter (pH Meter, *Model HM-25R*, *DKK-TOA Corporation*, Tokyo, Japan). The CEC of the root CW was calculated at each corresponding NaCl concentration as:

$$S = [(10^{(-pHf)} - 10^{(-pHin)}) \times 1\,000 \times V]/g$$

where S = cation-exchange capacity of isolated cell wall at corresponding NaCl concentration (µmol g^{-1}) dry weight of cell wall; pH^{in} and pH^{f} = initial and final pH of the solution; V = volume of the solution (mL); g = dry weight of the sample (g).

Statistical analysis: The data were subjected to analysis of variance (*ANOVA*) using the *Genstat* (8th ed., Rothamsted Experimental Station, Harpenden, UK) statistical software package. Treatment means were compared using *Duncan*'s Multiple Range Test (DMRT) at the 5% level of probability ($P \leq 0.05$). In addition, the functions *fviz_pca* in *R* software (*version 4.0.5*) was used for principal component analysis.

Principal component analysis: The principal component analysis (PCA) was conducted using the experimental dataset comprising factorial combinations of the two cultivars (V1 = *Daniela* and V2 = *Naomi*) and six salinity concentrations (S0 = 0.00 dS m^{-1} NaCl; S1 = 0.00 dS m^{-1} NaCl + 0.20 dS m^{-1} $CaCl_2$; S2 = 4.00 dS m^{-1} NaCl; S3 = 4.00 dS m^{-1} NaCl + 0.20 dS m^{-1} $CaCl_2$; S4 = 8.00 dS m^{-1} NaCl; S5 = 8.00 dS m^{-1} NaCl + 0.20 dS m^{-1} $CaCl_2$) resulting in 12 treatment combinations each of which was replicated thrice for a total of 36 observations. The PCA was used to visualize the effect of salinity level on root elongation (0 - 5 mm) and mature root zones (5 - 10 mm).

Results

Principal component analysis: At the root elongation zone (0 - 5 mm), the PC1 explained 55.4% of the total variation in CW composition and was mostly associated with pectin, UA_Pectin, Hem.2, Hem.1, and cellulose (Fig. 1*A*,*C*). Similarly, PC2 accounted for an additional 20.1% of the total variations and appeared to be related to UA_Hem.2, UA_Cellulose, and UA_Hem.1 (Fig. 1*B*,*D*). However, at the root mature zone (5 - 10 mm), the PC1 explained 61.1% of the total variations and was mostly associated with UA_Pectin, pectin, Hem.2, cellulose, and Hem.1 (Fig. 1*C*), whereas PC2 which accounted for 16.8% of the total variations in CW composition appeared to be related with UA_Cellulose and UA_Hem.1 (Fig. 1*D*). Root mature zone explained 61.1% of variation, while root elongation zone explained 55.4% of variation (Fig. 1*E*,*F*).

Fig. 2 shows the contribution of CW constituents to PC1 and PC2. In the root elongation zone (0 - 5 mm), V1S5_3 (*Daniela* at 8.0 $NaCl_2$ + $CaCl_2$ in replication 3), V1S5_2 (*Daniela* at 8.0 $NaCl_2$ + $CaCl_2$ in replication 2), V1S5_1 (*Daniela* at 8.0 $NaCl_2$ + $CaCl_2$ in replication 1), V2S2_3 (*Naomi* at 4.0 $NaCl_2$ in replication 3), and V2S1_3 (*Naomi* at 0.0 $NaCl_2$ + $CaCl_2$ in replication 3) had the highest contributions, while in the root mature zone (5 - 10 mm) V2S5_3 (*Naomi* at 8.0 $NaCl_2$ + $CaCl_2$

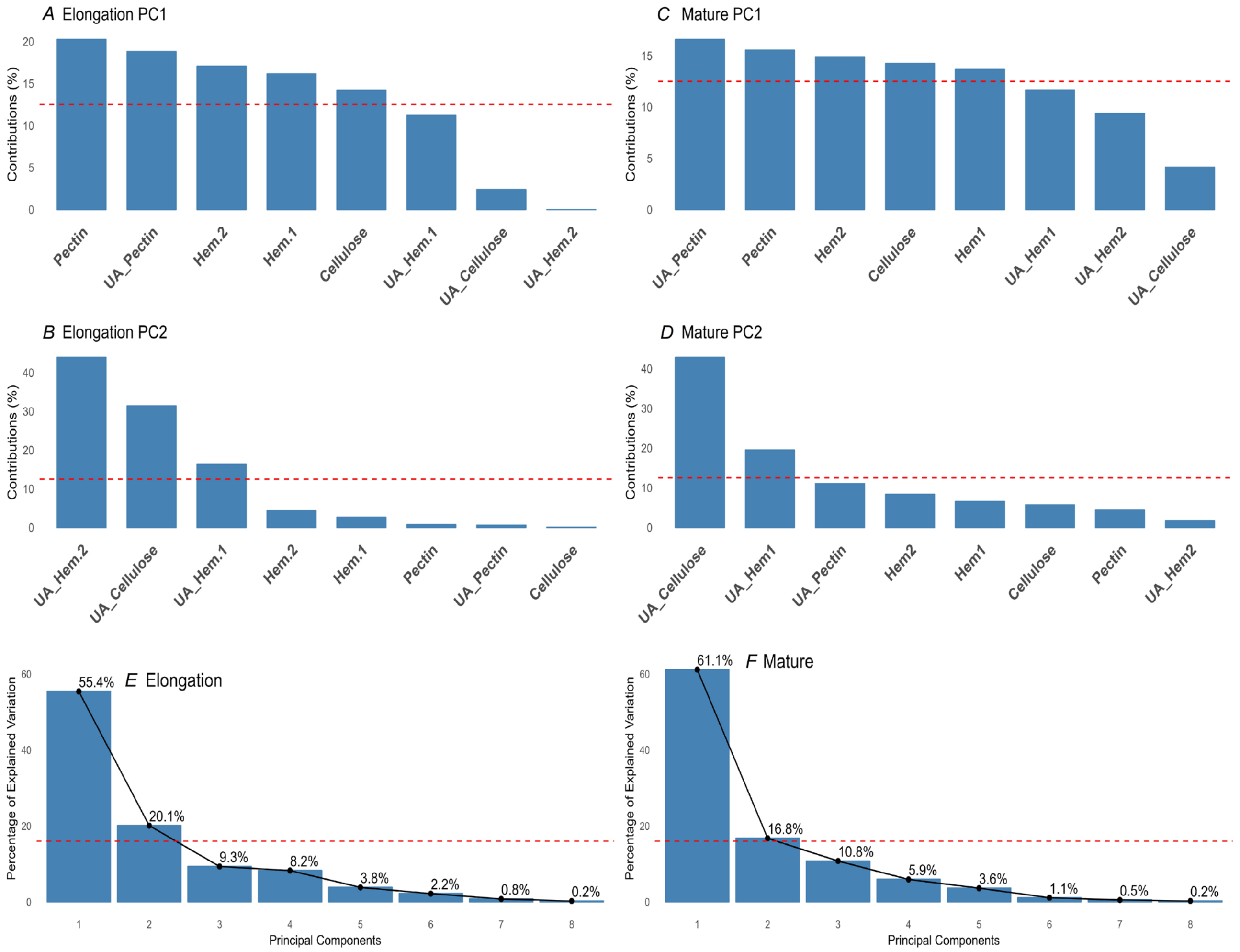

Fig. 1. Principal component analysis. Contribution of top variables to PC1 and PC2 in (*A* and *B*) root elongation zone (0 - 5 mm) and (*C* and *D*) root mature zone (5 - 10 mm) and proportion of variance (%) in (*E*) root elongation zone (0 - 5 mm) and (*F*) root mature zone (5 - 10 mm). The red dotted lines in the barplots denote reference lines and the variable bars above the reference lines are considered as important in contributing to the dimension.

in replication 3), V2S5_2 (*Naomi* at 8.0 NaCl$_2$ + CaCl$_2$ in replication 2), V1S5_3 (*Daniela* at 8.0 NaCl$_2$ + CaCl$_2$ in replication 3), V2S5_1 (*Naomi* at 8.0 NaCl$_2$ + CaCl$_2$ in replication 1) and V1S5_2 (*Daniela* at 8.0 NaCl$_2$ + CaCl$_2$ in replication 2) had the most contribution. Fig. 2*C*,*D* and Fig. 3 showed the PCA biplot with variables and observations represented as vectors and points, respectively. The biplot provides a visual representation of the relationships among CW constituents, including UA_Cellulose, UA_Pectin, Hem.1, UA_Hem.1, Hem.2, and UA_Hem.2 at root elongation zone (0 - 5 mm) and root mature zone (5 - 10 mm) under varying salinity conditions. Each of these properties is represented as a vector, and the angle and length of the vector indicate the correlation and strength of the property in relation to the principal components. At root elongation zone (0 - 5 mm), UA_Hem.2 was negatively correlated with all the variables except UA_Hem.1 and UA_Cellulose. The length of the vectors for each property corresponds to the property contributions of the respective principal component. The dark orange longer arrows indicate a higher contribution, while the dark blue shorter arrows indicate a lower contribution of the variables (Fig. 3). Clusters of variables on the biplot (*e.g.*, cellulose, UA_Pectin, Hem.1, pectin, and Hem.2) suggest that these groups of variables were affected similarly by any given salinity condition and will require similar management practices. However, at the root mature zone (5 - 10 mm), all the variables were positively correlated with each other, and cellulose, UA_Pectin, Hem.1, pectin, and Hem.2 formed clusters. The UA_Hem.2, UA_Hem.1, and UA_Cellulose also formed clusters.

Growth and biomass yield: The effects of NaCl and CaCl$_2$ treatments on plant height, above ground dry weight (AGDW) and root dry weight (RDW) of the two tomato cultivars are presented in Table 1, showing significant ($P \leq 0.05$) growth inhibition by the treatments. However, CaCl$_2$ significantly enhanced the growth compared with sole NaCl treatment. The salt-tolerant *Daniela* grew much taller than the salt-sensitive *Naomi*. For both cultivars, CaCl$_2$ treatment significantly increased plant height

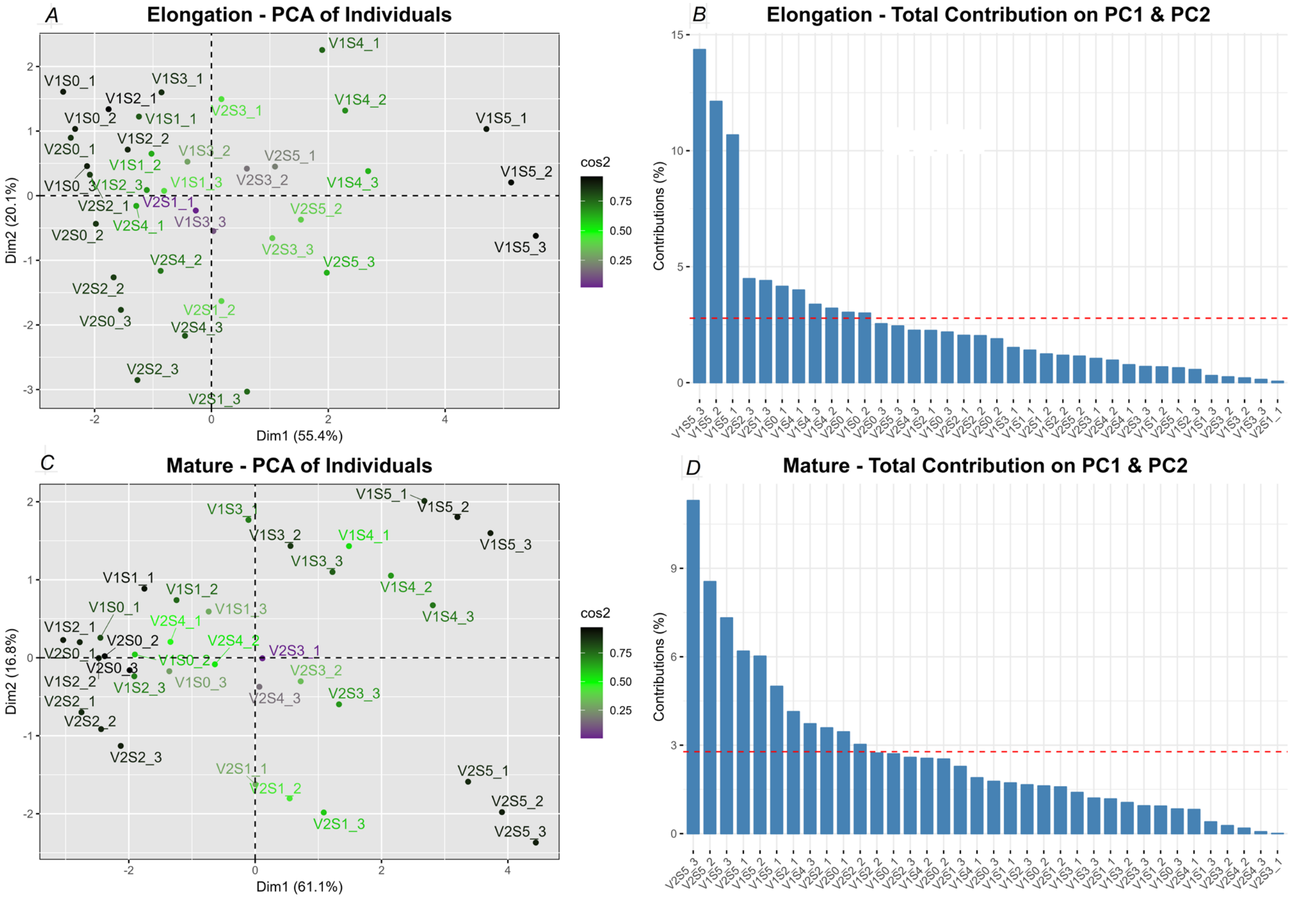

Fig. 2. Principal component analysis. Contribution of top individual to PC1 and PC2 in root elongation zone (0 - 5 mm) and root mature zone (5 - 10 mm), and PCA biplot of individual root elongation zone (0 - 5 mm) and root mature zone (5 - 10 mm). The red dotted lines in the barplots denote reference lines, while the individuals above the reference lines are considered as important in contributing to the dimension. S - salinity concentrations; V - tomato cultivars; S0 - 0.00 dS m^{-1} NaCl; S1 - 0.00 dS m^{-1} NaCl + $CaCl_2$; S2 - 4.00 dS m^{-1} NaCl; S3 - 4.00 dS m^{-1} NaCl + $CaCl_2$; S4 - 8.00 dS m^{-1} NaCl; S5 - 8.00 dS m^{-1} NaCl + $CaCl_2$; V1 - *Daniela* tomato cultivar; V2 - *Naomi* tomato cultivar.

compared with sole NaCl treatment (Table 1). Plants under 4.0 and 8.0 dS m^{-1} NaCl, or under 8.0 dS m^{-1} NaCl + $CaCl_2$ had much lower ($P \leq 0.05$) AGDW than those grown without salt treatment (S0) and *Daniela* had much higher readings than *Naomi*. The salt treatment significantly reduced the RDW but at each NaCl concentration, $CaCl_2$ treatment considerably ($P \leq 0.05$) improved the RDW of both cultivars (Table 1).

Chemical composition of the root cell wall: For both tomato cultivars, plants grown in salt treated media had more ($P \leq 0.05$) cellulose, pectin, Hem.1, and Hem.2 contents in the cell wall of their root elongation zone than the control medium (Fig. 4). Addition of $CaCl_2$ significantly increased the concentration of the above-mentioned CW components than the untreated plants. For both tomato cultivars, the highest concentration of NaCl in combination with $CaCl_2$ produced plants with the highest concentration of cellulose and pectin. The same trend was observed in *Daniela* for Hem.1 and Hem.2. However, for *Naomi*, plants grown at 4.00 dS m^{-1} NaCl + $CaCl_2$ had the highest contents of Hem.1 and Hem.2. For *Daniela*, the highest salt concentration with or without $CaCl_2$ had tomato plants with the highest UA_Pectin, but for *Naomi*, plants under $CaCl_2$ had more UA_Pectin than those exposed to NaCl alone. *Naomi* had higher contents of cellulose and UA_Cellulose than *Daniela*, while *Daniela* had higher contents of pectin, UA_Pectin, Hem.1, and Hem.2. Salt treatment had no significant effect on UA_Hem.1 and UA_Hem.2.

For both tomato cultivars, plants grown in salt medium significantly had higher contents of cellulose, pectin, Hem.1, and Hem.2 except 4.00 dS m^{-1} (S2) in the root mature zone (Fig. 5) relative to no salt (control). In all, the addition of $CaCl_2$ to NaCl produced plants with higher concentration of these CW constituents than NaCl alone. In most cases, the highest concentration of NaCl in combination with $CaCl_2$ (S5) had plants with the highest concentration of the CW constituents. Addition of $CaCl_2$ significantly increased UA_Cellulose in *Naomi* but not in *Daniela*, while the highest NaCl concentration in combination with $CaCl_2$ (S5) produced *Naomi* plants with the highest concentration of UA_Pectin and UA_Hem.1. The UA_Cellulose and UA_Pectin content in the roots of

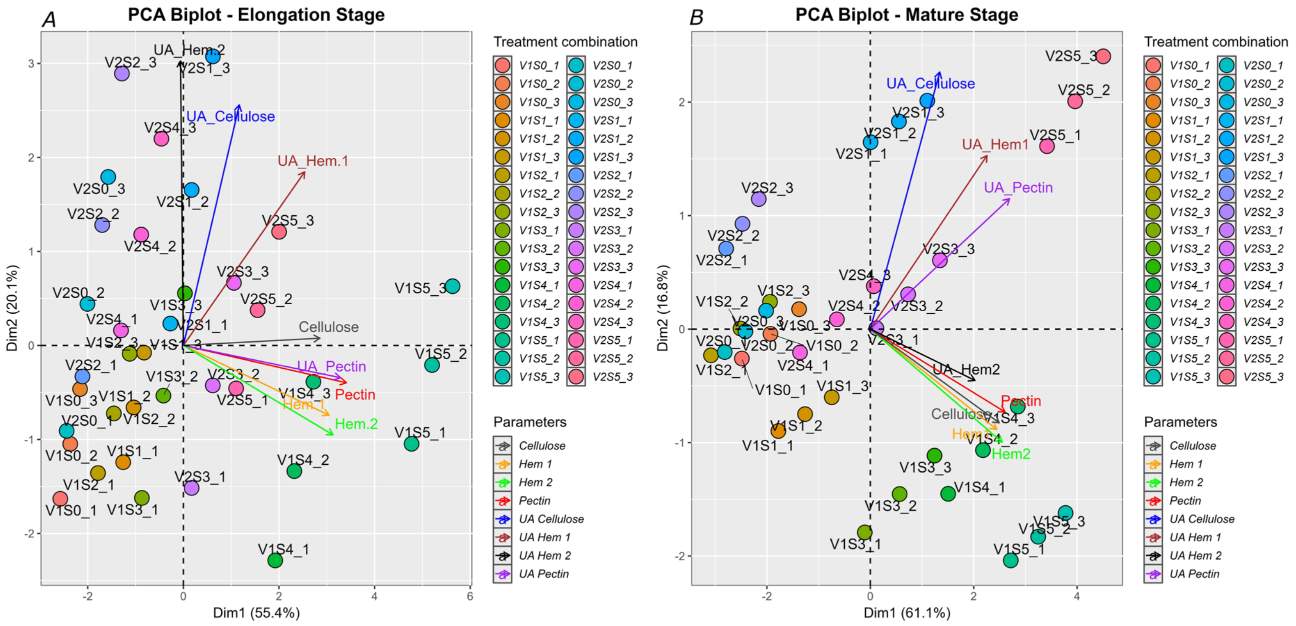

Fig. 3. Principal component analysis biplot showing effects of salinity levels in the root elongation zone (0 - 5 mm) and in the root mature zone (5 - 10 mm). UA_Cellulose - uronic acid in cellulose; UA_Pectin - uronic acid in pectin; Hem.1 - hemicellulose 1; UA_Hem.1 - uronic acid in hemicellulose 1; Hem.2 - hemicellulose 2; UA_Hem.2 - uronic acid in hemicellulose 2; S - salinity concentrations; V - tomato cultivars; S0 - 0.00 dS m^{-1} NaCl; S1 - 0.00 dS m^{-1} NaCl + $CaCl_2$; S2 - 4.00 dS m^{-1} NaCl; S3 - 4.00 dS m^{-1} NaCl + $CaCl_2$; S4 - 8.00 dS m^{-1} NaCl; S5 - 8.00 dS m^{-1} NaCl + $CaCl_2$; V1 - *Daniela* tomato cultivar; V2 - *Naomi* tomato cultivar.

Naomi was significantly higher than that of *Daniela*, while the reverse was the case for Hem.1, pectin, and Hem.2 contents.

Cation-exchange capacity (CEC) of the root cell wall: The CEC of the root cell wall under salt treatment increased remarkably, especially under 8.0 dS m^{-1} NaCl or 8.0 dS m^{-1} NaCl + $CaCl_2$ (Table 2). The CEC increased significantly with increases in the ionic concentration of the media, especially for *Daniela*.

The interaction effects of tomato cultivar, ionic concentration of the external solution and salinity levels on the root CW CEC are shown in Table 3. For both cultivars, increases in the ionic strength of the external solution up to 2.0 dS m^{-1} (N2) had marginal effects on the CEC of the root CW even as salinity increased to 8.0 dS m^{-1}, with or without $CaCl_2$ application. For *Daniela*, a media concentration of 4.0 dS m^{-1} (N3) or more significantly increased the CEC compared with the other lower ionic concentrations. Also, the 12.0 dS m^{-1} (N5) treatment showed higher root cell wall CEC than the 8.0 dS m^{-1} (N4). *Daniela* under 8.0 dS m^{-1} NaCl (S2) or 8.0 dS m^{-1} NaCl + $CaCl_2$ (S5) showed the highest CEC. For *Naomi*, the CEC was significantly highest under the 12.0 dS m^{-1} solution compared with other concentrations. Similarly, the media with highest external solution ionic concentration (N5) plus 8.0 dS m^{-1} NaCl (S2) or 8.0 dS m^{-1} NaCl + $CaCl_2$ (S5) showed the highest root CW CEC ($P \leq 0.05$).

Table 1. Effects of NaCl and $CaCl_2$ concentrations on plant height (cm), above ground dry weight (AGDW), and root dry weight (RDW) of two tomato cultivars. Means within a column (n = 3) bearing *different letters* ($P \leq 0.05$) differ significantly as determined by *Duncan*'s Multiple Range Test.

Treatments Salinity concentrations (S)	Plant height (cm)	AGDW (g)	RDW (g)
0.0 $NaCl_2$ (S0)	160.00^{a}	17.00^{a}	1.16^{a}
0.0 $NaCl_2$ + $CaCl_2$ (S1)	157.00^{a}	14.50^{a}	1.19^{a}
4.0 $NaCl_2$ (S2)	124.00^{c}	10.50^{b}	0.48^{d}
4.0 $NaCl_2$ + $CaCl_2$ (S3)	153.00^{b}	13.50^{ab}	0.87^{b}
8.0 $NaCl_2$ (S4)	88.00^{e}	6.00^{c}	0.44^{d}
8.0 $NaCl_2$ + $CaCl_2$ (S5)	99.00^{d}	6.83^{c}	0.64^{c}
Tomato cultivar (V)			
Daniela (V1)	133.00	12.50	0.88
Naomi (V2)	127.33	10.28	0.71
LSD $_{(0.05)}$	1.38	1.41	0.02
S × V			
S0V1	164.00^{a}	18.00^{a}	1.25^{b}
S1V1	162.00^{a}	15.00^{a}	1.45^{a}
S2V1	128.00^{c}	13.00^{a}	0.45^{hi}
S3V1	154.00^{b}	14.00^{a}	0.92^{d}
S4V1	92.00^{f}	7.00^{a}	0.51^{gh}
S5V1	98.00^{e}	8.00^{a}	0.72^{f}
S0V2	156.00^{b}	16.00^{a}	1.06^{c}
S1V2	152.00^{b}	14.00^{a}	0.92^{d}
S2V2	120.00^{d}	8.00^{a}	0.51^{gh}
S3V2	152.00^{b}	13.00^{a}	0.81^{e}
S4V2	84.00^{g}	5.00^{a}	0.38^{i}
S5V2	100.00^{e}	5.67^{a}	0.55^{g}

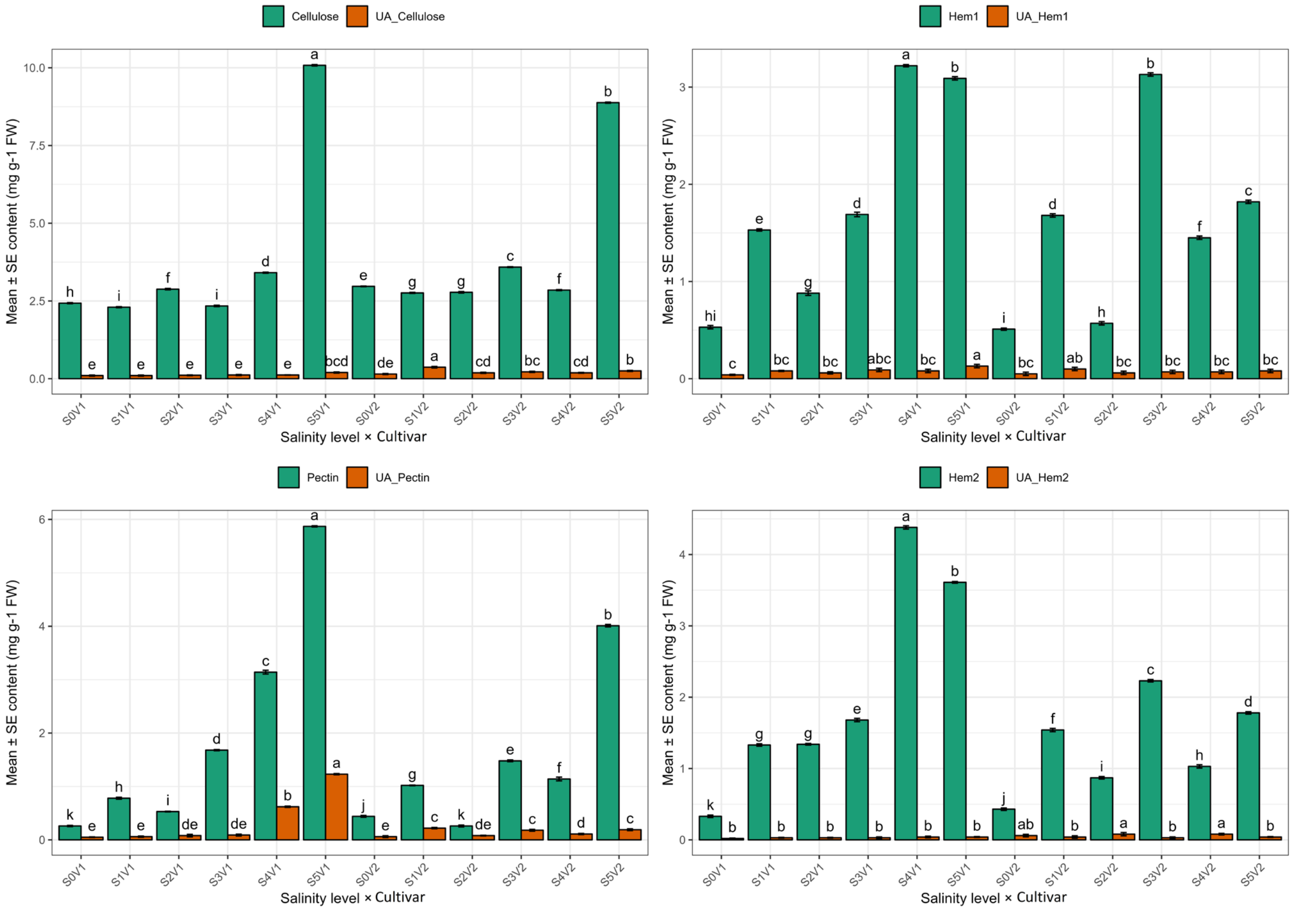

Fig. 4. Effects of NaCl and $CaCl_2$ concentrations on cellulose, uronic acid in cellulose (UA_Cellulose), pectin, uronic acid in pectin (UA_Pectin), hemicellulose 1 (Hem.1), uronic acid in hemicellulose 1 (UA_Hem.1), hemicellulose 2 (Hem.2), and uronic acid in hemicellulose 2 (UA_Hem.2) contents in the root elongation zone (0 - 5 mm) of two tomato cultivars. Means ± SE (n = 3) bearing *different letters* ($P \leq 0.05$) differ significantly as determined by *Duncan*'s Multiple Range Test. S - salinity concentrations; V - tomato cultivars; S0 - 0.00 dS m^{-1} NaCl; S1 - 0.00 dS m^{-1} NaCl + $CaCl_2$; S2 - 4.00 dS m^{-1} NaCl; S3 - 4.00 dS m^{-1} NaCl + $CaCl_2$; S4 - 8.00 dS m^{-1} NaCl; S5 - 8.00 dS m^{-1} NaCl + $CaCl_2$; V1 - *Daniela* tomato cultivar; V2 - *Naomi* tomato cultivar; FW - fresh root weight.

Discussion

Unlike several plant species highly tolerant of salt stress (halophytes), sensitive species (glycophytes) including most arable crops can only survive in low salt media (Cheeseman, 2015; Ismail and Horie, 2017). High salt concentrations in soil do inhibit plant growth and development. The need for finding physiological and genetic solutions to minimize the impact of soil salinity on crop yield has since been identified (Munns et al., 2020). In this study, two tomato cultivars with contrasting tolerance of salinity stress were monitored for changes in CW components as indicators of salt tolerance. The salt stress led to several negative changes in their CW structure and constituents. However, the higher content of CW components in *Daniela*, which may have protected the stability of its cell structure, could explain its superior salt tolerance under stress. Also, the lower Na^+ concentrations in the salt-tolerant *Daniela* was possibly due to its ability for rapid cell elongation and repairs, thereby preserving its cell wall integrity (CWI) in response to the stress (Zaki and Yokoi, 2016). To maintain the CWI during the reorganization of CWs, plants do constantly rely on the chemical and mechanical properties of the CWs and are able to repair the CW once it is seriously disrupted.

Principal components analysis (PCA) approach has been adopted for screening of salt stress-tolerant plants, including tomato (Sivakumar et al., 2020). The use of PCA in salt tolerance studies is well documented (Alam et al., 2021; Otie et al., 2022). In this study, V1 (*Daniela*) under varying salt concentrations contributed more to the variations in the principal components and was more tolerant than V2 (*Naomi*). At the root elongation zone, high values of UA_Hem.2 were observed for V2S1 and V2S4 relative to others. Similarly, high value of UA_Cellulose was observed for V1S3. Also, UA_Hem.1 and Hem.2 had high values for V2S5 and V2S3, while those of UA_Pectin, pectin, Hem.1, and cellulose were recorded for V1S4. At the root mature zone, high values of UA_Cellulose were recorded for V2S1 and V2S4, while that of UA_Pectin was for V2S3. The UA_Hem.2, pectin, cellulose, Hem.1, and Hem.2 had higher values in V2S3 and V1S4, respectively. The high content of CW components in *Daniela* could be due to its salt tolerance mechanisms which may have

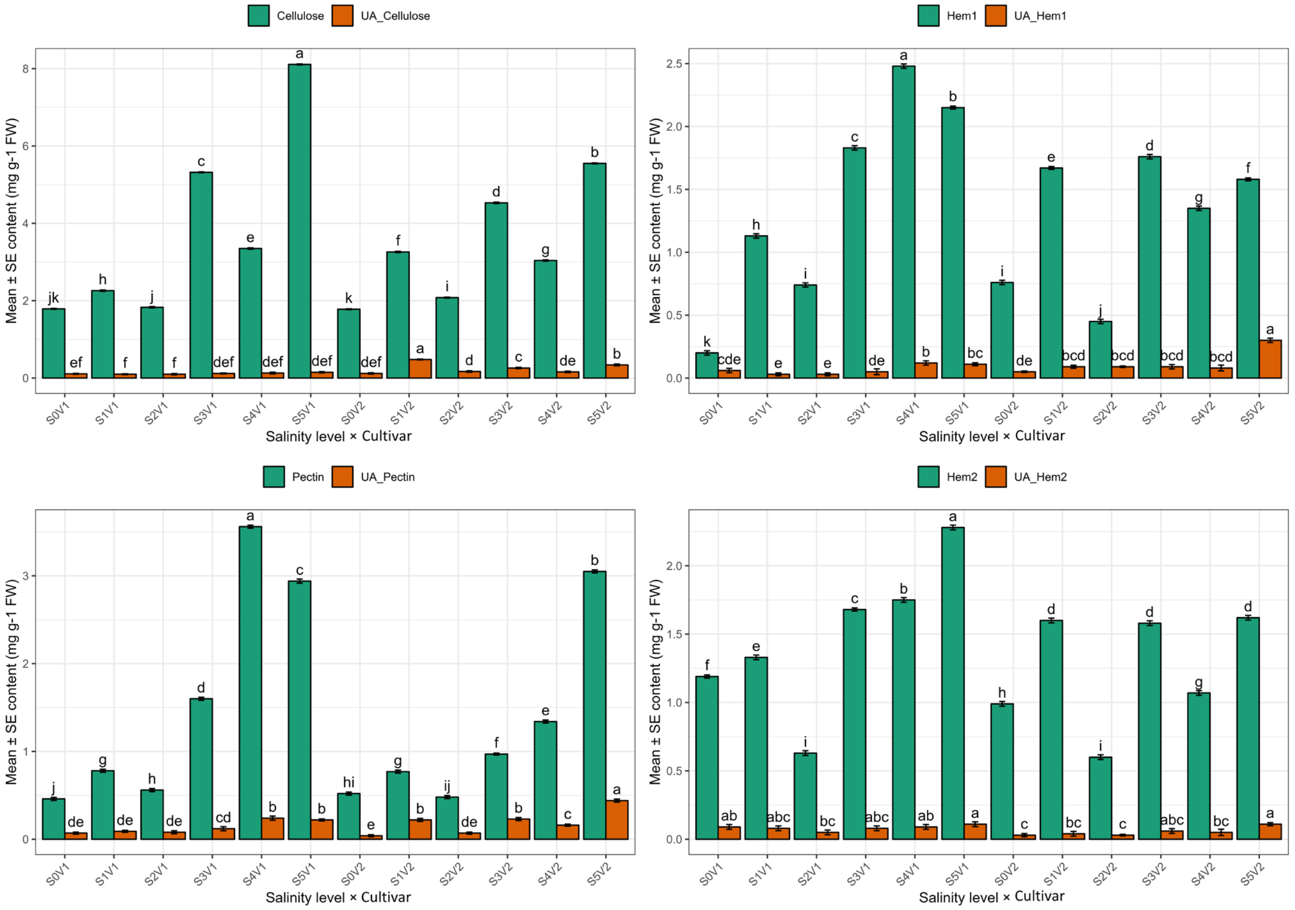

Fig. 5. Effects of NaCl and CaCl$_2$ concentrations on cellulose, uronic acid in cellulose (UA_Cellulose), pectin, uronic acid in pectin (UA_Pectin), hemicellulose 1 (Hem.1), uronic acid in hemicellulose 1 (UA_Hem.1), hemicellulose 2 (Hem.2), and uronic acid in hemicellulose 2 (UA_Hem.2) contents in the root mature zone (5 - 10 mm) of two tomato cultivars. Means ± SE ($n = 3$) bearing *different letters* ($P \leq 0.05$) differ significantly as determined by *Duncan*'s Multiple Range Test. S - salinity concentrations; V - tomato cultivars; S0 - 0.00 dS m^{-1} NaCl; S1 - 0.00 dS m^{-1} NaCl + CaCl$_2$; S2 - 4.00 dS m^{-1} NaCl; S3 - 4.00 dS m^{-1} NaCl + CaCl$_2$; S4 - 8.00 dS m^{-1} NaCl; S5 - 8.00 dS m^{-1} NaCl + CaCl$_2$; V1 - *Daniela* tomato cultivar; V2 - *Naomi* tomato cultivar; FW - fresh root weight.

protected the cell structure's stability under the salt stress. Previous studies identified PCA as a potent tool for the assessment of stress conditions in crop species, including tomato (Alam et al., 2021), maize (Huqe et al., 2021), soybean (Otie et al., 2022) and wheat (Rana et al., 2015).

Pectin is a group of acidic polysaccharides that accounts for up to 40% of the dry weight of higher plant CWs and plays critical roles in plant growth and development, leaf senescence, as well as biotic and abiotic stress responses (Liu et al., 2022a). Although pectin played important roles in both tomato cultivars, its effect was more pronounced in *Naomi*, possibly because of its lower salt tolerance (An et al., 2005).

The soil environment is variable and complex, thus, to grow in soil, the development of roots needs to be flexible in response to many stress factors. Overall, the growth of the salt-tolerant *Daniela* under NaCl stress was better than that of the sensitive *Naomi* and this may be due to its ability to detect multiple soil environmental stimuli and coordinating its cellular stress responses by re-orientating its root for better uptake (Lamers et al., 2020). For both cultivars, calcium chloride (CaCl$_2$) improved the root biomass relative to sole treatment with NaCl. This is consistent with previous reports (Choi et al., 2014) in which some cellular pathways, including calcium signaling, were generated in response to Na$^+$ ions and plant survival.

The plant CW, which mainly consists of polysaccharides and structural proteins, are essential for the establishment of plant structure and protection against adverse environmental changes. During plant growth and development or in response to environmental stresses, the CW composition and structure are dynamically modulated, allowing rapid cell elongation and increased stress tolerance (Liu et al., 2022b). Upon exposure to high salinity, several changes in the CW have been identified, including the reduction of cellulose content (Byrt et al., 2018), disruption of the cross-linking of pectins (Peaucelle et al., 2012) and accumulation of lignin (Le Gall et al., 2015). Studies have shown that the plants that are defective in cell wall biosynthesis are hypersensitive to salt stress, suggesting that maintenance of CWI is important for the adaptation of plants to high salinity (Feng et al., 2018). In this study, NaCl plus CaCl$_2$ increased the cellulose contents in the root elongation zones of tomato.

Table 2. Effects of NaCl, $CaCl_2$, and ionic strength of the external solution on cation exchange capacity (CEC, µmol g^{-1} DW) of root cell wall of two tomato cultivars differing in tolerance to salinity stress. Means within a column (n = 3) bearing *different letters* ($P \leq 0.05$) differ significantly as determined by *Duncan*'s Multiple Range Test.

Treatments Salinity concentrations (S)	CEC of root cell wall (µmol g^{-1} DW)
0.00 NaCl (S0)	4.66^{d}
4.00 NaCl (S1)	5.79^{c}
8.00 NaCl (S2)	8.17^{a}
0.00 NaCl + $CaCl_2$ (S3)	4.64^{d}
4.00 NaCl + $CaCl_2$ (S4)	6.33^{b}
8.00 NaCl + $CaCl_2$ (S5)	8.41^{a}
Ionic concentration of external solution (dS m^{-1}) (N)	
0.5 NaCl (N1)	1.13^{e}
2.0 NaCl (N2)	1.54^{d}
4.0 NaCl (N3)	4.99^{c}
8.0 NaCl (N4)	9.64^{b}
12.0 NaCl (N5)	14.37^{a}
Tomato cultivar	
Daniela (V1)	7.26^{a}
Naomi (V2)	5.40^{b}

The cellulose content increased for both cultivars, especially under 8 dS m^{-1} NaCl + $CaCl_2$, relative to the treatments without $CaCl_2$. Salt stress causes significant CW stiffening, which is detrimental to root cell division and expansion in the elongation zone. However, *Daniela* had a more stable CW structure under salt stress which may have contributed to its overall salt tolerance (Rozema and Schat, 2013). Calcium chloride application significantly increased the cellulose level under salinity and *Naomi* root growth was enhanced as reflected in the dry weight. We suggest that one way calcium ameliorates salt toxicity may be by maintaining the composition of the CW. The ameliorative effect was more conspicuous in the salt-tolerant cultivar, *Daniela*, than the salt-sensitive *Naomi* because of the significant deposition of pectins on the CW (An et al., 2014). The UA_Cellulose was much higher in *Naomi* than *Daniela* suggesting that it is an important functional constituent that plays a significant role in plant tolerance and adaptation to adverse stress conditions.

Pectin is also known to exhibit many other important functions in plant meristems, such as ion binding, ion homeostasis, pH adjustment, water retention, and electrochemical balance (Hocq et al., 2017). Its contents in the root CW increased directly with salt concentration. *Daniela* had higher pectin and UA_Pectin contents than Naomi, and the highest concentration was recorded under 8.00 dS m^{-1} NaCl stress. The increased proportion of UA_Pectin under NaCl treatment indicated that NaCl enhanced UA synthesis, which could be related to its cation binding ability, and thus likely to affect the structural integrity and mechanical properties of the CW (Byrt et al., 2018). For both cultivars, the NaCl + $CaCl_2$ treatment significantly increased pectin contents relative to sole NaCl treatment. However, *Naomi* under $CaCl_2$ had much higher content of UA_Pectin than under NaCl alone. This may be attributed to the inhibitory role of NaCl in pectin synthesis that affects root growth due to changes in CW synthesis (Liu et al., 2022a). The marked increase in Hem.1 and Hem.2 under NaCl + $CaCl_2$, and at the highest salt concentrations (S4 and S5) did indicate that a reduction in extensibility of CW could have occurred. *Daniela* had higher Hem.1 and Hem.2 than *Naomi*. As a tolerant cultivar, with stable CW architecture under salt stress, the mechanical properties and chemical composition of *Daniela* cell wall may have been improved (Liu et al., 2022b). Salt treatment caused little changes in the contents of UA_Hem.1 and UA_Hem.2, except at the highest salt level (S5). The increased UA-Hem may have conferred some salinity tolerance on *Naomi* enough to maintain turgor pressure and structural integrity, thereby improving the water-holding capacity of its CW. The increase in UA in halophytes was slower and could terminate at lower levels, unlike in glycophytes (Uddin et al., 2013).

Calcium chloride ($CaCl_2$) treatment increased the cellulose contents in both tomato cultivars at the root mature zone (5 - 10 mm). However, with the combined salt treatments, *Daniela* had a higher content than *Naomi*, except at the S1 = 4.00 dS m^{-1} NaCl salt level. Cellulose is the most abundant organic component in the CW of vascular plants (Endler and Persson, 2011). It is synthesized in both primary and secondary CWs. *Daniela* had superior cellulose content which could have conferred on it the inherent ability to tolerate the adverse effect of salt at the mature root zone (Zhang et al., 2016). Uronic acid in cellulose (UA_Cellulose) was higher in plants treated with only $CaCl_2$ compared to the control. The salt effect on the UA_Cellulose of *Daniela* was negligible, but for *Naomi* all $CaCl_2$ treatments increased the UA_Cellulose, possibly because of the non-impairment of the synthesis of cellulose and pectin to maintain the structural integrity of cells in support of growth. Salt treatment significantly increased the pectin content in the mature root zone, except at the 4.00 dS m^{-1} level. For both cultivars, $CaCl_2$ produced higher pectin content relative to sole treatment with NaCl.

The sole and mixed salt treatments enhanced the UA_Pectin in *Daniela*, compared with no salt. This further validates the fact that UA_Pectin could have accounted for the superior root growth in the salt tolerant cultivar. Unlike NaCl, $CaCl_2$ significantly increased the content of UA_Pectin in *Naomi*. This may be attributed to the ability of Ca^{2+} to form stronger cross-links with the pectin molecules. A previous report showed that it increased the cation binding sites that trapped Na^{+} to reduce cellular damage at the mature root zone and improve root growth (Cosgrove, 2016). There was a remarkable increase in Hem.1 content of the mature root zone under salt treatment. Hemicellulose 1 plays a vital role in promoting the structural integrity of plant CW and contributes to its flexibility and hydration properties (Rahman et al., 2021). *Daniela* may have developed compatible solutes, in the form of sugars and sugar alcohols, which could have

Table 3. Interaction effects of salinity, ionic concentration of external solution and tomato cultivars on cation-exchange capacity of root cell wall (CEC, µmol g^{-1} DW). Means within a column ($n = 3$) bearing *different letters* ($P \leq 0.05$) differ significantly as determined by *Duncan*'s Multiple Range Test. V - tomato cultivar; N - ionic concentration of external solution; S - NaCl2 + $CaCl_2$; V1 - *Daniela*; V2 - *Naomi*; N1 - 0.5 NaCl; N2 - 2.0 NaCl; N3 - 4.0 NaCl; N4 - 8.0 NaCl; N5 - 12.0 NaCl; S0 - 0.00 NaCl; S1 - 4.00 NaCl; S2 - 8.00 NaCl; S3 - 0.00 NaCl + $CaCl_2$; S4 - 4.00 NaCl + $CaCl_2$; S5 - 8.00 NaCl + $CaCl_2$.

Treatment V × N × S	Root cell wall CEC (µmol g^{-1} DW)	Treatment V × N × S	Root cell wall CEC (µmol g^{-1} DW)
V1 N1 S0	0.87^{wx}	V2 N1 S0	0.91^{vwx}
V1 N1 S1	0.04^{x}	V2 N1 S1	0.97^{t-x}
V1 N1 S2	1.00^{t-x}	V2 N1 S2	2.96^{qrs}
V1 N1 S3	0.96^{t-x}	V2 N1 S3	0.92^{u-x}
V1 N1 S4	1.00^{t-x}	V2 N1 S4	1.08^{t-x}
V1 N1 S5	1.03^{t-x}	V2 N1 S5	1.87^{r-w}
V1 N2 S0	1.22^{s-x}	V2 N2 S0	1.39^{r-x}
V1 N2 S1	1.47^{r-x}	V2 N2 S1	1.39^{r-x}
V1 N2 S2	1.04^{t-x}	V2 N2 S2	3.04^{pqr}
V1 N2 S3	1.12^{t-x}	V2 N2 S3	1.39^{r-x}
V1 N2 S4	1.28^{s-x}	V2 N2 S4	1.37^{r-x}
V1 N2 S5	1.11^{t-x}	V2 N2 S5	2.65^{q-v}
V1 N3 S0	4.80^{nop}	V2 N3 S0	2.71^{q-t}
V1 N3 S1	7.09^{mn}	V2 N3 S1	2.67^{q-u}
V1 N3 S2	6.72^{n}	V2 N3 S2	5.74^{no}
V1 N3 S3	4.79^{nop}	V2 N3 S3	2.47^{r-w}
V1 N3 S4	6.88^{n}	V2 N3 S4	2.55^{r-w}
V1 N3 S5	9.08^{jkl}	V2 N3 S5	4.35^{opq}
V1 N4 S0	6.83^{n}	V2 N4 S0	5.78^{no}
V1 N4 S1	11.18^{ghi}	V2 N4 S1	6.55^{n}
V1 N4 S2	17.51^{b}	V2 N4 S2	6.85^{n}
V1 N4 S3	7.26^{mn}	V2 N4 S3	7.40^{lmn}
V1 N4 S4	10.63^{hij}	V2 N4 S4	10.44^{h-k}
V1 N4 S5	15.80^{bcd}	V2 N4 S5	9.48^{i-k}
V1 N5 S0	13.43^{ef}	V2 N5 S0	8.71^{klm}
V1 N5 S1	14.05^{de}	V2 N5 S1	12.49^{efg}
V1 N5 S2	21.34^{a}	V2 N5 S2	15.47^{cd}
V1 N5 S3	10.12^{h-k}	V2 N5 S3	9.97^{h-k}
V1 N5 S4	16.41^{bc}	V2 N5 S4	11.69^{fgh}
V1 N5 S5	21.83^{a}	V2 N5 S5	16.87^{bc}

helped to maintain its cellulose osmotic balance and prevent water loss under saline conditions (Guo et al., 2022). Salt treatment at S5 = 8.00 NaCl + $CaCl_2$ boosted UA_Hem.1, and more so in *Naomi* than *Daniela*. This differential response could be due to the distinct strategies used by the cultivars to cope with high salt levels (Corrêa-Ferreira et al., 2019). *Naomi* exhibited a higher demand for UAs, possibly because it could develop compatible solutes for osmotic balance maintenance during salinity stress. The highest content of Hem.2 occurred under the highest salt concentration (S5 = 8.00 NaCl + $CaCl_2$). Under high salt conditions, plants often increase the synthesis and deposition of Hem.2, particularly xyloglucan in their CWs for water retention, CW reinforcement and ion sequestration, thereby contributing to the maintenance of CWI and functioning. For both cultivars, treatment with $CaCl_2$ increased Hem.2 significantly relative to sole treatment with NaCl. Overall, *Daniela* had much higher levels of Hem.2 than *Naomi* which could be typical of their specialized adaptation to high salt concentrations.

Cation transport *via* cell membrane is the basic factor determining salinity tolerance in plants (Meychik et al., 2021). Ion fluxes control ion concentrations which are essential for salinity tolerance. Halophytes are usually considered like sodium tolerant plants. Indeed, NaCl is the main source of salinity in most areas, though chloride, sulfate, calcium, magnesium, and other ions are involved, constituting sometimes the main salt for soil and water salinization (Shabala, 2013). Tomato plants grown in salt solution had much higher root CW CEC than those grown in solution without NaCl stress. A remarkable increase in the root CW CEC was observed with increasing ionic concentration of the external solution. The CW CEC of *Daniela* roots was higher than that of *Naomi* given its ability to bind and store salt ions and maintain a more balanced ion concentration in its tissues (Shabala, 2013). Interestingly, plants with an ionic concentration of the external solution of 12.0 dS m^{-1} had considerably higher root CW CEC than those with 8.0 dS m^{-1} at each corresponding salinity level. In both cultivars, the highest ionic concentration of the external solution, in combination with salinity level of 8.0 dS m^{-1} NaCl or 8.0 dS m^{-1} NaCl + $CaCl_2$ showed the highest CEC. Under increased NaCl media, the number of active sites capable of exchanging a proton for Na^+ in the CW increases. This may lead to an increase in Ca^{2+} transport to the root cells and contribute to Ca^{2+} signaling, an important step in plant response to salt stress (Meychik et al., 2010a; 2010b). Ion-exchange reactions in root CWs are an important component of plant response to salt stress, which affects sodium distribution and transport, ionic and water balance of plants, and in some cases a part of salt tolerance mechanisms. This adaptation could allow a regulation of osmotic balance and ultimately increase salinity tolerance (Volkov, 2015).

This study showed significant reductions in the CW-constituting polysaccharides of *Naomi* (salt-sensitive) relative to *Daniela* (salt-tolerant) tomato cultivars, whether at the 8.00 dS m^{-1} NaCl treatment or its combination with $CaCl_2$. The CW viscosity was better enhanced under NaCl + $CaCl_2$ salinity, but the contents of UA across the CW constituents increased under sole treatment with $CaCl_2$ at the mature root zone of *Naomi* relative to *Daniela*. The root CW CEC varied directly with the ionic concentration of the external solution. Salt concentrations at 8.0 dS m^{-1} NaCl or 8.0 dS m^{-1} NaCl + $CaCl_2$ significantly increased the CEC of the CW, especially for *Daniela*. The CW constituents played important roles in regulating the root growth of the tomato cultivars under salinity stress and the salt-tolerant *Daniela* with higher CW and ionic contents showed superior stability in cell structure under salt stress. Cation-exchange reactions in root CWs are important events in the response of tomato to salinity, through alterations in sodium distribution, and $CaCl_2$ transport, and ionic balance, especially in the more tolerant cultivar.

References

Alam, M.S., Tester, M., Fiene, G. & Mousa, M.A.A. (2021) Early growth stage characterization and the biochemical responses for salinity stress in tomato. *Plants*, 10, 712.

Albersheim, P., Darvill, A., Roberts, K., Sederoff, R. & Staehelin, A. (Eds.) (2010) Plant Cell Walls: From Chemistry to Biology. New York: Garland Science, pp. 430.

An, P., Inanaga, S., Li, X.J., Eneji, A.E. & Zhu, N.W. (2005) Interactive effects of salinity and air humidity on two tomato cultivars differing in salt tolerance. *Journal of Plant Nutrition*, 28, 459-473.

An, P., Li, X., Zheng, Y. et al. (2014) Effects of NaCl on root growth and cell wall composition of two soya bean cultivars with contrasting salt tolerance. *Journal of Agronomy and Crop Science*, 200, 212-218.

Annunziata, M.G., Ciarmiello, L.F., Woodrow, P., Maximova, E., Fuggi, A. & Carillo, P. (2017) Durum wheat roots adapt to salinity remodeling the cellular content of nitrogen metabolites and sucrose. *Frontiers in Plant Science*, 7, 2035.

Blumenkrantz, N. & Asboe-Hansen, G. (1973) New method for quantitative determination of uronic acids. *Analytical Biochemistry*, 54, 484-489.

Byrt, C.S., Munns, R., Burton, R.A., Gilliham, M. & Wege, S. (2018) Root cell wall solutions for crop plants in saline soils. *Plant Science*, 269, 47-55.

Cheeseman, J.M. (2015) The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytologist*, 206, 557-570.

Choi, W.-G., Toyota, M., Kim, S.-H., Hilleary, R. & Gilroy, S. (2014) Salt stress-induced Ca^{2+} waves are associated with rapid, long-distance root-to-shoot signaling in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 6497-6502.

Corrêa-Ferreira, M.L., Viudes, E.B., de Magalhães, P.M. et al. (2019) Changes in the composition and structure of cell wall polysaccharides from *Artemisia annua* in response to salt stress. *Carbohydrate Research*, 483, 107753.

Cosgrove, D.J. (2016) Catalysts of plant cell wall loosening. *F1000Research*, 5, 119.

Endler, A. & Persson, S. (2011) Cellulose synthases and synthesis in *Arabidopsis*. *Molecular Plant*, 4, 199-211.

Feng, W., Kita, D., Peaucelle, A. et al. (2018) The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca^{2+} signaling. *Current Biology*, 28, 666-675.

Guo, M., Wang, X.-S., Guo, H.-D. et al. (2022) Tomato salt tolerance mechanisms and their potential applications for fighting salinity: a review. *Frontiers in Plant Science*, 13, 949541.

Guo, Y., Liu, Y., Zhang, Y. et al. (2021) Effects of exogenous calcium on adaptive growth, photosynthesis, ion homeostasis and phenolics of *Gleditsia sinensis* Lam. plants under salt stress. *Agriculture*, 11, 978.

Hocq, L., Pelloux, J. & Lefebvre, V. (2017) Connecting homogalacturonan-type pectin remodeling to acid growth. *Trends in Plant Science*, 22, 20-29.

Hodge, J.E. & Hofreiter, B.T. (1962) Determination of reducing sugars and carbohydrates. In: Whistler, R.L. & Wolfrom, M.L. (Eds.): Methods in Carbohydrate Chemistry. New York: Academic Press, pp. 380-394.

Huqe, M.A.S., Haque, M.S., Sagar, A. et al. (2021) Characterization of maize hybrids (*Zea mays* L.) for detecting salt tolerance based on morpho-physiological characteristics, ion accumulation and genetic variability at early vegetative stage. *Plants*, 10, 2549.

Ismail, A.M. & Horie, T. (2017) Genomics, physiology, and molecular breeding approaches for improving salt tolerance. *Annual Review of Plant Biology*, 68, 405-434.

Lamers, J., van der Meer, T. & Testerink, C. (2020) How plants sense and respond to stressful environments. *Plant Physiology*, 182, 1624-1635.

Le Gall, H., Philippe, F., Domon, J.-M., Gillet, F., Pelloux, J. & Rayon, C. (2015) Cell wall metabolism in response to abiotic stress. *Plants*, 4, 112-166.

Liu, H., Pan, L., Ahmad, I. et al. (2025) Study on the mechanism of exogenous $CaCl_2$ regulating cell growth and development to alleviate salt tolerance of alfalfa (*Medicago sativa*). *Frontiers in Plant Science*, 16, 1565723.

Liu, J., Otie, V., Matsuura, A. et al. (2022a) Pectin characteristics affect root growth in spinach under salinity. *Plants*, 11, 3130.

Liu, J., Shao, Y., Feng, X. et al. (2022b) Cell wall components and extensibility regulate root growth in *Suaeda salsa* and *Spinacia oleracea* under salinity. *Plants*, 11, 900.

Loix, C., Huybrechts, M., Vangronsveld, J., Gielen, M., Keunen, E. & Cuypers, A. (2017) Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. *Frontiers in Plant Science*, 8, 1867.

Meychik, N., Nikolaeva, Y. & Kushunina, M. (2021) The significance of ion-exchange properties of plant root cell walls for nutrient and water uptake by plants. *Plant Physiology and Biochemistry*, 166, 140-147.

Meychik, N.R., Lyubimova, E.G. & Yermakov, I.P. (2010a) Ion-exchange properties of the cell wall of reindeer lichen *Cladonia rangiferina*. *Russian Journal of Plant Physiology*, 57, 260-266.

Meychik, N.R., Nikolaeva, J.I. & Yermakov, I.P. (2005) Ion exchange properties of the root cell walls isolated from the halophyte plants (*Suaeda altissima* L.) grown under conditions of different salinity. *Plant and Soil*, 277, 163-174.

Meychik, N.R. & Yermakov, I.P. (2001) Ion exchange properties of plant root cell walls. *Plant and Soil*, 234, 181-193.

Meychik, N.R., Yermakov, I.P., Khonarmand, S.D. & Nikolaeva, Y.I. (2010b) Ion-exchange properties of cell walls in chickpea cultivars with different sensitivities to salinity. *Russian Journal of Plant Physiology*, 57, 620-630.

Meychik, N.R., Yermakov, I.P. & Savvateeva, M.V. (1999) Ionogenic groups in the cell wall of wheat roots. *Russian Journal of Plant Physiology*, 46, 742-747.

Munns, R., Day, D.A., Fricke, W. et al. (2020) Energy costs of salt tolerance in crop plants. *New Phytologist*, 225, 1072-1090.

Munns, R. & Tester, M. (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59, 651-681.

Otie, V., Ibrahim, A., Udo, I. et al. (2022) Foliarly applied 24-epibrassinolide modulates the electrical conductivity of the saturated rhizospheric soil extracts of soybean under salinity stress. *Plants*, 11, 2330.

Peaucelle, A., Braybrook, S. & Höfte, H. (2012) Cell wall mechanics and growth control in plants: the role of pectins revisited. *Frontiers in Plant Science*, 3, 121.

Rahman, M.M., Mostofa, M.G., Keya, S.S. et al. (2021) Adaptive mechanisms of halophytes and their potential in improving salinity tolerance in plants. *International Journal of Molecular Sciences*, 22, 10733.

Rana, V., Ram, S., Sendhil, R., Nehra K. & Sharma, I. (2015) Physiological, biochemical and morphological study in wheat (*Triticum aestivum* L.) RILs population for salinity tolerance. *Journal of Agricultural Science*, 7, 119-128.

Raza, M.A., Saeed, A., Munir, H. et al. (2017) Screening of tomato genotypes for salinity tolerance based on early growth attributes and leaf inorganic osmolytes. *Archives of Agronomy and Soil Science*, 63, 501-512.

Reina-Sánchez, A., Romero-Aranda, R. & Cuartero, J. (2005) Plant water uptake and water efficiency of greenhouse tomato cultivars irrigated with saline water. *Agricultural Water Management*, 78, 54-66.

Rozema, J. & Schat, H. (2013) Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. *Environmental and Experimental Botany*, 92, 83-95.

Shabala, S. (2013) Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. *Annals of Botany*, 112, 1209-1221.

Sivakumar, J., Prashanth, J.E.P., Rajesh, N., Reddy, S.M. & Pinjari, O.B. (2020) Principal component analysis approach for comprehensive screening of salt stress-tolerant tomato germplasm at the seedling stage. *Journal of Biosciences*, 45, 141.

Tanveer, K., Gilani, S., Hussain, Z., Ishaq, R., Adeel, M. & Ilyas, N. (2019) Effect of salt stress on tomato plant and the role of calcium. *Journal of Plant Nutrition*, 43, 28-35.

Uddin, M.N., Hanstein, S., Leubner, R. & Schubert, S. (2013) Leaf cell-wall components as influenced in the first phase of salt stress in three maize (*Zea mays* L.) hybrids differing in salt resistance. *Journal of Agronomy and Crop Science*, 199, 405-415.

Volkov, V. (2015) Salinity tolerance in plants. Quantitative approach to ion transport starting from halophytes and stepping to generic and protein engineering for manipulating ion fluxes. *Frontiers in Plant Science*, 6, 873.

Zaki, H.E.M. & Yokoi, S. (2016) A comparative *in vitro* study of salt tolerance in cultivated tomato and related wild species. *Plant Biotechnology*, 33, 361-372.

Zhang, S.-S., Sun, L., Dong, X., Lu, S.-J., Tian, W. & Liu, J.-X. (2016) Cellulose synthesis genes *CESA6* and *CSI1* are important for salt stress tolerance in *Arabidopsis*. *Journal of Integrative Plant Biology*, 58, 623-626.

Zhao, C., Zayed, O., Zeng, F. et al. (2019) Arabinose biosynthesis is critical for salt stress tolerance in *Arabidopsis*. *New Phytologist*, 224, 274-290.

Zhong, H. & Lauchli, A. (1993) Changes of cell wall composition and polymer size in primary roots of cotton seedlings under high salinity. *Journal of Experimental Botany*, 44, 773-778.