

## MULTISTAGE SCREENING OF TOMATO (*SOLANUM LYCOPERSICUM*) GERMPLASM FOR SALT STRESS TOLERANCE USING MORPHOLOGICAL AND IONIC TRAITS

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### Abstract

Soil salinity is a major abiotic stress limiting global tomato (*Solanum lycopersicum* L.) production, highlighting the need to develop salt-tolerant genotypes for sustainable cultivation in saline environments. In this study, 101 tomato accessions were evaluated under controlled conditions using a sand-based system supplemented with Hoagland solution. Salinity stress was induced by applying NaCl to achieve electrical conductivity (EC) levels of 6 and 12 dS m<sup>-1</sup> at both seedling and reproductive stages. Morphological and ionic traits were assessed, and genotypic responses were analyzed using principal component analysis, correlation, and cluster analysis. Significant genotypic variability in salt tolerance was observed. At the seedling stage, accessions such as CLN-2498-A, Picdenato, AUT-318, and 17263 exhibited superior seedling performance under 12 dS m<sup>-1</sup>, with moderate reductions in shoot dry weight (up to 53%) and root dry weight (up to 48%). Ionic analysis revealed that these accessions maintained lower increases in Na<sup>+</sup>/K<sup>+</sup> ratios, up to 41% in roots and 50% in leaves, indicating effective ion regulation under stress. At the reproductive stage, CLN-2498-A and 17256 demonstrated superior performance at 12 dS m<sup>-1</sup>, with fruit yield reductions limited to 42% and average fruit weight declined up to 82% compared to the control. Despite considerable increases in Na<sup>+</sup>/K<sup>+</sup> ratios (62% in roots and over 99% in leaves), both accessions sustained better ionic balance than sensitive accessions. These findings identify CLN-2498-A and Picdenato as promising genetic resources for breeding salt-tolerant tomato cultivars, providing valuable insights for enhancing tomato resilience in saline environments and supporting global food security.

**Key words:** Climate change; Abiotic stress; Crop improvement; Sustainable agriculture; Precision agriculture

### Introduction

Salinity is a key abiotic stress threatening global crop production and food security, especially in arid and semi-arid regions (Julkowska & Testerink, 2015; Fatima *et al.*, 2022a). Over 1,125 million hectares of land are salt-affected globally, with 76 million hectares impacted by anthropogenic salinization and approximately 1.5 million hectares becoming unfit for agriculture annually due to salinity buildup (Harper *et al.*, 2021; Stavi *et al.*, 2021; Atta *et al.*, 2023). This stress affects nearly 20% of all cultivated lands and 33% of irrigated areas worldwide (Roşca *et al.*, 2023). Globally, over 50% of salt-affected soils are classified as sodic or saline-sodic, with Asia accounting for 327 million hectares, including 4.2 million hectares in Pakistan (Rehman *et al.*, 2021).

Salinity impairs plant growth through osmotic stress, ion toxicity, and nutrient imbalance, mainly due to excessive sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ions (Zhao *et al.*, 2020; Guo *et al.*, 2022). This ionic imbalance disrupts physiological processes such as photosynthesis, enzyme activity, and nutrient uptake (Julkowska & Testerink, 2015; Alexopoulos *et al.*, 2023; Kumar *et al.*, 2023; Seymen *et al.*, 2023), ultimately reducing biomass and yield. Salinity stress particularly reduces potassium (K<sup>+</sup>) uptake, and the Na<sup>+</sup>/K<sup>+</sup> ratio is widely used as an indicator of salt tolerance for its impact on membrane stability and enzyme regulation (Wu *et al.*, 2020). Plant adaptation to salinity involves three

key mechanisms: osmotic tolerance, ion exclusion, and tissue tolerance (Munns & Tester, 2008; Roşca *et al.*, 2023). Osmotic tolerance helps maintain growth by minimizing water loss and preserving leaf expansion and stomatal function. Ion exclusion limits Na<sup>+</sup> accumulation in shoots and leaves. Tissue tolerance protects cells by removing Na<sup>+</sup> from the cytosol and sequestering it into vacuoles (Roşca *et al.*, 2023).

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop worldwide in terms of economic value and dietary contribution (Quinet *et al.*, 2019). However, it is moderately sensitive to salinity. Yield declines by approximately 7.2% for every unit increase in EC beyond 2.5 dS m<sup>-1</sup>, with marked reductions above 5 dS m<sup>-1</sup> (Zhang *et al.*, 2016; Fatima *et al.*, 2022b). While wild relatives of tomato *Solanum pimpinellifolium*, *Solanum cheesmaniae* and *Solanum peruvianum*, have inherent salt tolerance, most cultivated varieties lack these traits due to domestication bottlenecks (Pailles *et al.*, 2020; Saeed & Fatima, 2021). This highlights the need for identifying and utilizing genetic variation for salt tolerance in breeding programs.

Assessing salinity tolerance at a single growth stage may not fully capture the dynamic nature of plant responses to salt stress. Therefore, screening at both the seedling and reproductive stages is crucial, as genotypes may exhibit differential tolerance depending on developmental timing and salt exposure (Choudhary *et al.*, 2021; Dustgeer *et al.*, 2021). Traits like low Na<sup>+</sup>/K<sup>+</sup> ratios and high biomass

support seedling vigor under salinity. However, these traits may not directly lead to better performance at the reproductive stage. Reproductive success is more closely linked to fruit yield, flowering traits, and stable ionic balance (Shimul *et al.*, 2014). Several studies in tomato and other crops have demonstrated that comprehensive, stage-specific evaluation enhanced the identification of stable, salt-tolerant genotypes suitable for sustained performance under stress (Pailles *et al.*, 2020; Alam *et al.*, 2021; Zafar *et al.*, 2022). This approach is especially important in tomato breeding, where reproductive traits are directly tied to commercial yield. Various screening techniques have been employed to assess salinity tolerance, including hydroponic systems (Rosadi *et al.*, 2014; Ntanasi *et al.*, 2023), sand culture (Kakar *et al.*, 2019; Kayess *et al.*, 2020), and lysimeters under a field rain (Gao *et al.*, 2025). Unlike hydroponics, pot culture better mimics soil-root interactions while allowing controlled saline irrigation. Here, we used pot-based screening at 6 and 12 dS m<sup>-1</sup> to reliably evaluate genotype performance under realistic salinity conditions.

Quantifying genotype responses to salinity requires integrated screening tools. Multivariate techniques such as Principal Component Analysis (PCA), hierarchical clustering, and correlation analysis enable the identification of salt-tolerant genotypes based on multiple traits. PCA, in particular, simplifies data interpretation by highlighting the most influential variables under stress (Sivakumar *et al.*, 2020; Sinha *et al.*, 2021). Complementary methods like correlation analysis help reveal trait relationships, while cluster analysis groups genotypes based on performance. The integration of these tools enables a more comprehensive understanding of trait relationships and genotype classification under stress conditions, collectively offering a robust approach to assess complex traits associated with

salinity tolerance (Shushay *et al.*, 2014; Kissoudis *et al.*, 2015; Oyiga *et al.*, 2016). Despite the availability of salt-tolerant wild relatives, breeding efforts are hindered by the lack of comprehensive phenotypic data across multiple growth stages. Moreover, few studies have integrated multivariate statistical approaches to jointly evaluate seedling vigor and reproductive performance under salinity (Villalta *et al.*, 2008; Rosadi *et al.*, 2014; Rehman *et al.*, 2019). Therefore, this study addresses a critical gap by screening a large panel of tomato genotypes at both developmental stages using PCA, correlation, and cluster analysis to identify genotypes with consistent tolerance.

In this study, 101 tomato accessions were evaluated under moderate and high salinity (6 and 12 dS m<sup>-1</sup>) to assess their responses at seedling and reproductive stages. Morphological traits and ionic parameters, particularly Na<sup>+</sup>/K<sup>+</sup> ratios in roots and leaves, were measured to assess tolerance levels. Data were analyzed using PCA, correlation, and cluster analysis to identify stable salt-tolerant genotypes for future breeding. This research aims to contribute in developing salt-resilient tomato cultivars suitable for saline environments.

## Material and Methods

### Collection of germplasm and nursery establishment:

Tomato accessions (*Solanum lycopersicum* L.) 101 were collected from the Ayub Agricultural Research Institute, Faisalabad (AARI), Plant Genetic Resources Institute, Islamabad (PGRI), Pakistan, The Nuclear Institute for Agriculture and Biology, Faisalabad (NIAB), Pakistan and University of Agriculture Faisalabad (UAF), Pakistan (Table 1). The seeds were sown in nursery trays, regularly sprayed with distilled water to maintain adequate moisture level and incubated under controlled conditions.

Table 1. Names of tomato accessions.

Genotype name	institute name	Genotype name	Institute name	Genotype name	Institute name	Genotype name	Institute name
6234	PBG	17859	PGRI	19897	PGRI	Legend	AARI
7040	AARI	17862	PGRI	19898	PGRI	LO-4279	PBG
9065	AARI	17868	PGRI	19899	PGRI	LYP-1	NIAB
10120	AARI	17874	PGRI	19900	PGRI	M82	PBG
10160	AARI	17876	PGRI	19903	PGRI	Money Maker	NIAB
10173	AARI	17884	PGRI	19905	PGRI	Nadir	AARI
13202	AARI	17902	PGRI	19907	PGRI	Nagina	AARI
13205	AARI	17903	PGRI	19908	PGRI	Naqeeb	AARI
13215	AARI	18278	AARI	Aut-305	AARI	NIAB Gohar	NIAB
13246	AARI	18285	AARI	Aut-318	AARI	NIAB Jauhar	NIAB
16244	AARI	18290	AARI	BA-1079	PBG	Pakit	AARI
16245	AARI	18291	AARI	BGH-24	PBG	PB-017895	PBG
17254	AARI	18298	AARI	Cchaus	NIAB	PB-LO-017902	PBG
17255	AARI	19290	PGRI	CLN-2001-A	PBG	Peelo	NIAB
17256	AARI	19292	PGRI	CLN-2366	PBG	Peto 86	NIAB
17260	AARI	19842	PGRI	CLN-2413	PBG	Picdenato	NIAB
17261	AARI	19843	PGRI	CLN-2418	PBG	Pioneer-2761	PBG
17263	AARI	19844	PGRI	CLN-2498-A	PBG	Riogrande	AARI
17265	AARI	19850	PGRI	CLN2498-D	PBG	Roma	AARI
17266	AARI	19857	PGRI	CLN-3552-D	PBG	Saad-49	AARI
17268	AARI	19860	PGRI	Dominator	PBG	Sandal	PBG
17269	AARI	19865	PGRI	Galia	NIAB	ST-11	PBG,
17270	AARI	19888	PGRI	H-24	NIAB	Sub-Arctic	PBG
17271	AARI	19892	PGRI	Immune Prior Beta	PBG	Target-T-66	AARI
17272	AARI	19895	PGRI	Kanatoo	PBG	Zarnitza	AARI
17858	PGRI						

AARI: Ayub Agricultural Research Institute PBG Dept. of Plant Breeding and Genetics, UAF, PGRI: Plant Genetic Resource Institute  
NIAB: Nuclear Institute for Agriculture and Biology

**Experiment 1. Germplasm screening at seedling stage:** In the first year, tomato plants at the two-leaf stage were transplanted into polyethene bags containing 900 g of sterilized sand to ensure uniform growth conditions. To supply nutrients, 50 mL of half-strength Hoagland solution was applied every alternate day. The EC of water was less  $<2 \text{ dS m}^{-1}$ . After a seven-day acclimatization period, salinity treatments were initiated by irrigating the plants with sodium chloride (NaCl) solutions at electrical conductivity (EC) levels of  $6 \text{ dS m}^{-1}$  and  $12 \text{ dS m}^{-1}$ . Control plants were maintained under identical conditions (temperature  $20 \pm 3^\circ\text{C}$ , humidity 60-70%, photoperiod  $12 \pm 1$  hours) without NaCl supplementation. Plants were subjected to these treatments for 28 days to simulate salt stress. At the end of the treatment period, seedlings from both salt-treated and control groups were harvested carefully to prevent tissue damage for morphological and ionic analyses. Each treatment group included three biological replicates to ensure accuracy and reproducibility.

**Experiment 2. Confirmation of selected genotypes at the reproductive stage:** Experiment 2 was designed to validate the salt tolerance observed at the seedling stage and to compare the responses of both tolerant and susceptible genotypes under prolonged stress. Seven accessions with the lowest  $\text{Na}^+/\text{K}^+$  ratios, highest biomass retention, and a few susceptible genotypes identified at the seedling stage were further evaluated at the reproductive stage to confirm the genotype selection against salt stress. Plants were transplanted into pots (30 cm top diameter  $\times$  45 cm height), containing 8 kg of soil ( $\text{EC} < 1 \text{ dS m}^{-1}$ ), and standard cultural practices, including irrigation (water  $\text{EC} < 1.5 \text{ dS m}^{-1}$ ), weeding, and fertilization, were applied to ensure optimal growth. Each pot was supplemented with the recommended dose of NPK (58:23:32). Fertilizer was uniformly applied during plant transplantation to ensure consistent nutrient availability. Ropes were used to support the plants and minimize physical stress. Throughout the experiment, plants were maintained under controlled environmental conditions with a temperature of  $26 \pm 3^\circ\text{C}$ , relative humidity of 60-70%, and a photoperiod of  $12 \pm 1$  hours. Two weeks post-transplantation, salinity treatments were applied using NaCl solutions at EC levels of  $6 \text{ dS m}^{-1}$  and  $12 \text{ dS m}^{-1}$ , along with a control group that received no additional salt. Each treatment group consisted of three biological replicates. To maintain consistent salinity levels ( $6$  and  $12 \text{ dS m}^{-1}$ ), the EC of the soil solution was regularly monitored using a portable EC meter, and NaCl solutions were adjusted accordingly throughout the experiment.

**Morphological attributes:** At the seedling stage, root and shoot lengths were measured using a ruler, and fresh and dry weights were recorded with an electronic balance (Ors *et al.*, 2021; Ntanasi *et al.*, 2023). At the reproductive stage, days to first flowering and first fruit initiation were recorded from the date of sowing. The number of trusses per plant, flowers per truss, and leaf area were measured, while individual fruit weight, width, and length were also assessed. Total fruit yield per plant was recorded at the red-ripe stage (Oztekin & Tuzel, 2011; Wu *et al.*, 2021; Eynizadeh *et al.*, 2023).

**Ionic attributes:** Quantification of sodium ( $\text{Na}^+$  ppm) and potassium ( $\text{K}^+$  ppm) in root and leaf tissues was conducted using a digestion-based protocol (Wolf, 1982). Dried samples (0.5 g) of leaf and root tissues at the seedling and

reproductive stage were digested with 5 mL of a 3:1 mixture of nitric acid ( $\text{HNO}_3$ ) and perchloric acid ( $\text{HClO}_4$ ) in digestion flasks. The flasks were sealed with aluminum foil and left undisturbed overnight. The next day, the samples were heated on a hotplate until fume production indicated digestion completion, and the solution turned colourless. The resulting extract was diluted with distilled water to a final volume of 50 mL and filtered through Whatman filter paper. Sodium and potassium concentrations in the filtrate were then measured using a flame photometer (Jenway Model, UK, 1998).

## Statistical analysis

Both experiments were analyzed as two-factor CRD designs with three replications. Descriptive statistics were computed using Microsoft Excel and Statistix 8.1. Data were subjected to principal component analysis (PCA) for each salinity treatment using the R “FactoMineR” package, and biplots were generated. **Correlation analysis** (R “corrplot”) and **cluster analysis** (R “agricolae”) were performed to reveal trait relationships and group genotypes by performance. All analyses were conducted in R Studio.

## Results

### Seedling stage

**a. Descriptive statistics:** Descriptive statistics from the two-factor factorial analysis indicated significant genotypic variation ( $p < 0.01$ ) among tomato accessions under salinity stress at the seedling stage. Increasing salinity levels (0, 6, and  $12 \text{ dS m}^{-1}$ ) significantly reduced seedling growth. At  $12 \text{ dS m}^{-1}$ , shoot length, root length, shoot fresh weight, and root fresh weight declined by up to 56%, 76%, 50%, and 92%, respectively, compared to the control. Shoot and root dry weights exhibited slightly lower reductions (up to 75%). A significant genotype  $\times$  salinity interaction ( $p < 0.01$ ) confirmed genotype-specific responses to salinity stress. Accessions CLN-2498-A, 17256, AUT-318, and 17263 exhibited superior seedling performance under salinity stress, showing relatively moderate reductions in shoot fresh weight (up to 35%), root fresh weight (up to 59%), shoot dry weight (up to 53%), and root dry weight (up to 59%) at  $12 \text{ dS m}^{-1}$ . Ionic analysis further revealed that CLN-2498-A, Picdenato, and AUT-318 maintained lower increases in  $\text{Na}^+/\text{K}^+$  ratios, up to 41% in roots and 50% in leaves-suggesting effective  $\text{Na}^+$  exclusion and  $\text{K}^+$  retention mechanisms (Table 2).

**b. PCA:** Principal Component Analysis (PCA) biplots (Fig. 1A–C) illustrated genotypic variation in morpho-ionic traits under control, moderate ( $6 \text{ dS m}^{-1}$ ), and high ( $12 \text{ dS m}^{-1}$ ) salinity. Accessions near biomass-related vectors (e.g., shoot/root fresh and dry weights) were identified as salt-tolerant, exhibiting both high biomass and low  $\text{Na}^+/\text{K}^+$  ratios. These accessions showed strong ion regulation and efficient physiological responses under stress. In contrast, the accessions aligning with high  $\text{Na}^+$  and  $\text{Na}^+/\text{K}^+$  vectors were sensitive. Notably, CLN-2498-A, 17263, Picdenato, 17256, and AUT-318 consistently clustered near biomass traits under 6 and  $12 \text{ dS m}^{-1}$ . These accessions showed strong ion regulation and efficient

physiological responses under stress. In contrast, Nadir, 19908, and 17255 clustered with traits associated with ionic toxicity and reduced biomass.

- PCA variance explanation:
- Control: Dim1 = 27.2% (ionic traits), Dim2 = 16.5% (biomass).
- 6 dS m<sup>-1</sup>: Dim1 = 32.2% (ionic traits), Dim2 = 16.8% (biomass).
- 12 dS m<sup>-1</sup>: Dim1 = 29.7% (ionic traits), Dim2 = 17.0% (biomass).

**c. Cluster analysis:** Cluster analysis (Fig. 3A–C) grouped the 101 accessions into 5 clusters across salinity levels based on morpho-ionic traits (squared Euclidean distance). Under control conditions, cluster I included salt-tolerant accessions such as CLN-2498-A, 17256, Picendanto, AUT-318, 17260, and 17263, exhibiting higher root and shoot biomass, along with lower Na<sup>+</sup> accumulation and reduced Na<sup>+</sup>/K<sup>+</sup> ratios. In contrast, accessions like Nadir, 17255, and 19908 were grouped into separate clusters, characterized by higher Na<sup>+</sup> content and lower biomass, indicating baseline sensitivity. Under 6 dS m<sup>-1</sup> salinity, clustering again revealed five distinct groups. Accessions CLN-2498-A, 17256, Picendanto, AUT-318, 17263, and 17266 clustered together, maintaining their superior morpho-ionic performance under moderate salt stress. While 17256, CLN-2498-A and Picendanto at 12 dS m<sup>-1</sup>, remained in the same high-performing cluster, AUT-318 shifted to a different cluster, suggesting a variation in its tolerance mechanisms under severe stress. Meanwhile, Nadir, 17255, and 19908 consistently clustered with accessions exhibiting elevated Na<sup>+</sup>/K<sup>+</sup> ratios and reduced biomass across stress levels, confirming their high sensitivity to salinity.

**d. Correlation analysis:** At the seedling stage, Pearson's correlation analysis (Fig. 5) revealed several significant associations among morpho-ionic traits under both control and salinity stress conditions. Root length exhibited a highly significant ( $p < 0.01$ ) positive correlation with root fresh weight, shoot fresh weight, and dry weight, indicating its critical role in biomass accumulation under saline conditions. Leaf potassium (K<sup>+</sup>) concentration was strongly and negatively correlated with the leaf sodium-to-potassium ratio (Na<sup>+</sup>/K<sup>+</sup>), highlighting the importance of K<sup>+</sup> homeostasis and sodium exclusion. Furthermore, the root Na<sup>+</sup>/K<sup>+</sup> ratio showed a positive correlation with both root Na<sup>+</sup> concentration and leaf Na<sup>+</sup>/K<sup>+</sup> ratio, reflecting systemic ionic imbalance in sensitive accessions.

## Reproductive stage

**a. Descriptive statistics:** At the reproductive stage, salinity stress significantly affected key growth and yield parameters, with genotypic variation remaining highly significant ( $p < 0.01$ ) (Table 3). Increasing salinity levels delayed flowering, reduced fruit yield, and disrupted ion homeostasis across accessions. At 12 dS m<sup>-1</sup>, notable reductions were observed in fruit yield and fruit weight, while the number of fruits per truss and Na<sup>+</sup>/K<sup>+</sup> ratios varied significantly among accessions. Accessions such as CLN-2498-A and 17256 showed superior performance under stress conditions. Fruit yield in these accessions was reduced by only 42%, and average fruit weight by 82%, compared to the control. Interestingly, they exhibited decrease upto 14%

in the number of fruits per truss, suggesting a possible compensatory mechanism. Despite increases in root and leaf Na<sup>+</sup>/K<sup>+</sup> ratios by 31% and over 99%, respectively, they maintained relatively efficient ion regulation compared to sensitive accessions. In contrast, accessions like 17255 and Nadir exhibited heightened sensitivity under salinity stress. Their Na<sup>+</sup>/K<sup>+</sup> ratios in both root and leaf tissues increased by over 100%, corresponding with a 50% reduction in fruit yield. Although these accessions showed up to a 20% increase in the number of fruits per truss, the overall reproductive performance declined, likely due to cumulative ionic toxicity affecting fruit development and filling.

**b. PCA:** Principal Component Analysis (PCA) biplots (Fig. 2A–C) further illustrated genotypic variation in morpho-ionic traits under control, moderate (6 dS m<sup>-1</sup>), and high (12 dS m<sup>-1</sup>) salinity treatments. Accessions clustered near yield-related vectors such as fruit yield, fruit weight, number of fruits per truss, and plant height were identified as salt-tolerant. These accessions demonstrated strong ion regulation and morphological resilience under stress, reflected in their ability to maintain productivity and favorable Na<sup>+</sup>/K<sup>+</sup> ratios. In contrast, accessions clustered near vectors representing high Na<sup>+</sup> concentrations in leaf and root tissues and elevated Na<sup>+</sup>/K<sup>+</sup> ratios were classified as salt-sensitive, showing disrupted ion homeostasis and reduced yield. CLN-2498-A, 17256 and AUT-318 consistently clustered near yield-related vectors under both 6 and 12 dS m<sup>-1</sup>, reaffirming their tolerance. Meanwhile, sensitive accessions like Nadir, 19908, and 17255 grouped with vectors linked to high Na<sup>+</sup> and Na<sup>+</sup>/K<sup>+</sup> ratios, indicating ionic toxicity and diminished reproductive success.

PCA variance explanation:

- Control: Dim1 = 37.7% (ionic traits), Dim2 = 20.3% (yield traits).
- 6 dS m<sup>-1</sup>: Dim1 = 48.1% (ionic traits), Dim2 = 18.8% (yield traits).
- 12 dS m<sup>-1</sup>: Dim1 = 61.0% (ionic traits), Dim2 = 13.2% (yield traits).

**c. Cluster analysis:** At the reproductive stage, 10 selected tomato accessions were classified into two main clusters under all three salinity treatments, control, 6 dS m<sup>-1</sup>, and 12 dS m<sup>-1</sup>, based on fruit yield and ion accumulation traits (Fig. 4A–C). Under control conditions, first group comprised of accessions 17255 and Nadir, both of which exhibited higher baseline Na<sup>+</sup>/K<sup>+</sup> ratios and comparatively lower fruit yield than the rest of the accessions. Group second contained the remaining 8 accessions, which maintained better ionic balance and yield performance. Under 6 dS m<sup>-1</sup> and 12 dS m<sup>-1</sup> salinity stress, first group comprised of accessions Nadir, 17255, and 19908. These accessions showed a sharp increase in Na<sup>+</sup> accumulation and Na<sup>+</sup>/K<sup>+</sup> ratios in both roots and leaves, accompanied by substantial yield losses. Their grouping indicated a shared sensitivity to salinity stress. In contrast, second group comprised of accessions CLN-2498-A, 17256, and AUT-318, which consistently maintained lower Na<sup>+</sup>/K<sup>+</sup> ratios and experienced less reduction in fruit yield and weight, indicating stronger tolerance mechanisms. This consistent pattern of clustering across salinity levels demonstrated a distinct separation between tolerant and sensitive accessions, emphasizing the critical role of ion regulation and yield stability in determining salinity tolerance at the reproductive stage.

Table 2. Genotypic, treatment, and interaction effects on biomass and ionic balance in tomato seedlings.

Traits	Control Range	Control Mean $\pm$ SE	Stress 1 Range (6 dS m <sup>-1</sup> )	Stress 1 Mean $\pm$ SE	Stress 2 Range (12 dS m <sup>-1</sup> )	Stress 2 Mean $\pm$ SE	MSS (Genotype)	MSS (Treatment)	MSS (G $\times$ T Interaction)
Shoot length	14-66	41.57 $\pm$ 0.50	11.00-60.00	39.73 $\pm$ 0.53	16.00-61.00	39.72 $\pm$ 0.49	294.42**	409.56**	119.11**
Shoot fresh weight	1.03-11.00	4.75 $\pm$ 0.10	0.24-11.12	5.20 $\pm$ 0.12	0.92-10.17	4.73 $\pm$ 0.10	16.33**	21.40**	5.79**
Shoot dry weight	0.10-1.89	0.47 $\pm$ 0.01	0.05-1.25	0.48 $\pm$ 0.01	0.04-1.14	0.49 $\pm$ 0.01	0.24**	0.04**	0.10**
Root length	6.00-55	27.16 $\pm$ 0.49	3.00-70	22.73 $\pm$ 0.56	4.00-53.00	28.02 $\pm$ 0.54	252.42**	2439.89**	135.38**
Root fresh weight	0.15-6.25	1.88 $\pm$ 0.06	0.02-4.90	1.48 $\pm$ 0.06	0.13-4.56	1.75 $\pm$ 0.06	5.32**	12.42**	1.55**
Root dry weight	0.01-0.80	0.27 $\pm$ 0.01	0.0040-0.68	0.20 $\pm$ 0.01	0.01-0.79	0.20 $\pm$ 0.01	0.10**	0.45**	0.04**
K <sup>+</sup> Leaves	2224.91-57012.24	31510.92 $\pm$ 606.21	2296.37-70158.24	35118.28 $\pm$ 745.42	2147.33-67078.95	33691.63 $\pm$ 759.11	5.32 $\times$ 10 <sup>8</sup> **	9.90 $\times$ 10 <sup>8</sup> **	4.11 $\times$ 10 <sup>8</sup> **
K <sup>+</sup> Roots	2145.37-51201.41	12997.90 $\pm$ 472.23	805.76 - 66084.27	11862.87 $\pm$ 552.76	350.37-49065.28	12737.76 $\pm$ 581.20	8.69 $\times$ 10 <sup>8</sup> **	2.83 $\times$ 10 <sup>11</sup> **	7.94 $\times$ 10 <sup>8</sup> **
Na <sup>+</sup> Roots	1658.94-58071.08	14745.45 $\pm$ 564.5	2568.74-136203.63	40306.14 $\pm$ 1128.88	520.03-100440.45	47190.12 $\pm$ 1234.90	8.68 $\times$ 10 <sup>7</sup> **	2.19 $\times$ 10 <sup>10</sup> **	8.68 $\times$ 10 <sup>7</sup> **
Na <sup>+</sup> Leaves	460.38-30026.54	7405.22 $\pm$ 167.43	8057.23-189632.2	64363.54 $\pm$ 1314.84	17429.15-196328.77	67337.62 $\pm$ 1266.54	2.6 $\times$ 10 <sup>8</sup> 1**	2.47 $\times$ 10 <sup>8</sup> **	1.4 $\times$ 10 <sup>8</sup> **
Na <sup>+</sup> /K <sup>+</sup> Root	0.18-1.59	1.17 $\pm$ 0.01	0.29-20.48	5.28 $\pm$ 0.23	0.01-99.56	7.27 $\pm$ 0.69	2.78**	92.00**	2.26**
Na <sup>+</sup> /K <sup>+</sup> Leaves	0.02-0.95	0.26 $\pm$ 0.01	0.52-12.13	2.09 $\pm$ 0.08	0.75-8.74	2.26 $\pm$ 0.07	9.77 $\times$ 10 <sup>8</sup> **	2 $\times$ 10 <sup>11</sup> **	7 $\times$ 10 <sup>8</sup> **

\*\*Highly significant at 1% (p&lt;0.01), \*Significant at 5% (p&lt;0.05), MSS: Mean sum of square

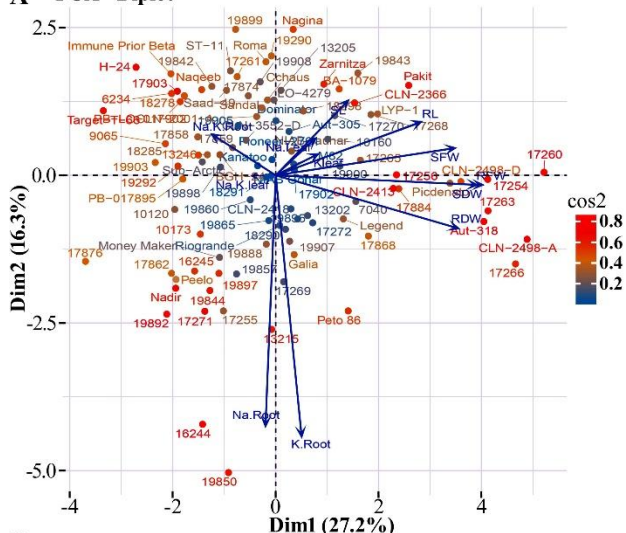
Table 3. Genotypic, treatment, and interaction effects on fruit yield and ionic balance in tomato plants.

Traits	Control Range	Control Mean $\pm$ SE	Stress 1 Range (6 dS m <sup>-1</sup> )	Stress 1 Mean $\pm$ SE	Stress 2 Range (12 dS m <sup>-1</sup> )	Stress 2 Mean $\pm$ SE	MSS (Genotype)	MSS (Treatment)	MSS (G $\times$ T Interaction)
Days to first flower	55.33-73.00	63.36 $\pm$ 0.85	59.33-81.33	67.26 $\pm$ 1.04	48.67-70.67	58.20 $\pm$ 1.16	258.55**	618.96**	12.1**
Days to first fruit	86.33-100.00	91.99 $\pm$ 0.63	85.67-107.67	94.99 $\pm$ 1.12	89.00-109.00	97.56 $\pm$ 1.06	196.49**	232.88**	24.43**
No. of truss per plant	3.67-20.00	8.44 $\pm$ 0.76	3.33-14.67	6.36 $\pm$ 0.49	1.67-10.67	4.90 $\pm$ 0.46	84.54**	95.23**	6.74**
No. of flowers per truss	5.00-6.67	5.72 $\pm$ 0.08	5.00-6.67	5.60 $\pm$ 0.07	5.00-6.00	5.42 $\pm$ 0.06	0.06**	0.68**	0.32**
Leaf area	13.13-28.13	19.20 $\pm$ 0.59	8.37-15.50	11.92 $\pm$ 0.32	4.67-13.93	7.35 $\pm$ 0.35	28.57**	1071.46**	8.15**
Fruit length	24.40-52.57	41.30 $\pm$ 1.33	18.83-45.23	31.26 $\pm$ 1.28	10.83-30.53	20.84 $\pm$ 0.98	355.03**	3139.95**	23.31**
Fruit width	21.33-43.63	34.58 $\pm$ 1.01	13.80-38.43	27.20 $\pm$ 0.18	7.37-29.57	18.91 $\pm$ 1.18	303.72**	1843.42**	27.5**
Fruit weight	6.23-28.93	18.64 $\pm$ 1.16	2.35-22.55	13.71 $\pm$ 1.02	0.50-10.56	4.08 $\pm$ 0.56	5152.56**	1644.22**	37.32**
Fruit yield per plant	0.52-2.06	1.25 $\pm$ 0.08	0.70-1.83	1.15 $\pm$ 0.06	0.60-1.29	0.98 $\pm$ 0.04	0.61**	0.59**	0.18**
Plant height	42.17-131.67	70.22 $\pm$ 4.63	37.50-89.40	49.66 $\pm$ 2.74	13.17-69.43	31.65 $\pm$ 2.86	3106.19**	11173.59**	225.34**
Na <sup>+</sup> Leaves	1375.34-2854.24	1910.86 $\pm$ 68.93	1856.26-3386.04	2390.89 $\pm$ 85.21	1944.06-6975.40	3386.20 $\pm$ 340.62	7343632**	1.7 $\times$ 10 <sup>7</sup> **	2432589**
K <sup>+</sup> leaves	2009.93-5347.89	3794.70 $\pm$ 152.74	1147.22-4592.14	3079.80 $\pm$ 132.63	700.23-3025.15	1980.85 $\pm$ 123.26	3847623.7**	2.5 $\times$ 10 <sup>7</sup> **	472448.95**
Na <sup>+</sup> /K <sup>+</sup> Leaves	0.27-1.30	0.55 $\pm$ 0.04	0.47-2.66	0.88 $\pm$ 0.09	0.71-9.78	2.52 $\pm$ 0.48	11.28**	33.28**	5.43**
Na <sup>+</sup> Roots	457.07-2312.96	1508.18 $\pm$ 94.35	826.50-3910.33	1960.08 $\pm$ 161.05	445.13-6128.87	2775.52 $\pm$ 359.62	9827786.13**	12376525.9**	2722056.26**
K <sup>+</sup> Roots	2002.87-4981.26	2941.18 $\pm$ 140.46	954.02-3125.67	2016.95 $\pm$ 114.04	695.26-2739.86	1578.76 $\pm$ 117.27	2973945.47**	14511997.9**	493902.82**
Na <sup>+</sup> /K <sup>+</sup> Roots	0.18-1.03	0.56 $\pm$ 0.05	0.36-2.27	1.06 $\pm$ 0.10	0.52-7.32	2.39 $\pm$ 0.45	10.76**	26.84**	4.9**

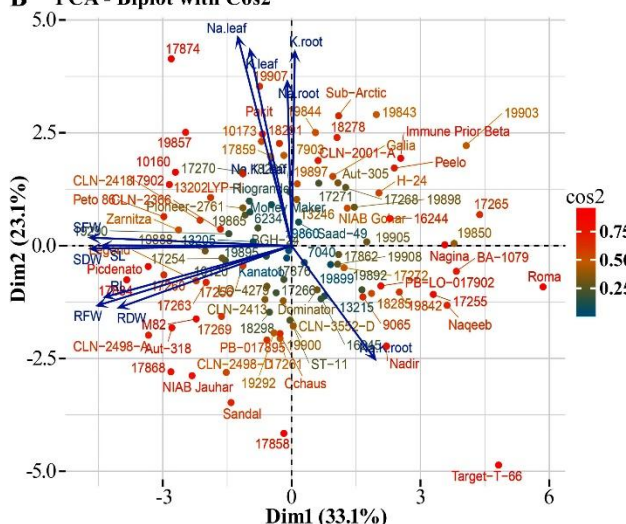
\*\*Highly significant at 1% (p&lt;0.01); \*Significant at 5% (p&lt;0.05); MSS: Mean sum of square



## A PCA - Biplot



## B PCA - Biplot with Cos2



## C PCA - Biplot with Cos2

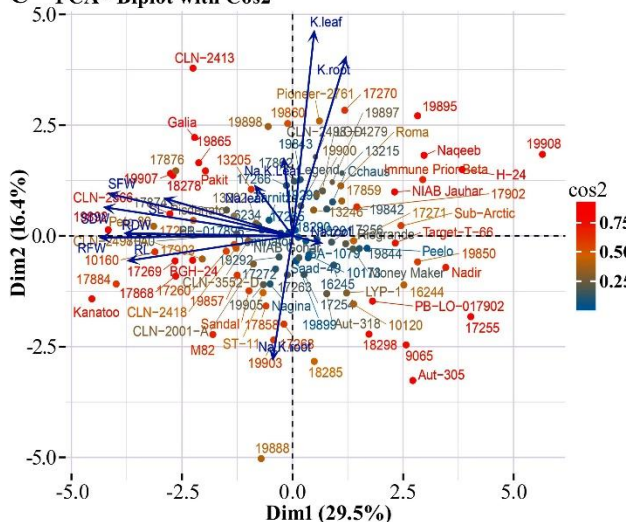
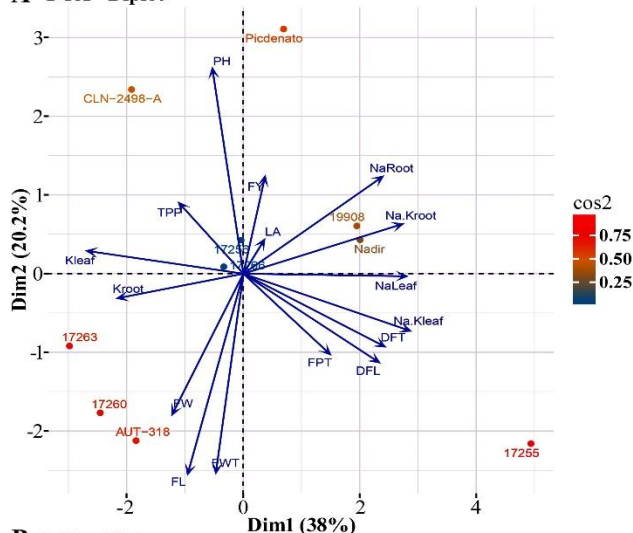


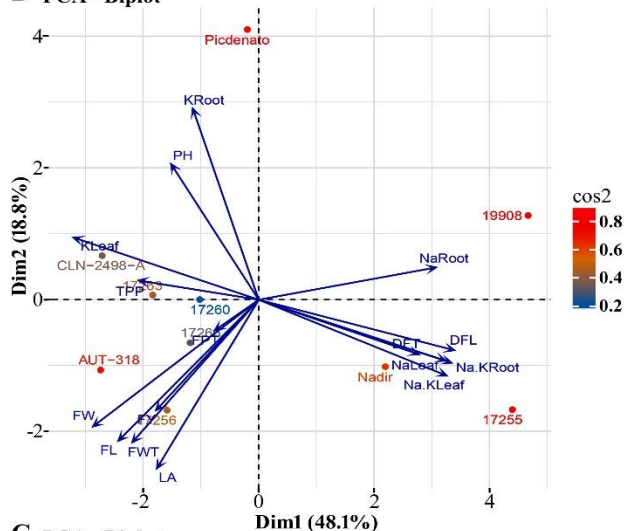
Fig. 1. PCA of tomato germplasm at seedling stage under different salinity levels. (A) Control, (B) 6 dS m<sup>-1</sup> NaCl, (C) 12 dS m<sup>-1</sup> NaCl. Traits: included:

- **Morphological:** SL (shoot length), RL (root length), SFW (shoot fresh weight), SDW (shoot dry weight), RFW (root fresh weight), RDW (root dry weight).
- **Ionic:** Na<sup>+</sup> (sodium), K<sup>+</sup> (potassium) content and Na<sup>+</sup>/K<sup>+</sup> ratio in leaf and root tissues

## A PCA - Biplot



## B PCA - Biplot



## C PCA - Biplot

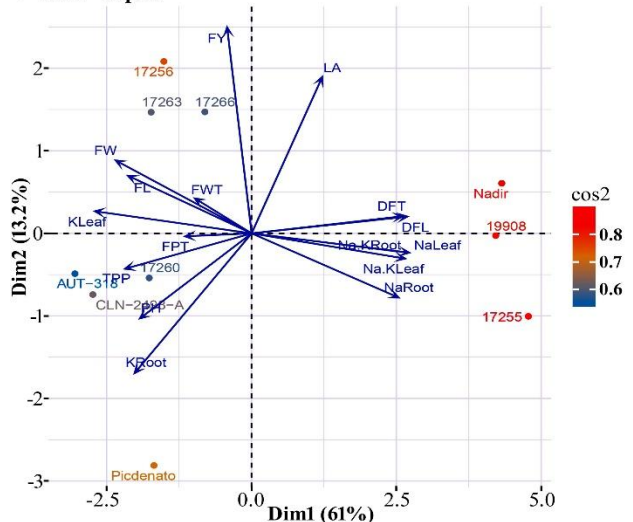


Fig. 2. PCA of tomato germplasm at reproductive stage under different salinity levels. (A) Control, (B) 6 dS m<sup>-1</sup> NaCl, (C) 12 dS m<sup>-1</sup> NaCl. Traits included:

- **Morphological:** FY (fruit yield per plant), LA (leaf area), DFT (days to first fruit), DFL (days to first flower), FW (fruit width), FL (fruit length), FWT (fruit weight), FPT (number of flowers per truss), TPP (number of truss per plant), PH (plant height)
- **Ionic:** Na<sup>+</sup> (sodium), K<sup>+</sup> (potassium) content and Na<sup>+</sup>/K<sup>+</sup> ratio in leaf and root tissues.

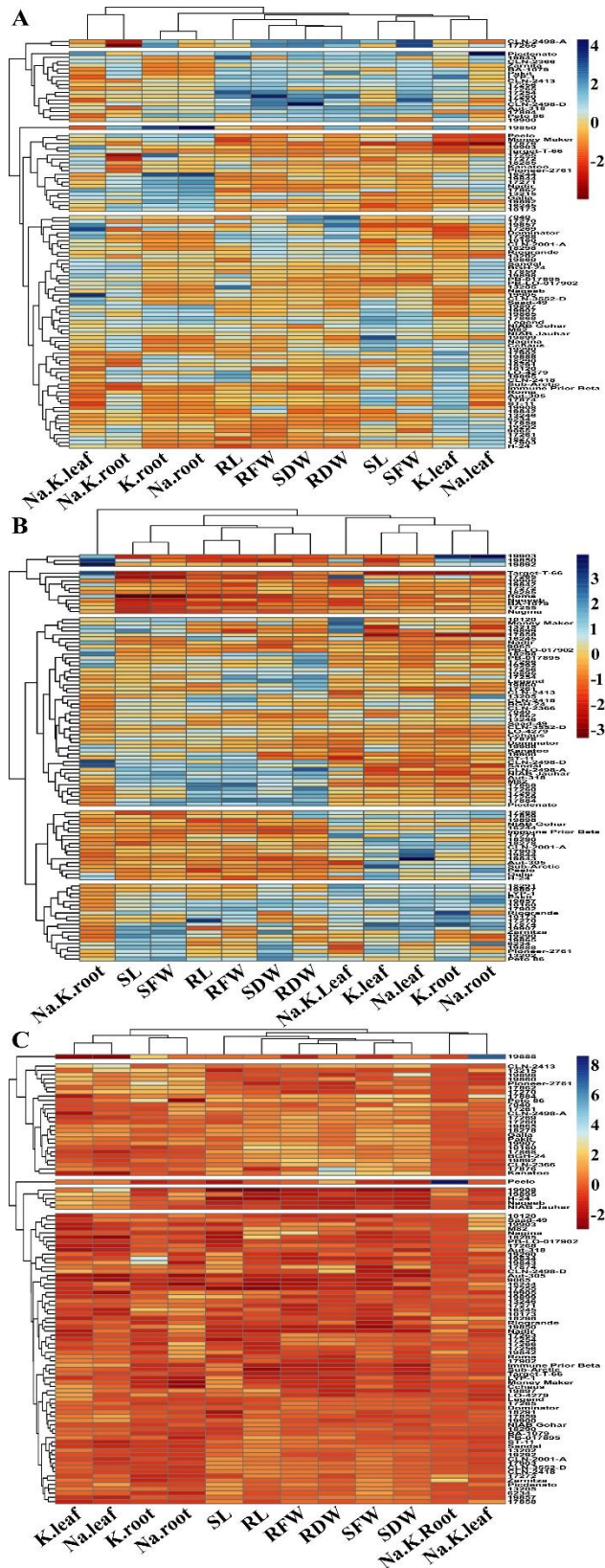


Fig. 3. Cluster analysis of tomato genotypes at seedling stage under different salinity levels: (A) Control (B) 6 dS m<sup>-1</sup> NaCl, (C) 12 dS m<sup>-1</sup> NaCl. Traits: included:

- **Morphological:** SL (shoot length), RL (root length), SFW (shoot fresh weight), SDW (shoot dry weight), RFW (root fresh weight), RDW (root dry weight)
- **Ionic:** Na<sup>+</sup> (sodium), K<sup>+</sup> (potassium) content and Na<sup>+</sup>/K<sup>+</sup> ratio in leaf and root tissues

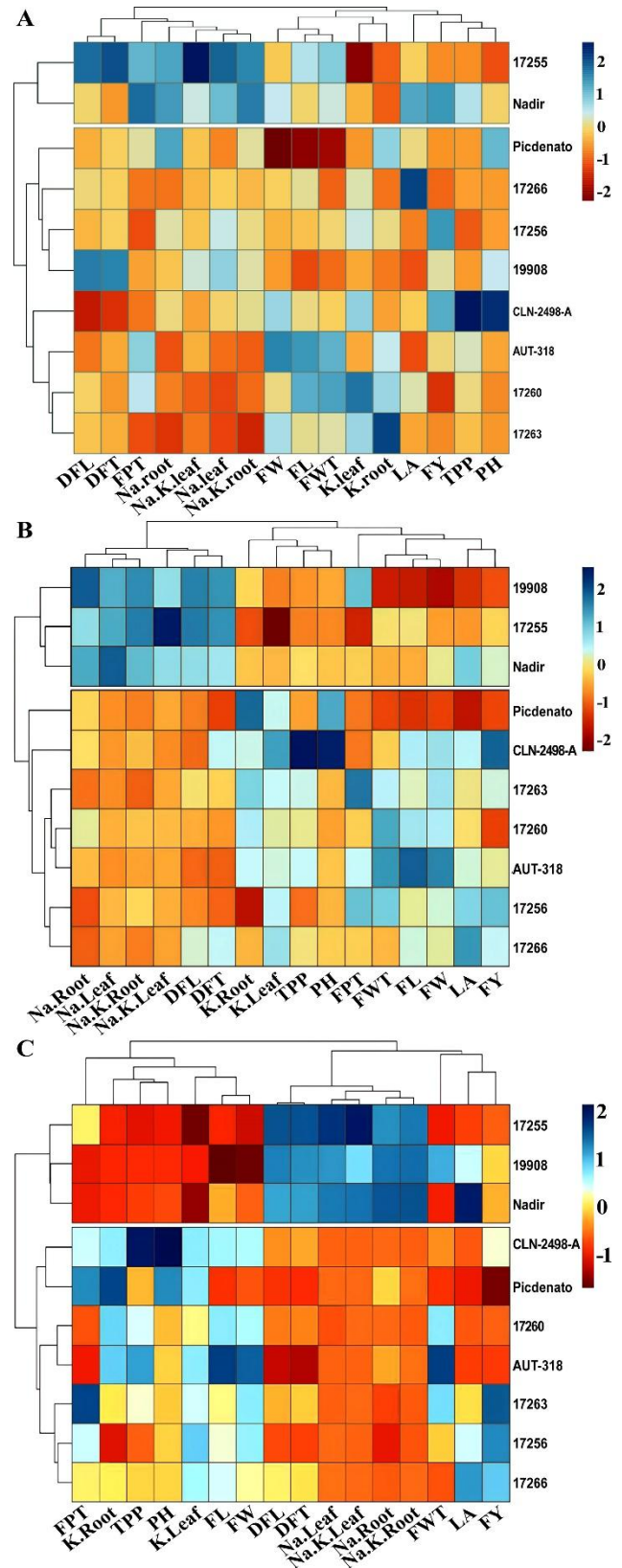


Fig. 4. Cluster analysis of tomato genotypes at reproductive stage under different salinity levels: (A) Control (B) 6 dS m<sup>-1</sup> NaCl, (C) 12 dS m<sup>-1</sup> NaCl. Traits: included:

- **Morphological:** FY (fruit yield per plant), LA (leaf area), DFT (days to first fruit), DFL (days to first flower), FW (fruit width), FL (fruit length), FWT (fruit weight), FPT (number of flowers per truss), TPP (number of truss per plant), PH (plant height)
- **Ionic:** Na<sup>+</sup> (sodium), K<sup>+</sup> (potassium) content and Na<sup>+</sup>/K<sup>+</sup> ratio in leaf and root tissues



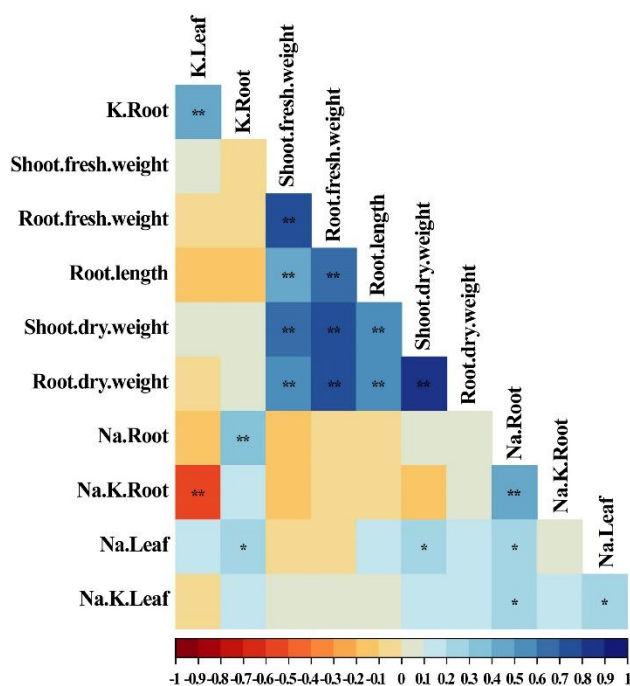


Fig. 5. Pairwise correlations among morphological and ionic traits of tomato germplasm at seedling stage under salinity stress conditions.

**d. Correlation analysis:** At the reproductive stage, correlation analysis (Fig. 6) revealed tightly coordinated relationships among reproductive and ionic traits. Days to first flowering showed a strong positive correlation with days to first fruit formation, indicating synchronized developmental timing. Fruit weight exhibited a positive correlation with fruit length and width, demonstrating their collective contribution to overall yield. Fruit yield per plant was strongly and positively associated with leaf area, emphasizing the influence of vegetative vigor on reproductive output. Notably, leaf and root  $K^+$  concentrations were negatively correlated with days to first fruit formation and the number of flowers per truss, suggesting a potential trade-off between stress adaptation and reproductive timing.

## Discussion

The findings demonstrate that salinity stress significantly affects both vegetative and reproductive growth in tomatoes, with considerable genotypic variation at both stages.

At the seedling stage, tolerant accessions like CLN-2498-A and Picdenato maintained superior biomass accumulation and lower  $Na^+/K^+$  ratios, suggesting effective osmotic adjustment and selective ion transport mechanisms. These findings aligned with earlier studies that highlighted the importance of  $Na^+$  exclusion and  $K^+$  retention in salinity tolerance (Almeida *et al.*, 2014; Oyiga *et al.*, 2016; Ali *et al.*, 2020). The relatively moderate increase in  $Na^+/K^+$  ratios was observed in these accessions reinforces the critical role of ionic homeostasis in conferring salt stress resilience.

Almeida *et al.*, (2014) and Oyiga *et al.*, (2016) similarly reported that maintaining lower  $Na^+/K^+$  ratios significantly contributed to enhanced stress tolerance across various crops, including tomato. In this study, significant genotypic variation was also observed in key morphological and ionic traits, such as root and shoot

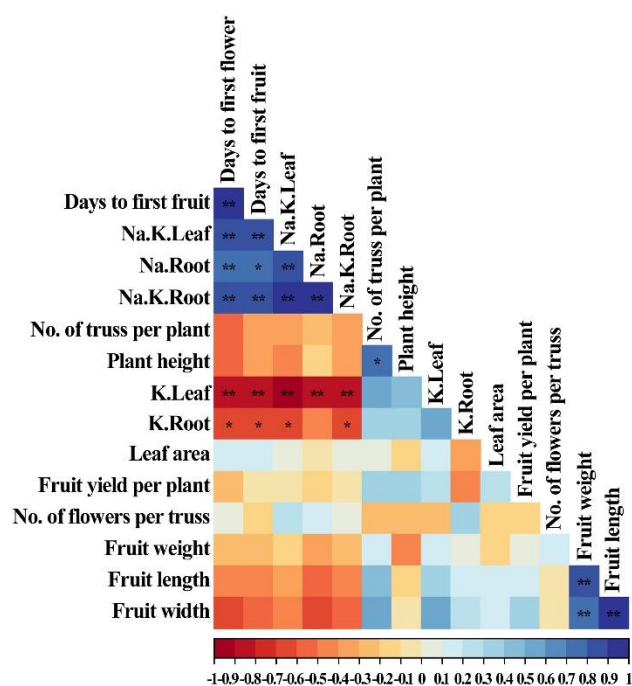


Fig. 6. Pairwise correlations among morphological and ionic traits of tomato germplasm at reproductive stage under salinity stress conditions.

length, fresh and dry biomass, and  $Na^+$  and  $K^+$  concentrations, which are widely recognized indicators of salinity tolerance (Wang *et al.*, 2011; Oyiga *et al.*, 2016; Choudhary *et al.*, 2021; Site *et al.*, 2021; Raziq *et al.*, 2022). Salinity tolerance in crop species is strongly associated with the plant's ability to exclude  $Na^+$  from shoots, thereby maintaining ionic balance (Almeida *et al.*, 2014). This process largely depends on the accession's capacity to restrict  $Na^+$  uptake and enhance  $K^+$  retention under saline conditions (Raziq *et al.*, 2022).

At the reproductive stage, the superior performance of CLN-2498-A and 17256 under 12 dS  $m^{-1}$  salinity further confirmed their tolerance across developmental stages. In contrast, accessions such as 17255 and Nadir, which performed moderately at the seedling stage, exhibited poor tolerance at the reproductive stage, reinforcing the need for stage-specific screening. These findings aligned with studies in other crops such as wheat (Choudhary *et al.*, 2021), maize (Dustgeer *et al.*, 2021), cotton (Zafar *et al.*, 2022), and rice (Kakar *et al.*, 2019; Site *et al.*, 2021), which highlighted the value of evaluating salt tolerance across different phenological stages. In tomato, Shimul *et al.*, (2014) reported that regulating  $Na^+$  accumulation in shoots was crucial for improving reproductive success under salinity.

The PCA biplots further supported these observations, demonstrating that tolerant accessions were clustered around biomass-related vectors, while sensitive ones like Nadir and 17255 aligned with high  $Na^+$  and  $Na^+/K^+$  ratio vectors. The separation of accessions in PCA space at both seedling and reproductive stages highlights the effectiveness of multivariate analysis in distinguishing tomato accessions based on their ionic and morphological responses to salinity. The observed clustering patterns reflected genotypic differences in ion homeostasis and biomass accumulation under stress. These findings aligned with earlier studies in tomato and other crops (Sivakumar *et al.*, 2020, 2023; Alam



*et al.*, 2021; Fatima *et al.*, 2022b; Zulfiqar *et al.*, 2024), where PCA was successfully employed to identify key traits, such as  $K^+$  uptake efficiency, low  $Na^+$  accumulation, and high shoot/root biomass, that contributed to salinity tolerance. In the present study, accessions clustering near biomass and yield-related vectors consistently exhibited lower  $Na^+/K^+$  ratios and better growth performance under salinity stress. This indicates the presence of efficient ionic regulation mechanisms, such as selective ion uptake and compartmentalization, which help mitigate  $Na^+$  toxicity. Traits like biomass yield, leaf area retention, and preferential  $K^+$  uptake are widely regarded as reliable physiological markers of salt tolerance in tomato (Alam *et al.*, 2021). These parameters are critical for maintaining metabolic activity and photosynthetic efficiency under stress and thus offer valuable selection criteria for tomato improvement programs. A notable contribution of this study is the emphasis on stage-specific expression of tolerance traits. The significant genotype  $\times$  salinity interaction was observed at both developmental stages underscores the dynamic nature of salt stress responses. Accessions such as CLN-2498-A and 17256 demonstrated superior adaptability, maintaining relatively stable  $Na^+/K^+$  ratios and minimal reductions in growth and yield across both seedling and reproductive stages. Their consistent performance under both moderate and high salinity levels highlights their potential as parental lines for developing salt-tolerant cultivars capable of sustained productivity throughout the crop cycle. In contrast, accessions such as 17255 and Nadir accumulated progressively higher  $Na^+$  concentrations in both root and leaf tissues with the increase of salinity levels. This was associated with substantial losses in both biomass and reproductive output, suggesting that impaired ionic regulation directly affects physiological functions and reproductive success. These findings reaffirm the role of  $Na^+/K^+$  homeostasis in maintaining cell function and underscore how ionic imbalance under salinity can lead to metabolic disruption and yield decline (Munns & Tester, 2008). Overall, the study highlights that effective salt tolerance in tomato involves a combination of traits, including high biomass retention, low  $Na^+$  accumulation, and favorable  $Na^+/K^+$  ratios, that operate at both early and later stages of growth. Accessions expressing these traits consistently across developmental phases are prime candidates for breeding programs focused on improving salt resilience. In particular, CLN-2498-A and 17256 emerged as robust performers, offering valuable genetic resources for developing tomato varieties suited to saline environments.

Building upon the PCA results, which highlighted key traits contributing to salinity tolerance, cluster analysis offered further resolution by grouping accessions with similar morpho-ionic responses under stress. This technique has been widely employed to categorize genotypic responses to salinity in tomatoes (Pailles *et al.*, 2020; Ur Rahman *et al.*, 2021; Eynizadeh *et al.*, 2023). The ability to sustain  $K^+$  uptake while maintaining a lower  $Na^+/K^+$  ratio emerged as a key determinant of salt tolerance (Chen *et al.*, 2007; Shabala & Cuin, 2008).  $K^+$  deficiency has been shown to impair photosynthesis (Cakmak, 2005) and disrupt cellular functions such as protein synthesis (Flowers & Dalmond, 1992). The superior performance of CLN-2498-A and 17256 suggests their ability to efficiently regulate  $K^+$  uptake under saline conditions. Ion homeostasis is a crucial determinant

of salinity tolerance, as excessive  $Na^+$  accumulation disrupts cellular metabolism. While  $Na^+$  can serve as an osmotic regulator, its excessive cytosolic accumulation leads to ionic imbalance (Hanin *et al.*, 2016) and competition with  $K^+$  for binding sites, impairing metabolic processes (Wei *et al.*, 2017). To protect itself under salt stress, a plant must either limit  $Na^+$  entry through the roots or regulate  $Na^+$  concentration and distribution once it has entered (Tester & Davenport, 2003; Hanin *et al.*, 2016).  $Na^+$  that enters root cells is expelled from the cytoplasm into the apoplast space and stored in the vacuole (Maggio *et al.*, 2007). This mechanism is known as tissue tolerance. One cluster from each species included tolerant accessions with high  $Na^+$  concentrations in their leaves, suggesting significant levels of tissue tolerance (Munns *et al.*, 2016).

These findings emphasize the pivotal role of  $K^+$  retention and  $Na^+$  exclusion in seedling-stage salinity tolerance. The negative correlation between  $K^+$  concentration and  $Na^+/K^+$  ratios aligned with earlier reports in wheat and barley, where efficient  $K^+$  uptake and minimal  $Na^+$  accumulation contributed to higher shoot biomass and stress resilience (Chen *et al.*, 2005; Chen *et al.*, 2007). Maintaining root integrity and biomass at early growth stages supports improved stress response, suggesting that traits such as root length and  $K^+$  content can serve as early selection markers in breeding programs targeting salinity tolerance.

The positive correlation between fruit yield and vegetative traits such as plant height and leaf area (Naveen *et al.*, 2017; Ghorbanpour *et al.*, 2018) highlights the integrated nature of growth and productivity under salinity stress. Similar trends have been observed in wheat, where grain yield positively correlated with  $Na^+$  exclusion from leaves (Chen *et al.*, 2007). Additionally, the inverse relationship between  $K^+$  levels and reproductive timing may suggest that accessions prioritizing ion homeostasis might delay flowering as a stress avoidance strategy. These findings underscore the complex interplay between ionic regulation and reproductive success, offering valuable targets for improving tomato performance under saline conditions.

## Conclusion

This study offers a thorough assessment of genetic variation in tomato germplasm under salinity stress at both seedling and reproductive stages. Significant genotypic differences were observed, particularly in traits related to biomass retention,  $Na^+/K^+$  balance, and yield stability under high salt levels. Two genotypes, CLN-2498-A and 17256, consistently outperformed others across both stages. Their strong ionic regulation and growth performance mark them as valuable resources for breeding salt-tolerant tomato varieties. These findings emphasize the importance of selecting tolerant genotypes that spans developmental stages. Future studies should integrate genomic approaches to further refine breeding strategies and enhance tomato resilience in salt-affected environments.

## Acknowledgements

This research formed part of my Ph.D. dissertation at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. I gratefully acknowledge the support and facilities provided by the University during the course of this study.

**Authors contribution:** Noreen Fatima (NF) conceived and designed the study, performed the experiments, analyzed the data, and wrote the manuscript. All authors read and approved the final version of the manuscript.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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