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Research Article

MULTIVARIATE ANALYSIS ON MORPHO-PHYSIOLOGICAL PARAMETERS UNDER SALINITY STRESS IN TOMATO (*SOLANUM LYCOPERSICUM L*)

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ABSTRACT
Using principal component analysis (PCA), the current study was conducted with the purpose of determining the salt tolerance of each of the ten tomato cultivars. The morphological and physiological parameters were measured at different concentration (mild to severe) of salinity stress in tomato cultivars. The experiment was conducted at 0, 25, 50, 75 and 100mM along with control condition. The tomato cultivars namely, Pusa Ruby, Pusa Rohini, Pusa Gourav, Pusa 120, Pusa Sadabahar, Pusa Early Dwarf, Hisar Arun, F1 WT-04, Pusa Sheetal and Pusa Uphar were used. Different morphological attributes such as root – shoot length, dry mass, fresh weight, leaf area and physiological parameters such as Relative water content, Chl fluorescence, Chl content, sodium (Na^+), and potassium (K^+) content recorded at flowering stages. All the cultivars have differential response towards different conc. of salt and showed reduction in growth, RWC, Chl pigment and ion homeostasis as compared with control plants. The overall performance of the cultivars Pusa ruby was tolerant and Pusa early dwarf is salt susceptible on the basis of above-mentioned parameters recorded at highest concentration of salinity while all other cultivars showed moderately sensitive. The PCA analysis of 16 morpho-physiological variables indicated the sequence of tolerance to sensitive cultivars under salinity stress i.e., Pusa Ruby > F1 WT-04 > Pusa Rohini > Hisar Arun > Pusa Gourav > Pusa Sheetal > Pusa Uphar > Pusa Sadabahar > Pusa 120 > Pusa Early Dwarf.

Keywords: *Principal Component Analysis, Tomato, Salinity*

INTRODUCTION
Tomatoes contain important nutrients such as vitamins C and E, carotenoids (β-carotenoids and lycopene), and bioactive phenolic compounds (naringenin, quercetin, kaempferol, and lutein). Bioactive phenolic compounds like caffeic, ferulic, and chlorogenic acids are also found in tomato fruits (Ali MY *et al.,* 2020; Kumar M *et al.,* 2021; Collins *et al.,* 2022). Salinity stress are significant and give rise to a series of alterations in morphology, physiology, molecular composition, and biochemical processes, ultimately leading to cellular harm and suppression of overall metabolic activity (Khalid MF *et al.,* 2019; Kapazoglou A. *et al.,* 2023). Maeda *et al*., 2020 concluded that, the detrimental effects of salinity stress on a variety of morpho-physiological parameters, such as the plant biomass, RWC, leaf area, Na+/K+ ratio, rate of photosynthesis, rate of transpiration, conductance in stomata, chlorophyll pigment, and nutrient utilization, have been the subject of recent research on tomato cultivars. The potential for increased soil salinity arises from the intensification of climate change, which may lead to the degradation of heavily watered arable soils through the use of diverse irrigation techniques. In addition, the problem of soil salinization is a significant concern in areas characterized by limited access to water resources or inadequate irrigation systems (Li A. *et al.,* 2023). Salt stress causes the osmotic stress and ionic toxicity while ion toxicity results from Na+ accumulation in the cytosol, causing an imbalance of Na+ and Cl− ions in tomato plants (Hanin *et al*., 2016; Bigot S. *et al*., 2023).Na^+ Ion presence in the environment leads to a decrease in water intake. Additionally, the accumulation of Na^+ over time hinders the process of photosynthesis in the shoots (Rose CR and Verhkhratsky A 2023). It is important to note that these two

stages, water intake reduction, and photosynthesis restriction, are geographically and temporally separated. Na+ concentration in the apoplast may increase physical interaction between positive and negative charges, affecting pH (Farooq *et al*., 2017; El-Badri AM *et al*., 2023). A reduction in water absorption also leads to a decline in turgid pressure within guard cells. Therefore, the stomata remain closed, resulting in modest rates of stomatal transpiration. The closure of stomata results in reduced availability of CO_2 for photosynthesis, which subsequently affects the productivity of the plant and the accumulation of ROS (Kashtoh H and Baek KH 2021). The osmotic imbalance resulting from elevated salinity in the vicinity of the roots is mitigated through two mechanisms: direct solute uptake by the roots and the production of certain tiny metabolic solutes (Gul Z *et al.*, 2022). The guard cells compensate for the reduced turgor pressure by altering their ion usage, specifically by substituting Na^+ ions for K^+ ions, in order to maintain the typical process of stomatal closure and opening

Principal component analysis (PCA) is one of the most important methods in multivariate analysis. It takes a group of correlated variables and turns them into a smaller group of orthogonal (not correlated), which are called principal components. The Eigen-decomposition of positive semi-definite matrices and the singular value decomposition (SVD) of rectangular matrices are what PCA is based on. PCA was used successfully on large datasets to find different types of tomato (Raza *et al*., 2018; Pailles *et al*., 2019), wheat (El-Hendawy *et al*., 2017), and rice (Chunthaburee *et al*., 2016) and barley (Chikha *et al*., 2016) that are sensitive or tolerant to salt. This study examined the diverse morpho-physiological responses exhibited by different cultivars of tomato under salinity stress, as well as the potential underlying mechanisms.

MATERIALS AND METHODS

Plant material

The seeds of ten tomato *(Solanum lycopersicum L.)* varieties namely Pusa Ruby, Pusa Rohini, Pusa Gourav, Pusa 120, PusaSadabahar, Pusa Early Dwarf, Hisar Arun, F1 WT-04, Pusa Sheetal and Pusa Uphar were procured from the Department of Horticulture IARI, New Delhi and Hisar Agricultural University (HAU) Hisar,Haryana.

Seed germination and salt treatment

The seeds were subjected to surface sterilization using a 1% sodium hypochlorite solution for duration of 2-minutes, after which they were washed three times with distilled water. Seeds were sown in germination trays (8 columns and 5 rows, total 40 wells) with duplicate tray for each treatment and experimental setup was kept for 48 days. Two seeds were sownper well, thus a total of 140 seeds were used for each treatment. After 14 days of sowing, germinated seeds were transplanted into pots. The pots were pre-filled with soil, coco peat and vermicompost in 3:2:1 ratio.Regular watering twice a day about 10-20ml for 35 days and then on alternate days different salt concentrations were applied i.e., 0mM, 25mM, 50mM, 75mM, 100mM, 125mM, 150Mm, and 200mM for 10 days.

Morphological growth analysis

On the 48th day after sowing, the seedlings that were treated with salt and the untreated seedlings (control group) were carefully removed from the pots and washed with distilled water. Then the root/shoot length; root/shoot fresh and dry weight were recorded. Leaf area was determined by the Millimeter Graph Paper method refers as non-destructive technique to calculate leaf area (Pandey SK and Singh H 2011) .For each treatment, a total of three replicated were used in completely randomized sampling.

Physiological growth analysis

Relative Water Content

The Relative water content (RWC) was determined by the method given as Flexas *et al.*, 2006 with some

modification. The leaf discs were cut and weighed. They were then settled by floating in sterile distilled water according to treatment on separate Petri dishes. After 3 hours, the discs were left to surface dry. Fresh weight was taken for RWC%, and dry weight was obtained by keeping them in an oven at 60°C for 48 hours.

RWC=Fresh weight –Dry weight/Turgid weight–Dry weight*100

Chlorophyll Fluorescence and Chlorophyll Content

The efficiency of PSII was measured through fluorescence analysis with Pulse amplitude modulated fluorometer (PAM). Plants were kept for 30 minutes in complete dark condition then fluorescence (*F*0) was determined by the measuring PAM fluorometer. Chlorophyll a and Chlorophyll b were extracted using the Hiscox and Israelstam (1979) method with some modification and calculation of the pigments were done using the Arnon (1949) method.

Chlorophylla=12.21×(A663)-2.81(A645) ×volume/weight

Chlorophyllb=20.13×(A645)-5.03(A663)×volume/weight

Na^+ and K^+ concentration in plant tissue

100 mg of the plant sample was dried in oven at 60ºC for 48 hours. The dried tissue was digested in di-acid (H_2SO_4& $HClO_4$: 4:1) mixture. The flask was gently heated on ahot plate to digest the plant material till dense white fumes were emitted. Na^+ and K^+contentwereestimated in the diluted acid digest on a Flame photometer.

Na^+ and K^+ content (µg) = O.D. × Dilution factor/weight of plant sample taken

Data analysis

A dataset consisting of 16 morpho-physiological parameters was collected for the purpose of conducting principal component analysis (PCA). The average values of the three replicates were utilised to construct the correlation matrix. This matrix consists of the values of 10 cultivars arranged in rows and 16 parameters arranged in columns.

RESULTS AND DISCUSSION

The tomato cultivars were evaluated for salinity tolerance by analyzing various morpho-physiological parameters under different salinity concentration (0, 25, 50, 75, 100, 150 and 200 mM NaCl).

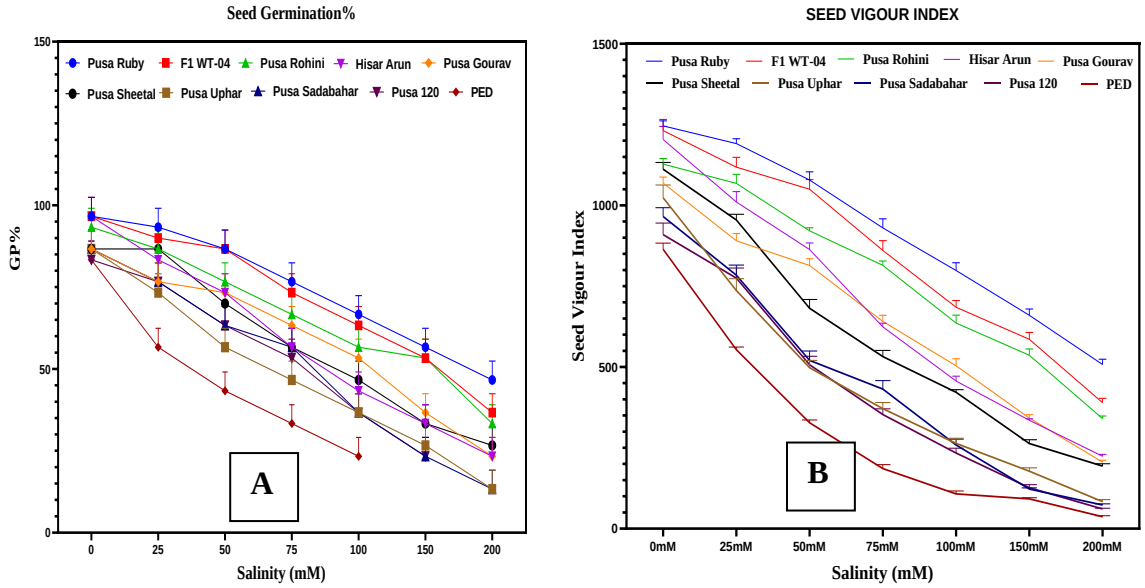
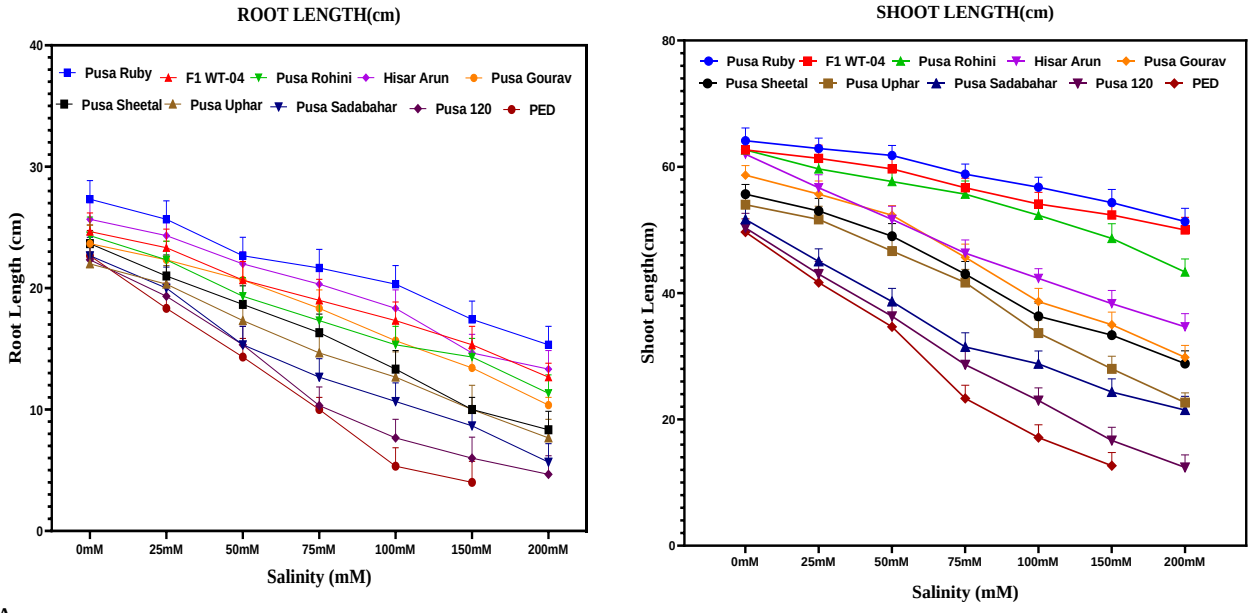


Figure-1: Effect of different concentration of salinity stress on(A) Seed germination percentage (B) Seed vigor index of 10 tomato cultivars.



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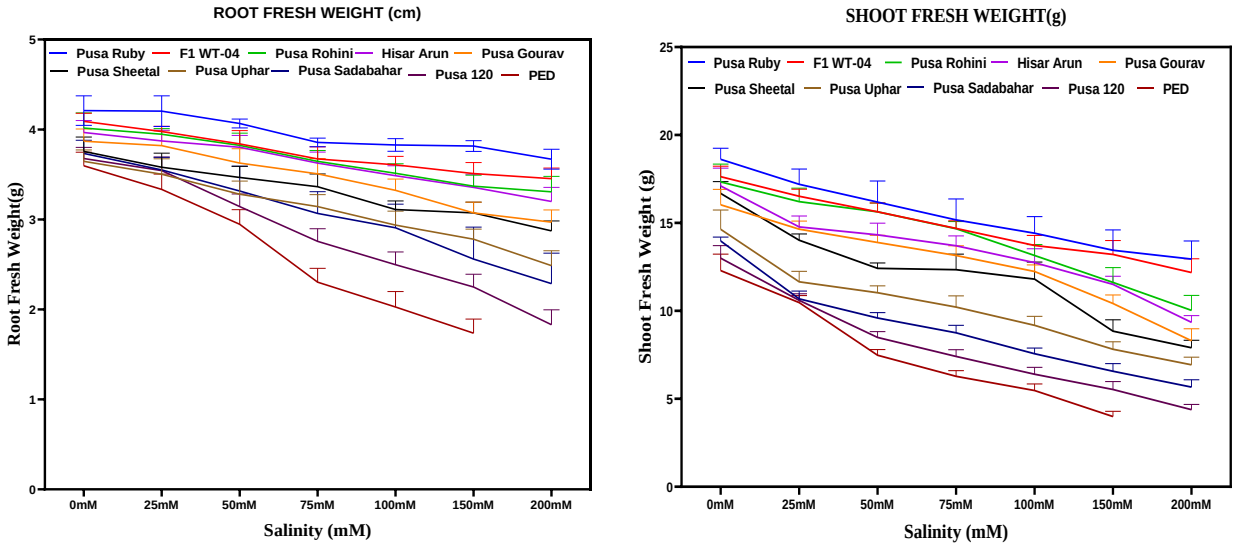
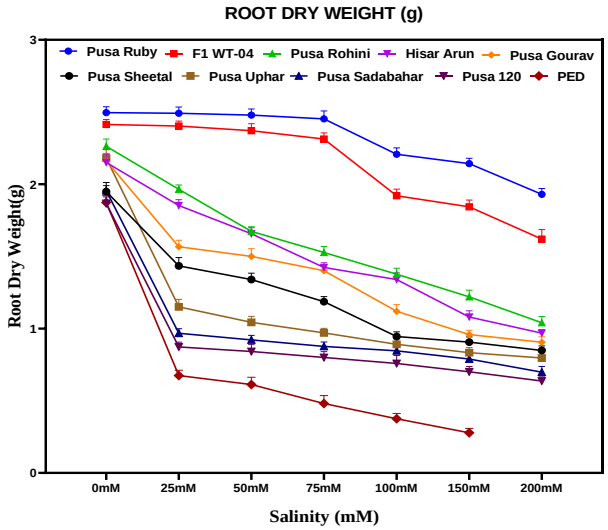


C D

Figure 2: Effects of different conc. of salinity stress on morphological growth in 10 tomato cultivars (A-B) Root and Shoot length (C-D)



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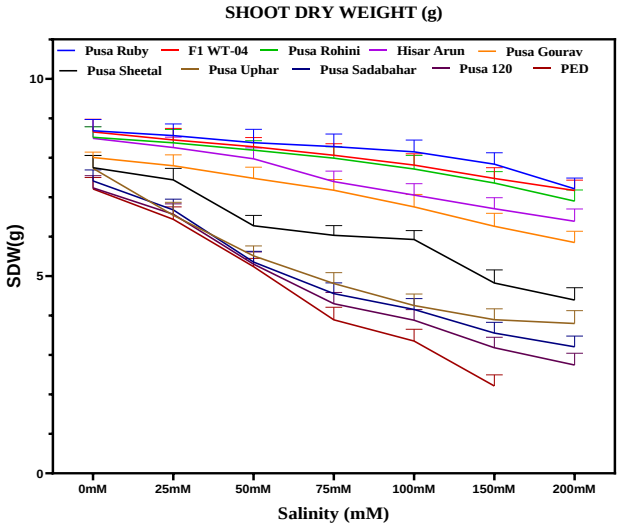


F

Figure 2 [Contd..]: Effects of different conc. of salinity stress on morphological growth in 10 tomato cultivars (E-F) Root and Shoot dry weight

Seed germination and Vigor index

The seed germination percentage and vigor index showed a declining trend with increasing salinity in all 10 cultivars.Seed germination percentage declined from 96.67% to 46.67% in Pusa Ruby, from 96.67% to 36.67% in F1 WT-04, and from 93.33% to 33.33% in Pusa Rohini as salinity increased from 0 to 200 mM NaCl**.** Pusa Ruby maintained the highest germination across the cultivars at all salinity levels. The vigor index also showed a similar decreasing trend with increasing salinity in all cultivars**.** Pusa Ruby had the highest vigor index of 1246 at control, which declined to 507 at 200 mM NaCl. In contrast, the vigor index of sensitive genotype PED decreased sharply from 864 at control to just 36 at 200 mM NaCl.Pusa Ruby maintained the highest germination percentage and vigor index across all salinity levels, indicating its superior seed germination ability under salt stress compared to other cultivars. The sensitive cultivars PED and Pusa 120 showed a sharp reduction in germination and vigor even at mild salinity of 25 mM NaCl.

Growth Parameters

The growth parameters, including root length, shoot length, fresh root weight, fresh shoot weight, dry root weight, and dry shoot weight, exhibited a gradual decline as the salinity levels increased across all 10 cultivars.The reduction was most prominent in the susceptible cultivars Pusa Sadabahar, Pusa 120 and PED, especially at higher salinities of 150 and 200 mM NaCl. In contrast, Pusa Ruby maintained superior root and shoot growth attributes across all salinity levels. Pusa Ruby maintained the highest root length (27 cm at 0 mM to 15 cm at 200 mM NaCl), shoot length (64 cm at 0 mM to 51 cm at 200 mM NaCl), and fresh and dry weights of root and shoot across salinity treatments compared to the other cultivars. Pusa Ruby had the highest root and shoot growth across salinity levels, suggesting its better salt tolerance during early vegetative growth.

Leaf Relative Water Content

Leaf relative water content, a measure of the amount of water in plant leaves, decreased significantly in all cultivars when exposed to higher levels of salinity. The leaf-relative water content exhibited a substantial decrease in response to salinity stress, with the most pronounced decline observed in Pusa Sadabahar. Pusa Ruby maintained high water content across all salt levels, while the sensitive cultivars Pusa Sadabahar, Pusa 120 and PED displayed severe water loss at higher salinities.Leaf relative water content showed a steep declining trend with increasing salinity, with the maximum drop observed in Pusa Sadabahar from 73% at 0 mM NaCl to just 44% at 200 mM NaCl.

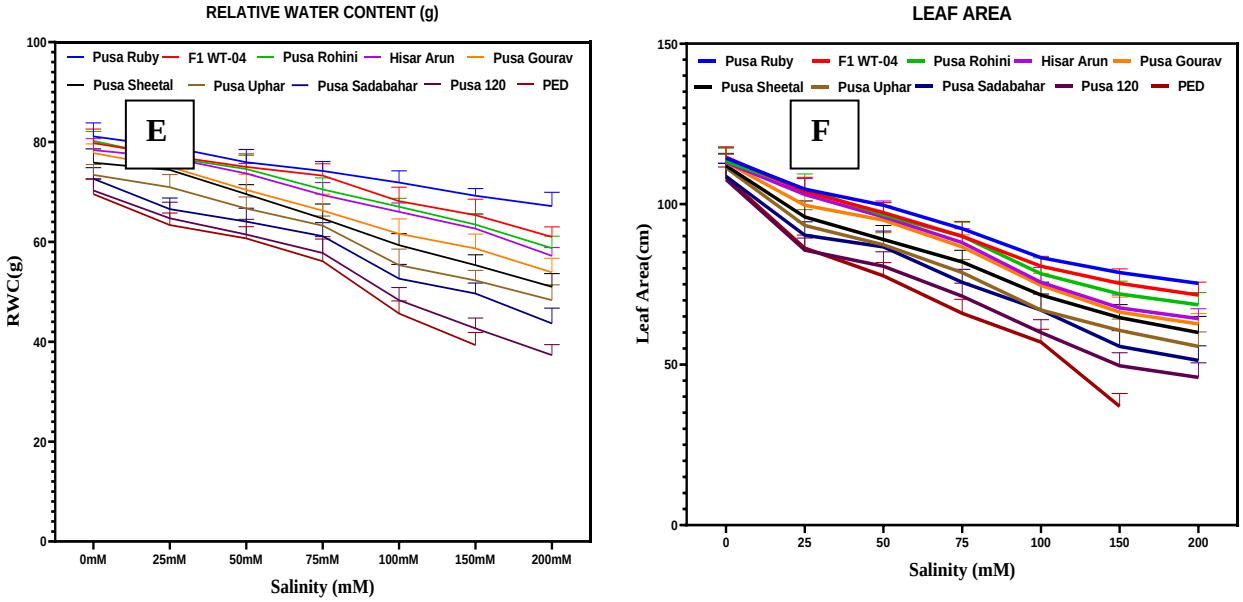


Figure 3: Effect of salinity stresses of different conc. on **(A)** Relative Water Content and (B) Leaf of 10 tomato cultivars

Chlorophyll Content and Fluorescence

The chlorophyll content (Chl a, Chl b, and total Chl) and fluorescence decreased significantly with salinity in all cultivars. The susceptible cultivars Pusa Sadabahar, Pusa 120 and PED showed the highest decline. Pusa Ruby retained higher chlorophyll levels and fluorescence across treatments compared to the other cultivars. The susceptible Pusa Sadabahar, Pusa 120 and PED showed a steep decline in chlorophyll parameters especially at salinities ≥100 mM NaCl. Pusa Ruby maintained the highest chl content and fluorescence across treatments. At 200 mM NaCl, the total chl content of Pusa Ruby (3.9 mg/g) was about 3 times higher than that of Pusa Sadabahar (1.3 mg/g).

Ion Content

Increasing salinity led to enhanced foliar Na^+ accumulation coupled with decreasing K^+ content in all 10 cultivars. Pusa Ruby maintained the lowest Na^+(34 mg/g at 200 mM NaCl) and highest K^+(25 mg/g at 200 mM NaCl), indicating effective regulation of ion homeostasis. The salt sensitive nature of Pusa Sadabahar, Pusa 120 and PED was also evident from the sharp increase in Na^+ accumulation coupled with a decrease in K^+ content in their leaves at higher salinities**.** PED showed a drastic 6-fold increase in Na^+ accumulation from 30 mg/g at control to 182 mg/g at 150 mM NaCl. Its leaf K+ content also dropped sharply from 28 mg/g at control to just 14 mg/g at 150 mM NaCl. In contrast, Pusa Ruby maintained lower Na^+ and higher K^+ levels across treatments, conferring salinity tolerance.

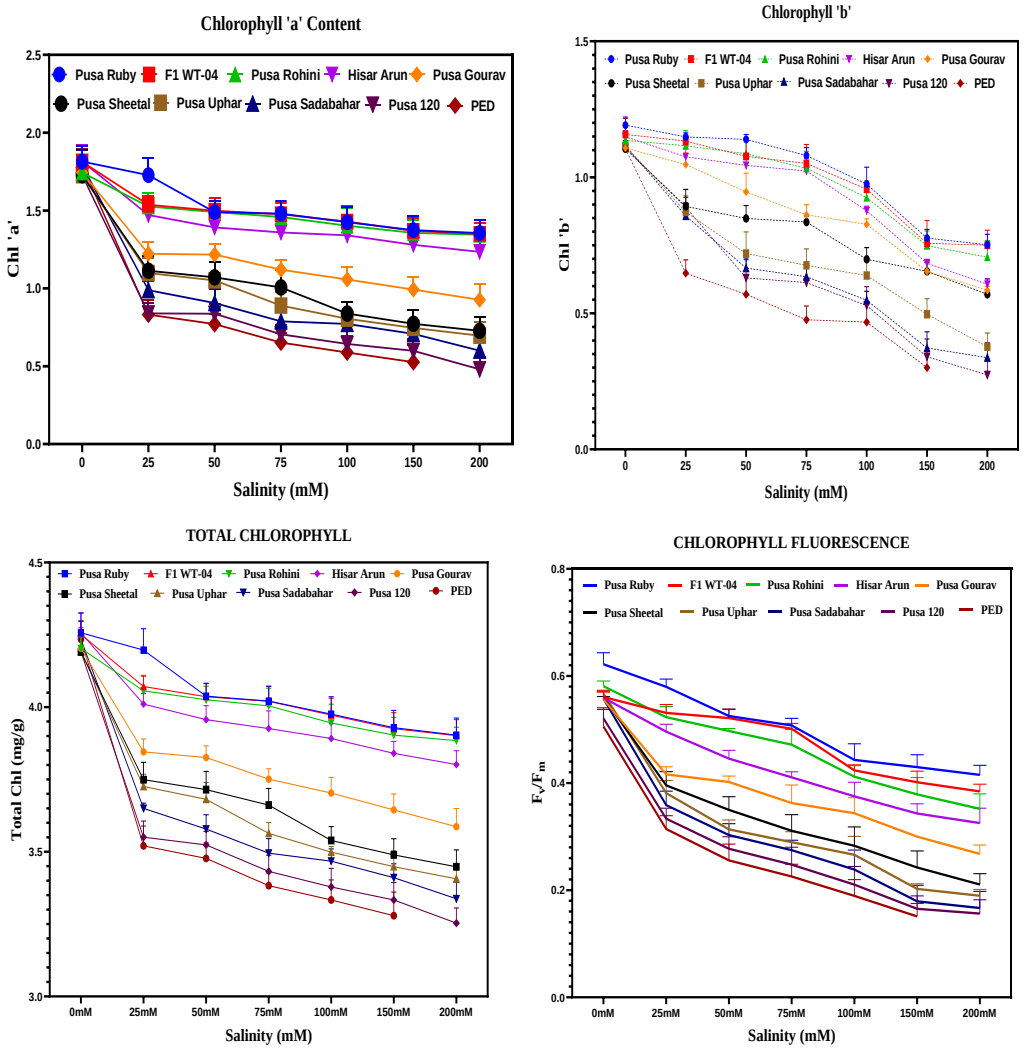
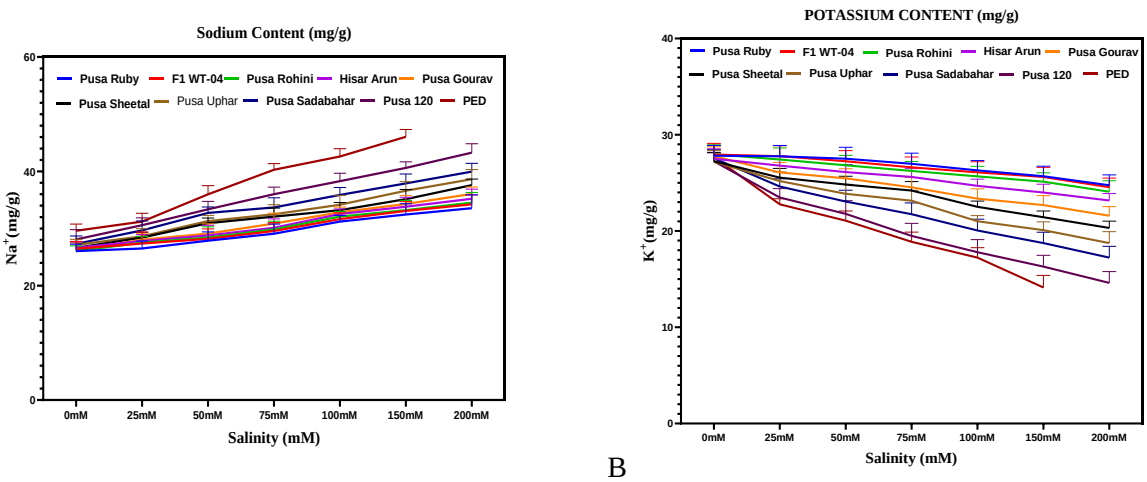


Figure 4: Effect of salinity stresses of different conc. on **(A)** Chl 'a' content (B) Chl 'b' content **(C)** Total Chl content (D) Fluorescence of 10 tomato cultivars**.**



A B

Figure 5: Effect of salinity stresses of different conc. on **(A)** Na^+ content and (B) K^+ content (mg/gm) accumulation of 10 tomato cultivars**.**

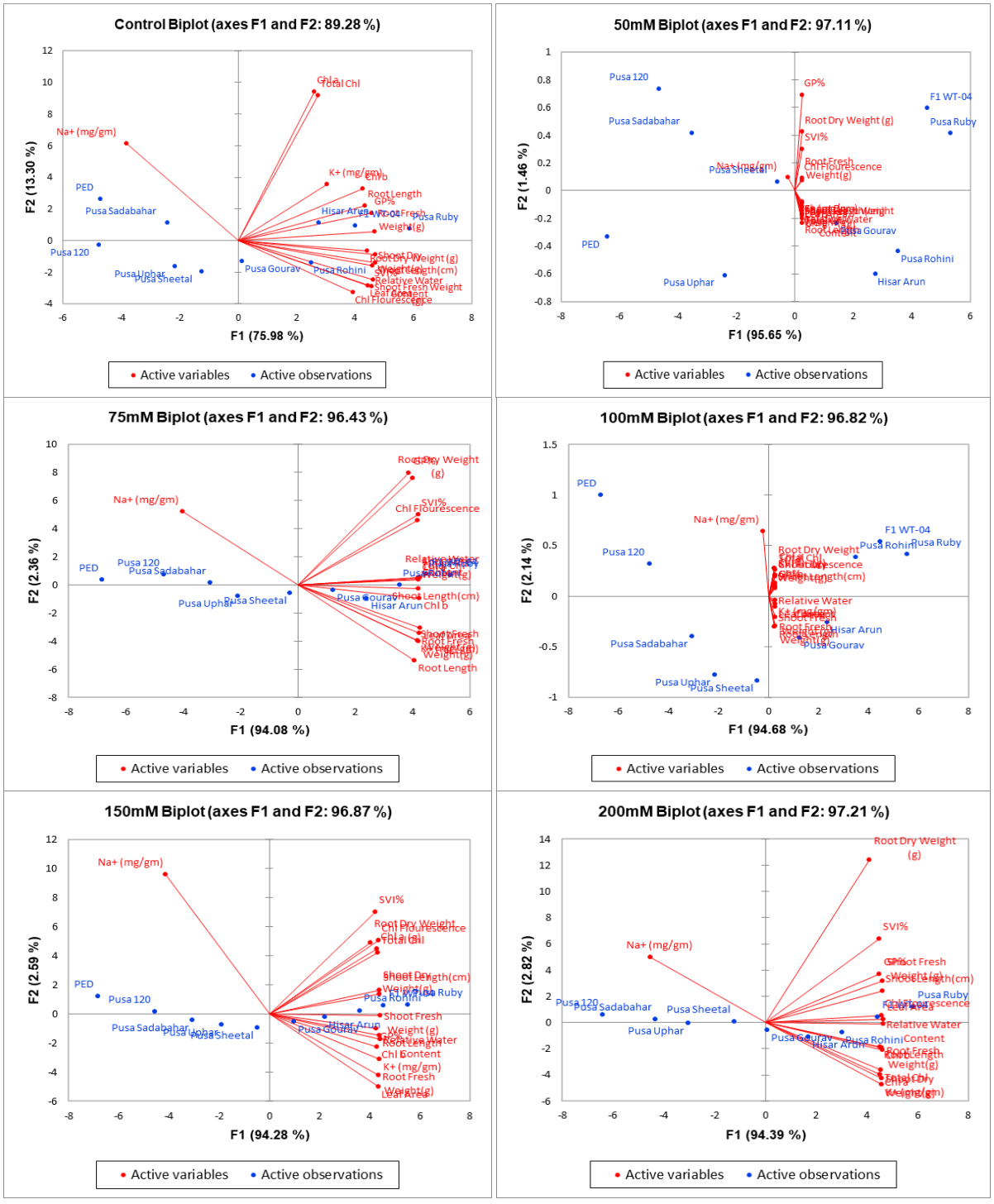


Figure 5: Bi-plots of PCA represents the strength correlations and variance between 16 parameters under salinity stress in ten tomato cultivars. (i) Control Bi-plot (ii)50mM salt stress Bi-plot (iii) 75mM salt stress Bi-plot (iv) 100mM salt stress Bi-plot (v) 150mM salt stress Bi-plot (vi) 200mM salt stress Bi-plot

PCA Analysis

Principal component analysis (PCA) effectively discriminated the 10 cultivars based on their salt tolerance ability at different salinity levels**.** Pusa Ruby, F1 WT-04 and Pusa Rohini were consistently ranked as the most salt tolerant cultivars across all treatments in PCA, while Pusa Sadabahar, Pusa 120 and PED were the most sensitive. Principal Component Analysis (PCA) was used to evaluate the salt tolerance potential of cultivars using sixteen variables from control, 25mM, 50mM, 75mM, 100mM, 150mM, and 200mM salt-stressed plants. The PCA scattered the cultivars across all four quadrants of the biplots. The F1 and F2 components had Eigenvalues greater than one.The PCA scattered the cultivars across all four quadrants of the biplots. In the PCA biplots, the F1 and F2 components exhibited Eigenvalues greater than one. Under control, 25mM, 50mM, 75mM, 100mM, 150mM and 200mM stress conditions, the biplots displayed 89.28%, 95.92%, 97.11%, 96.4%, 96.82%, 96.87% and 97.21% cumulative variability, respectively.

REFERENCES

Abdel-Farid, I. B., Marghany, M. R., Rowezek, M. M., & Sheded, M. G. (2020). Effect of Salinity Stress on Growth and MetabolomicProfiling of Cucumis sativus and Solanum lycopersicum. *Plants*, *9*(11), 1626.

Ali, M. Y., Sina, A. A. I., Khandker, S. S., Neesa, L., Tanvir, E. M., Kabir, A., ... & Gan, S. H. (2020). Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A review. *Foods*, *10*(1), 45.

Bigot, S., Fuksová, M., Martínez, J. P., Lutts, S., & Quinet, M. (2023). Sodium and chloride accumulation and repartition differed between the cultivated tomato (Solanum lycopersicum) and its wild halophyte relative Solanum chilense under salt stress. *Scientia Horticulturae*, *321*, 112324.

Chikha, M. B., Hessini, K., Ourteni, R. N., Ghorbel, A., & Zoghlami, N. (2016). Identification of barley landrace genotypes with contrasting salinity tolerance at vegetative growth stage. *Plant Biotechnology*, *33*(4), 287-295.

Chunthaburee, S., Dongsansuk, A., Sanitchon, J., Pattanagul, W., & Theerakulpisut, P. (2016). Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. *Saudi Journal of Biological Sciences*, *23*(4), 467-477.

Collins, E. J., Bowyer, C., Tsouza, A., & Chopra, M. (2022). Tomatoes: An extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. *Biology*, *11*(2), 239.

El-Badri, A. M., Batool, M., Mohamed, I. A., Agami, R., Elrewainy, I. M., Wang, B., & Zhou, G. (2023). Role of Phytomelatonin in Promoting Ion Homeostasis During Salt Stress. In *Melatonin: Role in Plant Signaling, Growth and Stress Tolerance: Phytomelatonin in normal and challenging environments* (pp. 313-342). Cham: Springer International Publishing.

El-Hendawy, S. E., Hassan, W. M., Al-Suhaibani, N. A., Refay, Y., & Abdella, K. A. (2017). Comparative performance of multivariable agro-physiological parameters for detecting salt tolerance of wheat cultivars under simulated saline field growing conditions. *Frontiers in plant science*, *8*, 255157.

Farooq, M., Gogoi, N., Hussain, M., Barthakur, S., Paul, S., Bharadwaj, N., ... & Siddique, K. H. (2017). Effects, tolerance mechanisms and management of salt stress in grain legumes. *Plant Physiology and Biochemistry*, *118*, 199-217.

Gul, Z., Tang, Z. H., Arif, M., & Ye, Z. (2022). An insight into abiotic stress and influx tolerance mechanisms in plants to cope in saline environments. *Biology*, *11*(4), 597.

Hanin, M., Ebel, C., Ngom, M., Laplaze, L., & Masmoudi, K. (2016). New insights on plant salt tolerance mechanisms and their potential use for breeding. *Frontiers in plant science*, *7*, 209523.

Kapazoglou, A., Gerakari, M., Lazaridi, E., Kleftogianni, K., Sarri, E., Tani, E., & Bebeli, P. J. (2023). Crop wild relatives: A valuable source of tolerance to various abiotic stresses. *Plants*, *12*(2), 328.

Kashtoh, H., & Baek, K. H. (2021). Structural and functional insights into the role of guard cell ion channels in abiotic stress-induced stomatal closure. *Plants*, *10*(12), 2774.
Khalid, M. F., Hussain, S., Ahmad, S., Ejaz, S., Zakir, I., Ali, M. A., ... & Anjum, M. A. (2019). Impacts of abiotic stresses on growth and development of plants. In *Plant tolerance to environmental stress* (pp. 1-8). CRC Press.
Kumar, M., Tomar, M., Bhuyan, D. J., Punia, S., Grasso, S., Sa, A. G. A., ... & Mekhemar, M. (2021). Tomato (Solanum lycopersicum L.) seed: A review on bioactives and biomedical activities. *Biomedicine & Pharmacotherapy*, *142*, 112018.
Li, A., Wu, Y. Z., & Cao, S. Z. (2024). Effects of land use-land cover on soil water and salinity contents. *Ecological Frontiers*, *44*(2), 307-314.
Maeda, K., Johkan, M., Tsukagoshi, S., & Maruo, T. (2020). Effect of salinity on photosynthesis and distribution of photosynthates in the Japanese tomato 'CF momotaro york'and the Dutch tomato 'Endeavour'with low node-order pinching and a high-density planting system. *The Horticulture Journal*, *89*(4), 454-459.
Pailles, Y., Awlia, M., Julkowska, M., Passone, L., Zemmouri, K., Negrão, S., ... & Tester, M. (2020). Diverse traits contribute to salinity tolerance of wild tomato seedlings from the Galapagos Islands. *Plant physiology*, *182*(1), 534-546.
Pandey, S. K., & Singh, H. (2011). A simple, cost-effective method for leaf area estimation. *Journal of botany*, *2011*(1), 658240.
Raza, M. A., Saeed, A., Munir, H., Munawar, A., Kamran, A., Rehman, F., & Riaz, A. (2018). Improving salt tolerance and weight percent reduction in tomato by exploiting physio-agronomic seedling traits. *African Journal of Agricultural Research*, *13*(13), 607-616.