



RESEARCH ARTICLE

Comparative Morphological Responses of Maize Genotypes at the 3-leaf Stage to Drought and Salinity Stress

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ABSTRACT

Major abiotic stressors on maize (*Zea mays* L.) production are drought and salinity stress, so tolerant genotypes are needed. Under drought and salt, this study examined the morphological reactions of two maize genotypes, Gohar-19 and Pop-1. Under drought, Gohar-19 displayed a growth maintenance strategy marked by better root elongation, consistent shoot development, and increased plant vigor, therefore implying a greater potential for osmotic adjustment and antioxidant defense. Indicative of greater Osmo protectant accumulation and stress-adaptive metabolism, Pop-1 followed a biomass retention strategy under salinity and displayed higher fresh and dried weight, and improved water retention. Principal component analysis (PCA) and cluster analysis verified the several stress-adaptation strategies; Gohar-19 clustering with growth-related traits under drought and Pop-1 aligning with biomass conservation traits under salinity. These results provide important new perspectives for breeding initiatives aiming at producing maize varieties with increased tolerance to certain abiotic stresses since they highlight genotype-specific resilience mechanisms.

Key words: Maize, Drought stress, Salinity tolerance, Growth maintenance, Biomass retention.

INTRODUCTION

As a key source of food, fodder, and forage, maize (*Zea mays* L.) is a major cereal crop belonging to the Poaceae family and ranks among the most farmed crops globally (Ranum et al., 2014). Maestro under warm regions with enough sunlight (Tariq and Iqbal 2010), maize shows great photosynthetic efficiency as a C₄ crop. Its versatility over several agro-climatic zones has resulted in extensive farming with notable yields in both irrigated and rain-fed areas. Originally from Central America and Mexico (Hossain et al. 2016), maize

has quickly spread over the world and is today a staple food crop in many nations, including Pakistan, where maize output reached 9847224 tons in 2023 (FAOSTAT 2025). Though its agronomic importance, abiotic factors such drought and salinity seriously restrict maize output, both of which compromise world food security (Daryanto et al., 2016; Shao et al. 2021).

For germination and early development, maize needs a temperature range ideal between 15 and 20°C (Orhun, 2012). Extreme environmental circumstances, especially water shortage and soil salinization, greatly hinder plant development and hence lower yield

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potential. Drought stress is now mostly responsible for declining crop output, which results in worldwide yield losses of up to 50%. Research shows that drought lowers kernel count, postpones germination, and stunts vegetative development (Lobell et al. 2014). The result of some studies showed that drought reduces root elongation at the seedling stage, therefore influencing water intake and postponing early plant establishment (Aslam et al. 2012; Harrison et al. 2014; Sangakkara et al., 2010; Wang et al. 2023). Fascinatingly, maize genotypes vary in their drought adaptation tactics; some varieties preserve water by stomatal control and osmotic adjustment while others maintain growth by raising root-to-shoot ratios.

Likewise, salinity stress mostly causes osmotic stress and ionic toxicity, so influencing maize development and metabolism (Negrão et al., 2017; Zhong et al. 2020). Maestros seedling development is lowered by around 50% at a salt concentration of 100 mmol L⁻¹ NaCl (Cao et al. 2019). Further compromising plant vigor is salinity's reduction of germination rates, disturbance of root physiology, lower transpiration, and nutritional imbalances (Hu et al. 2022; Iqbal et al. 2020). Long-term salinity also affects carbon assimilation (Feng et al. 2021; Zhu et al. 2022). These impacts become more noticeable in areas where climate change aggravates both salinity and drought, therefore endangering maize output (Naumann et al. 2018; Raposo et al., 2023).

Maize has evolved multiple physiological, biochemical, and genetic adaptations to resist environmental stressors. Maestros maize plants control osmotic equilibrium, build suitable solutes such as proline and glycine betaine, and strengthen antioxidant defenses to minimize reactive oxygen species (ROS) damage (Farooq et al. 2015). While the AP2/ERF gene family controls drought and salinity stress responses (Makarevitch et al. 2015; Meng et al., 2024), the overexpression of stress-responsive genes including *ZmMAPKKK18* increases drought tolerance. Furthermore, linked to salt tolerance are *Z. mays* *SAG4* and *SAG6*, suggesting their possible usage in lines of salt-tolerant maize. By means of lignin production, the phenylalanine ammonia-lyase (PAL) enzyme increases stress tolerance and strengthens plant cell walls (Schubert et al. 2009).

Deep root systems, improved water absorption efficiency, and lower leaf area to restrict water loss define morphologically drought-resistant genotypes (Sheoran et al. 2022). Tolerant maize varieties limit Na⁺ buildup in leaf tissues and maintain higher K⁺/Na⁺ ratios to prevent ionic toxicity under salinity stress, therefore acting as a fundamental adaptation mechanism (de Azevedo Neto et al. 2006; Kaya et al., 2010). Additionally found to be advantageous is the stay-green phenotype since it delays leaf senescence and maintains photosynthetic activity under stress (Song et al., 2019). Maintaining maize output in conditions marked by soil salinization and water shortage depends

on these physiological and genetic adaptations.

While much study has been done on mature maize plants, seedling-stage reactions to salinity and drought remain understudied (Ali et al. 2023). Early development phases are especially susceptible to abiotic stress since poor root formation and germination failure greatly lower plant establishment and yield potential. Drought stress during germination alters later shoot elongation, reduces radicle emergence, and delays seed imbibition (Aslam et al. 2012). Similar high osmotic imbalance brought on by salinity stress in early growth phases limits water absorption and increases seedling death (Cao et al. 2019; Shahzad et al. 2012). Breaching programs aiming at improving stress tolerance in current cultivars depends on an awareness of how various maize genotypes react at the seedling level.

This work attempted to examine the reactions of two maize genotypes, "Gohar-19" and "Pop-1," at the seedling stage under controlled drought and salinity circumstances given the rising frequency of drought and salinity stress. We tried to identify which genotype shows better stress adaptation and which mechanisms support stress tolerance by combining morphological, physiological, and biochemical investigations. This work will give important information for programs of maize breeding, therefore enabling the development of robust maize varieties with improved resilience to climatic conditions brought about by climate change.

MATERIALS AND METHODS

Study Site

This study was conducted in 2023 at the University of Agriculture, Faisalabad, Punjab, Pakistan, under controlled pot conditions. Faisalabad has a semi-arid climate, characterized by hot summers ($\geq 40^{\circ}\text{C}$), mild winters, and an average annual rainfall of 350–500 mm. The experimental period spanned March to May 2023, coinciding with the maize seedling stage. The soil texture was sandy loam, and to enhance organic content, 40% soil was mixed with 60% peat moss. The soil pH ranged between 7.0 and 8.0, indicating slightly alkaline conditions.

Plant Material and Growth Conditions

Two maize (*Zea mays* L.) genotypes, 'Gohar-19' and 'Pop-1', were selected based on their contrasting responses to abiotic stress. Seeds were obtained from local certified sources. Each genotype was subjected to two treatments (drought and salinity stress) along with a control, arranged in a randomized complete block design (RCBD) with three replications per treatment. A total of 18 pots per genotype ($n = 36$ pots) were used, each containing 10 g of maize seeds. The growth medium consisted of 40% sand and 60% peat moss, ensuring adequate aeration and water retention. The pots were maintained in a greenhouse with a controlled temperature of 22–30°C, relative humidity of

70–80%, and natural photoperiod conditions. The sowing date was March 23, 2023. Seeds were watered every 48 hours with distilled water (pH 6.5–7.5) until the plants reached the three-leaf stage (~13 days after emergence).

Experimental Design

A randomized complete block design (RCBD) with three replications was used to evaluate the response of maize genotypes under drought and salinity stress. The treatments included: Control (Well-watered conditions): Plants received regular irrigation throughout the experiment. Drought stress: Water was withheld for seven days after plants reached the three-leaf stage. Salinity stress: Plants were irrigated with 100 mM NaCl solution daily for seven days after reaching the three-leaf stage. Each treatment was replicated three times for each genotype, ensuring statistical robustness. To impose drought stress, the soil was fully saturated before planting to ensure uniform seed germination. After seedling establishment, normal irrigation continued until the three-leaf stage (~13 days after sowing). Water was then completely withheld for seven days (Efeoğlu et al., 2009). Control plants continued to receive adequate irrigation throughout the experiment. Visual signs of drought stress, such as leaf rolling, reduced growth rate, and lower turgidity, were observed from day 2 of stress application. After seven days of drought stress, plant samples were collected for morphological and physiological measurements. Salinity stress was induced using a 100 mM NaCl solution prepared with analytical-grade NaCl dissolved in distilled water (Zahra et al., 2020). Starting from the three-leaf stage, plants were irrigated daily with 100 mL of 100 mM NaCl solution for seven consecutive days (Vennam et al. 2024). Control plants were watered with equal amounts of distilled water to eliminate differences in soil moisture. Symptoms of salt stress, including chlorosis, leaf wilting, and white salt deposits on the soil surface, were first observed on day 3 of treatment. After seven days of salinity stress, morphological and physiological data were recorded.

Data Collection

After seven days of stress application, data were recorded for each genotype and treatment. The following parameters were measured: Germination percentage (%): The number of emerged seedlings was counted and expressed as a percentage. Root length (cm): The length of the primary root was measured using a ruler. Shoot length (cm): The length from the base of the stem to the tip of the longest leaf was recorded. Leaf length (cm): The length of the largest leaf per plant was measured. Fresh weight (g): Plants were harvested and immediately weighed. Dried weight (g): Samples were oven-dried at 70°C for 48 hours before weighing. Plant vigor index (PVI): calculated using the formula:

$$PVI = (\text{Shoot length} + \text{Root length}) \times \text{Germination percentage}$$

Statistical Analysis

All recorded data were analyzed using Statistix 10 and R Studio for Analysis of variance (ANOVA), cluster, and Principal component Analysis (PCA) (Zafar et al., 2023). A p-value < 0.05 was considered statistically significant. The ANOVA results indicated significant differences among genotypes for most traits, except for shoot length (p = 0.065, nearing significance) and leaf growth (p = 0.542, non-significant). The variation in germination percentage and biomass accumulation suggests that Pop-1 exhibits better salt stress tolerance, whereas Gohar-19 shows superior drought resilience.

RESULTS

Effect of Drought Stress on Studied Traits

A genotype's capacity to resist unfavorable conditions-especially drought stress-is crucially revealed by its germination percentage (Fig. 1a). Pop-1 showed the highest germination percentage (88%) in control conditions; Gohar-19 came second at 78.5%. The variation implies that, under ideal conditions, Pop-1 naturally had a better seed viability and germination efficiency. Under drought stress, however, germination percentage dropped in both genotypes; Pop-1 suffered a more loss (from 88% to 82.7%) than Gohar-19 (from 78.5% to 77%). While Gohar-19 preserved a rather constant germination rate despite water stress, the relative decrease in germination under stress suggests that Pop-1 was more sensitive to water limitation during seed germination. Superior seed moisture retention, improved protective seed coat properties, or more efficient activation of stress-responsive metabolic pathways during early seedling establishment could help to explain this stability in Gohar-19.

Drought tolerance was much influenced by root length since longer and deeper roots help to absorb water from lower soil layers (Fig. 1b). Gohar-19 showed noticeably longer roots (14.24 cm) under control conditions than Pop-1 (8.96 cm), suggesting that under ideal growth conditions Gohar-19 has a more developed root system. Root length in both genotypes dropped under drought stress; Gohar-19 dropped to 12.6 cm and Pop-1 dropped to 6.68 cm. In Pop-1 (25.4%) the percentage decrease in root length was more noticeable than in Gohar-19 (11.5%). This finding implies that Gohar-19 had more capacity to sustain root development under drought conditions, hence maybe helping to preserve water intake during extended stress. Retention of root elongation in Gohar-19 points to either increased osmotic adjustment to maintain cell expansion under water-limited conditions, upregulation of drought-responsive genes, or activation of mechanisms including increased root-to-shoot ratio.

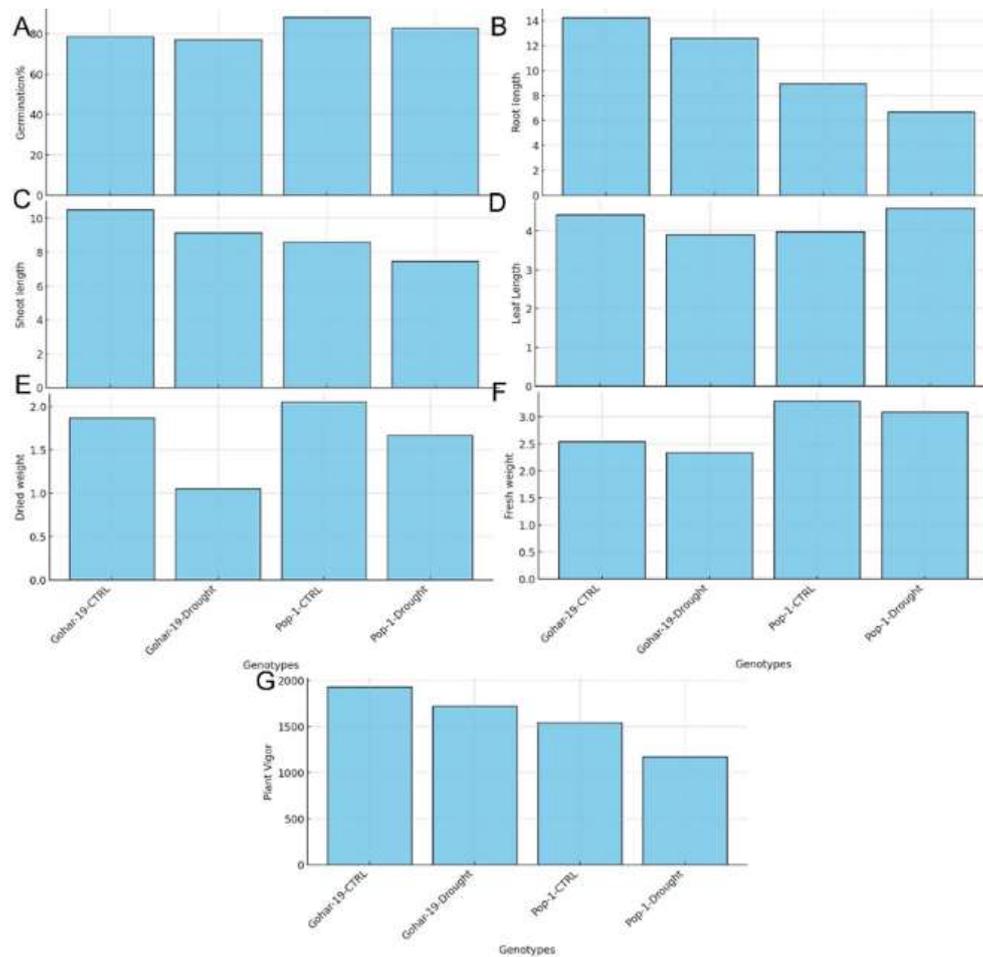


Fig. 1: The effect of drought stress on morphological traits. a) Germination%, b) root length, c) shoot length, d) leaf length, e) dried weight, f) fresh weight, g) plant vigor index.

Another important factor reflecting plant development under stress is shoot length (Fig. 1c). Gohar-19 showed a longer shoot (10.5 cm) than Pop-1 (8.6 cm) in well-watered conditions, suggesting that, generally speaking, Gohar-19 has a more active aerial development pattern in ideal conditions. Both genotypes showed declines in shoot length under drought stress; Gohar-19 dropped to 9.16 cm (a 12.8% loss) and Pop-1 dropped to 7.46 cm (a 13.3% reduction). The somewhat larger decrease in Pop-1 points to this genotype's increased sensitivity to limits in shoot elongation brought on by dryness. This reaction could be explained by a decrease in cell division and expansion when water shortage limits turgor pressure, so restricting the development of the shoots. Gohar-19 maintained a longer shoot under stress despite the reduction, implying that it has mechanisms to reduce drought-induced shoot growth inhibition, either by better stomatal control, higher water-use efficiency, or synthesis of growth regulators like cytokinin's supporting shoot elongation.

An important quality affecting the ability of the plant for photosynthesis and transpiration is leaf length (Fig. 1d). Gohar-19 showed a rather longer leaf length (4.42 cm) under control conditions than Pop-1 (3.98 cm). Fascinatingly, Gohar-19 showed a drop in leaf

length (to 3.9 cm) under drought conditions while Pop-1 showed a rise in leaf length (to 4.58 cm). This surprising reaction in Pop-1 points to a possible adaptation mechanism wherein the plant elongates its leaves under stress, maybe to maximize light capture or preserve photosynthetic efficiency despite water constraints. On the other hand, Gohar-19 responded more conventionally—that is, with less leaf elongation—which is sometimes a tactic to limit water loss through transpiration. This variation in genotypes implies that Pop-1 might give sustained leaf expansion top priority while Gohar-19 might use a more cautious approach by cutting leaf size to help to preserve water under stress.

Dried weight captures the capacity of the plant to collect biomass (Fig. 1e), which is directly related to metabolic efficiency and water availability. Pop-1 displayed a higher dried weight (2.05 g) than Gohar-19 (1.87 g), showing that under well-watered settings it has more biomass accumulation potential under control conditions. Dried weight dropped significantly in Gohar-19 (from 1.87 g to 1.05 g) under drought stress, while Pop-1 showed a rather lower drop (from 2.05 g to 1.67 g). The more Gohar-19 reduces indicates that this genotype is more vulnerable in terms of dry matter accumulation to drought. On the other hand, Pop-1

seems to sustain biomass production better under stress, which could be ascribed to more effective carbon assimilation, improved osmotic control, or better nutrient absorption under drought circumstances.

Direct indication of plant water content and general turgidity, fresh weight affects physiological activities and growth (Fig. 1f). Pop-1 showed the highest fresh weight (3.29 g) under control conditions; Gohar-19 (2.54 g) came second. Fresh weight in both genotypes dropped under drought stress; Gohar-19 dropped to 2.34 g and 'Pop-1' dropped to 3.09 g. Gohar-19 (7.87%) showed more marked decrease in fresh weight than Pop-1 (6.07%), implying that under drought conditions Pop-1 maintains more water and biomass. Better osmotic adjustment, which helps the plant to retain water and continue cell development despite the decrease in external water availability, may be related to the capacity of Pop-1 to maintain greater fresh weight under stress circumstances.

Plant vigor combines several growth criteria: seedling biomass, root and shoot development, and general physiological state (Fig. 1g). Under control conditions (1929.72), Gohar-19 displayed the maximum plant vigor; followed by Pop-1 (1545.28). In both genotypes, drought stress caused a notable drop in plant vigor; Gohar-19 dropped to 1719.04 and 'Pop-1' dropped to 1173.62. Pop-1 (24%), had a bigger percentage decrease in plant vigor than 'Gohar-19' (10.9%), suggesting that under drought stress, Gohar-19 preserves more physiological activity than Pop-1. The improved plant vigor retention in Gohar-19 points to either increased root water absorption, improved stomatal control, or more effective antioxidant defense mechanisms against drought-induced oxidative stress as the genotype is better suited to sustain growth and metabolic activities under limited water availability.

With different degrees of decrease across the two genotypes, the data unequivocally show that drought stress negatively affects all evaluated parameters. With better resilience in germination %, root length, shoot length, and plant vigor, Gohar-19 genotype suggested superior drought tolerance mechanisms, especially in preserving root and shoot development. Conversely, under drought stress, Pop-1 showed better performance in dried weight, fresh weight, and leaf length indicating that it may adopt alternative drought tolerance strategies, such as better water retention, increased biomass accumulation efficiency, or a modified leaf morphology to maximize photosynthesis under stress conditions. The differing reactions of the two genotypes underline the complexity of drought tolerance mechanisms and imply that, while choosing for drought-resistant crop varieties, breeding programs should take several physiological and morphological characteristics into account.

Cluster Analysis

Integrated with genotypic treatments and trait responses, the hierarchical clustering analysis revealed

two main clusters (Fig. 2): one tied to biomass-related characteristics and drought stress and another connected with growth-related traits and control treatments. With "Gohar-19" excelling in root development and "Pop-1" in germination efficiency, "Gohar-19" and "Pop-1" grouped with germination percentage, root length, and shoot length showing that these qualities are more apparent under ideal conditions. By contrast, drought treatments ("Gohar-19-Drought" and "Pop-1-Drought") clustered with fresh weight, dried weight, plant vigor, and leaf length, implying that biomass conservation is an important drought adaptation strategy. Whereas 'Gohar-19-Drought' shown more divergence, suggesting a more dynamic drought adaptation strategy, 'Pop-1-Drought' demonstrated a closer relationship to its control, indicating a more stable response to drought. Under drought stress, the clustering of plant vigor and biomass features emphasizes how important structural integrity is rather than encouraging early-stage development in drought resistance. The significant correlation between root and shoot length in the control cluster underlines even more how co-regulated these features are and how mostly controlled by water availability. These results suggest that, in targeted drought adaptation, where biomass retention may be a preferred feature for extended drought conditions, breeding strategies should concentrate on selecting genotypes with optimal trait clustering; under intermittent water shortages, enhanced root and shoot growth may be beneficial. The cluster-based trait evaluation offers a whole framework for raising crop breeding drought resilience.

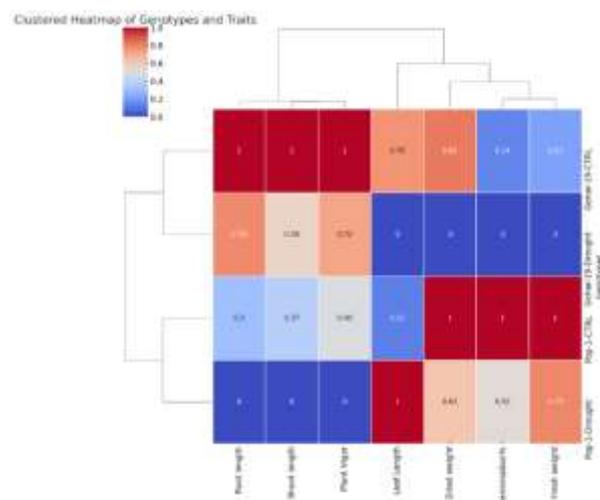


Fig. 2: Cluster analysis of studied traits and treatments under normal and drought stress conditions.

PCA Analysis

Direct comparison with the hierarchical clustering results was made possible by the clear visualizing of the correlations between genotypic treatments and traits made possible by the principal component analysis (PCA) (Fig. 3). With PC1 capturing most of the variation

among genotypes and characteristics, the first two main components—PC1 and PC2—explained a noteworthy fraction of the total variance.

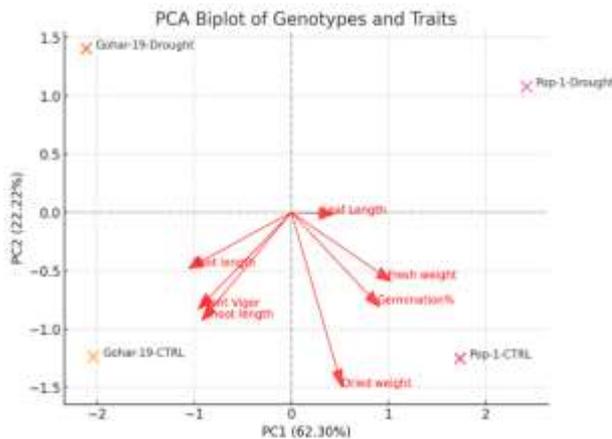


Fig. 3: PCA analysis of studied traits and treatments under normal and drought stress conditions.

Two separate clusters, one linked closely to biomass-related traits such as dried weight, fresh weight, leaf length, and plant vigor and another linked with growth-related traits such as germination percentage, root length, and shoot length, were revealed by the distribution of genotypic treatments in the PCA plot. While drought-treated genotypes ("Gohar-19-Drought" and "Pop-1-Drought") aligned more with biomass conservation traits, this clustering pattern is quite consistent with the hierarchical clustering results, where control treatments ("Gohar-19-CTRL" and "Pop-1-CTRL") grouped closely with growth-related traits. The PCA loadings revealed that 'Gohar-19-CTRL' was strongly linked with root and shoot length, while 'Pop-1-CTRL' showed a closer relationship with germination percentage, so confirming the earlier finding that 'Gohar-19' excels in root development while 'Pop-1' has superior seedling establishment under well-watered conditions. Whereas 'Pop-1-Drought' maintained a closer relationship with fresh and dried weight, 'Gohar-19-Drought' showed stronger relationships with plant vigor. Genotypes under drought stress moved their position in the PCA plot towards the biomass-conservation axis. This strengthens the theory that 'Pop-1' uses a biomass-retention strategy under drought stress, whereas 'Gohar-19' experiences a more dynamic physiological change to maintain vigor. Since both genotypes retained unique trait clustering even under stress conditions, the PCA results confirmed the hierarchical clustering conclusion that genotypic responses to drought are essentially driven by their underlying trait correlations rather than just treatment effects alone. Nonetheless, PCA gave greater understanding of the degree of similarity between genotypic responses by presenting a more continuous gradient of variation than the rigid clustering of hierarchical analysis. Although the cluster analysis clearly separated genotypes depending on general trait

similarity, PCA revealed finer-scale variations, showing that "Pop-1-Drought" showed fewer extreme changes in trait associations than "Gohar-19-Drought," in line with the earlier result showing "Pop-1" is more stable under stress. The close agreement between PCA and clustering results confirms the robustness of the observed drought-response patterns and suggests that both approaches essentially capture the underlying structure of genotype-trait relationships, so offering a complete framework for identifying potential drought-tolerant cultivars based on their phenotypic performance across many traits.

Effect of Salinity Stress on Studied Traits

Reflecting the negative effects of salt-induced osmotic stress on seed viability and early seedling establishment, the germination percentage showed a clear drop under salinity stress in both genotypes (Fig. 4a). 'Pop-1' showed the greatest germination percentage (80%) followed closely by 'Gohar-19' (79%). Under control conditions. But in 'Gohar-19,' germination declined to 74%; in 'Pop-1,' it dropped to 75%. Salinity affected both. While both genotypes showed a similar drop—about 5%—'Pop-1' kept a somewhat higher germination percentage, implying that it might have better seed tolerance to salt-induced stress or enhanced seed coat permeability, so allowing improved water absorption despite osmotic challenges. Often connected to ionic toxicity, especially the accumulation of Na^+ and Cl^- ions, which disturbs seed metabolic activities, is reduced germination in salinity conditions. The rather lesser decrease in 'Pop-1' suggests that this genotype may have superior seed vigor or a more efficient method for preventing too strong salt absorption during imbibition, so preserving germination rates.

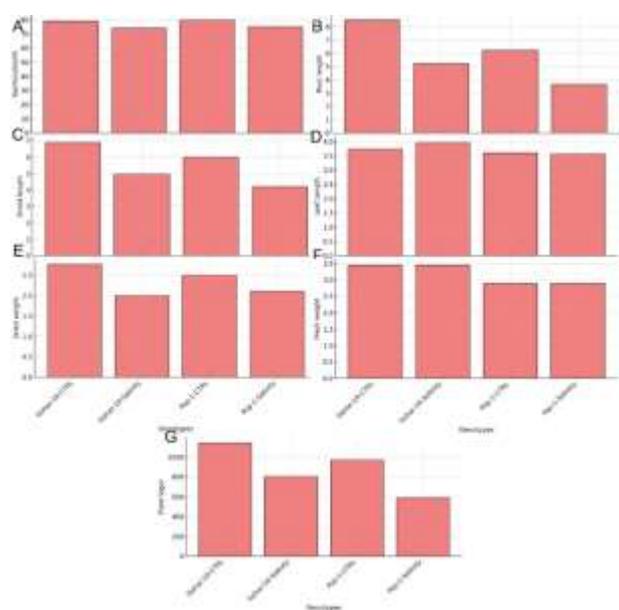


Fig. 4: The effect of salinity stress on morphological traits. a) Germination%, b) root length, c) shoot length, d) leaf length, e) dried weight, f) fresh weight, g) plant vigor index.

Plant tolerance to salinity stress depends critically on root length since it directly influences water and nutrient absorption efficiency (Fig. 4b). Suggesting a more active root system under non-stress conditions, 'Gohar-19' displayed a notably larger root length (8.52 cm) under control conditions than 'Pop-1' (6.22 cm). Salinity stress, however, substantially shortened root elongation in both genotypes; 'Gohar-19' dropped to 5.22 cm (a 38.7% reduction) and 'Pop-1' dropped even further to 3.64 cm (a 41.5% reduction). More severe drop in 'Pop-1' suggests that this genotype is more vulnerable to salinity-induced suppression of root development. This reaction can be ascribed to higher Na⁺ toxicity, which disturbs cellular osmotic equilibrium and causes shortened cell elongation and division in root meristems. The capacity of "Gohar-19" to sustain rather better root growth under salinity implies that it may have enhanced production of root-associated osmolytes including proline and glycine betaine, which help maintain cellular turgor or stronger salt exclusion mechanisms.

Under salt stress, shoot length showed considerable declines in line with root length (Fig. 4c). Under controlled conditions, 'Gohar-19' had a shoot length of 6.88 cm; under salt stress, this dropped to 4.96 cm, a 27.9% decrease. In terms of shoot elongation, "Pop-1" showed a somewhat higher sensitivity to salt stress as seen by a 29.9% reduction from 5.99 cm to 4.2 cm. Often linked with ion toxicity, decreased cell turgor, and hormonal imbalances—especially reductions in gibberellin levels and increased abscisic acid (ABA) accumulation—reduced shoot growth under salinity is sometimes related with The somewhat higher retention of shoot length in 'Gohar-19' indicates that this genotype might control its hormonal balance more effectively under stress conditions, therefore enabling better maintenance of shoot elongation than in 'Pop-1'. Furthermore, the larger root-to-shoot length ratio found in 'Gohar-19' under stress may point to a priority of root development to improve water absorption and maintain general plant growth.

With 'Gohar-19' displaying a modest rise under salinity stress and 'Pop-1' experiencing a minor decrease (Fig. 4d), leaf length shown a different response than root and shoot characteristics. 'Gohar-19' had a leaf length of 3.74 cm under control conditions; under salt stress, this grew to 3.96 cm. On the other hand, "Pop-1" showed a little drop in leaf length from 3.6 cm to 3.56 cm. The lengthening of the leaves in "Gohar-19" points to an adaptation mechanism perhaps used to maximize photosynthetic efficiency by maintaining leaf area growth under salinity stress. This could be connected to improved osmotic adjustment or higher water retention in leaf tissues, which supports cell proliferation independent of outside osmotic limitations. On the other hand, the little decrease in "Pop-1" points to a more traditional stress reaction in which plants limit leaf expansion to

lower transpiration losses under salinity.

A key measure of biomass accumulation, dried weight showed a marked decline under saline stress (Fig. 4e). 'Gohar-19' displayed a dry weight of 2.76 g under control conditions; under stress, this dropped to 2.0 g; whereas, 'Pop-1' dropped from 2.5 g to 2.1 g (a 16% reduction). These results imply that whilst 'Pop-1' preserves a rather superior biomass buildup, 'Gohar-19' endures a more loss of dry matter under salinity stress. The capacity of "Pop-1" to maintain a higher proportion of its dry weight under stress suggests better ion compartmentalization, in which case harmful Na⁺ and Cl⁻ ions are sequestered in vacuoles so minimizing their negative impact on cellular metabolism. Conversely, the larger decrease in 'Gohar-19' implies that, at the expense of biomass accumulation, this genotype may devote more energy toward stress tolerance mechanisms including the synthesis of osmolytes and stress-related proteins.

Under salinity stress, fresh weight—which represents plant water content and hydration state—remained constant in both genotypes (Fig. 4f). Fresh weights of 3.45 g and 2.89 g respectively for "Gohar-19" and "Pop-1" under control conditions were stable during salinity stress. This implies that, despite salt stress, both genotypes have efficient water retention systems most likely by osmotic adjustment including the buildup of suitable solutes such proline, carbohydrates, and glycine betaine. Although dried weight dropped, the fact that both genotypes kept fresh weight under stress suggests that water retention—a vital drought and salinity tolerance tactic—was compensatory for the loss of biomass.

Under salinity stress, plant vigor was the most significantly compromised quality displaying a significant drop in both genotypes (Fig. 4g). 'Gohar-19' displayed the highest plant vigor (1140.34) under control conditions; 'Pop-1' (969.6) came second. Salinity stress caused a notable drop; 'Gohar-19' dropped to 804.22 (a 29.5% reduction) and 'Pop-1' dropped to 589 (a 39.3% reduction). The larger decrease in 'Pop-1' implies that this genotype suffers more severe physiological and metabolic disturbances under salinity stress, maybe from less effective antioxidant defense systems or poorer ion homeostasis. By contrast, the somewhat higher plant vigor retention in 'Gohar-19' points to stronger tolerance mechanisms include improved Na⁺ exclusion, more efficient osmolyte storage, or enhanced stress-responsive gene expression.

Though with different degrees of intensity amongst genotypes, the data show that salt stress negatively affects all evaluated properties. 'Gohar-19' showed stronger mechanisms to deal with salinity since it retained higher plant vigor under stress and showed better resistance in root and shoot length retention. Conversely, 'Pop-1' showed superior biomass retention, especially in dried weight, which would suggest a distinct adaptation strategy stressing water retention

and ion compartmentalization. The varied reactions of the two genotypes emphasize that salinity tolerance is a complicated feature controlled by several physiological processes. These results offer important new perspectives for breeding initiatives meant to improve salinity tolerance in agricultural species, where genotypes with improved biomass conservation ('Pop-1') or stronger stress adaptation capacity ('Gohar-19') could be chosen depending on environmental conditions.

Cluster Analysis

Under salinity stress, the hierarchical clustering study along with the heatmap visualization gave a whole picture of the interactions between genotypic treatments and phenotypic responses (Fig. 5). Based on their trait correlations, the clustering pattern exposed two separate groups dividing salinity-stressed treatments from control. Closely linked to the control treatments ("Gohar-19-CTRL"), the first main cluster comprised growth-related variables including germination %, root length, and shoot length". This suggests that among the most sensitive to salinity stress and are more strongly expressed under ideal circumstances.

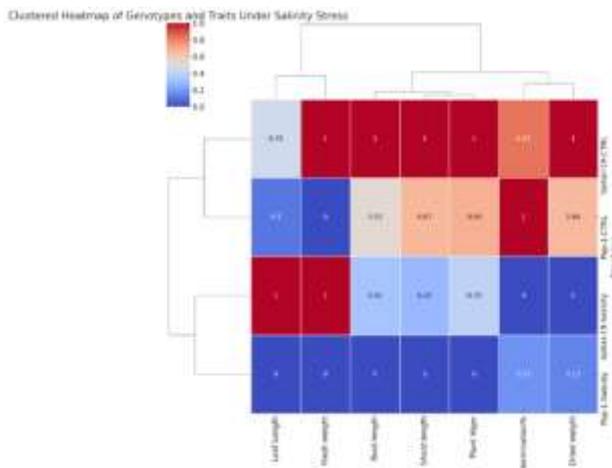


Fig. 5: Cluster analysis of studied traits and treatments under normal and salinity stress conditions.

Mostly connected with salinity-stressed genotypes ("Gohar-19-Salinity" and "Pop-1-Salinity"), the second significant cluster comprised biomass-related variables including dried weight, fresh weight, leaf length, and plant vigor. This implies that important markers of salinity tolerance mechanisms and play a major role in stress adaption related parameters are biomass-related ones.

Examining genotype clustering closely indicated that 'Gohar-19' and 'Pop-1' used different salinity adaption techniques. Positioned closer to root and shoot length, 'Gohar-19-CTRL' confirmed its exceptional development potential under controlled conditions. Under salinity stress, however, "Gohar-19" grouped more closely with plant vigor and leaf length, implying

that it gives physiological changes—perhaps through osmotic balance and stress-responsive mechanisms—top priority in order to sustain metabolic activity under stress. On the other hand, 'Pop-1-CTRL' indicated its first advantage in early-stage establishment and nutrient allocation under control settings since it was directly linked with germination percentage and biomass buildup features. On salinity stress, however, 'Pop-1' moved its cluster location toward dried weight and fresh weight, implying that it uses a biomass-conservation strategy rather than active growth maintenance, thereby enabling it to preserve structural integrity despite stress circumstances.

With notable reductions under salinity stress, the strong correlation of root and shoot length within the same cluster shows that these parameters are co-regulated and essentially driven by water availability. With genotype variations affecting the degree of biomass retention, the grouping of dried weight, fresh weight, and plant vigor in a separate cluster emphasizes their function in long-term response to stress. Especially, plant vigor was more strongly linked with "Gohar-19" under stress circumstances, therefore supporting the theory that this genotype has higher physiological resilience under salinity. While biomass retention and stress adaptation techniques define overall plant resilience, the general clustering pattern fits well with the PCA and bar chart data, thereby indicating that growth parameters are quite sensitive to salinity. These results imply that genotypic selection for salinity tolerance should take biomass conservation methods under stress into account as well as growth potential under control circumstances to maximize performance over several environmental variables.

PCA Analysis

Complementing the hierarchical clustering results, the Principal Component Analysis (PCA) biplot offered a comprehensive view of the interactions between genotypic treatments and their related features under salinity stress (Fig. 6). Strong variation among the genotypic responses to salinity was shown by the first two main components, PC1 and PC2, which explained a sizable fraction of the total variance. Growth-related traits (germination percentage, root length, and shoot length) were positioned in one direction, while biomass-related traits (dried weight, fresh weight, plant vigor, and leaf length) aligned in another, so reinforcing the earlier clustering results suggesting different regulation of these trait groups on the PCA biplot. Genotypic positioning in the PCA biplot confirmed the clustering analysis even more since 'Gohar-19-CTRL' and 'Pop-1-CTRL' matched root and shoot length but 'Gohar-19-Salinity' and 'Pop-1-Salinity' changed toward biomass traits, so showing their adaptation strategies under stress.

One important PCA biplot finding was the two genotypes' differing responses to salinity. Emphasizing its better development potential under ideal

conditions, "Gohar-19-CTRL" showed a greater link with shoot and root length. 'Gohar-19,' however, shifted toward plant vigor and leaf length under salinity stress, suggesting that this genotype gives physiological maintenance first priority over biomass preservation. Under salinity, 'Pop-1' changed toward dried weight, implying a biomass-conservation strategy rather than active growth maintenance. On contrast, 'Pop-1' had a closer correlation with germination % and fresh weight under control conditions. This fits very nicely with the results of hierarchical clustering, in which 'Pop-1-Salinity' was paired with biomass-related features while 'Gohar-19-Salinity' was better linked with plant vigor, so indicating various stress-response mechanisms. PCA showed how each treatment moves between growth and stress-adaptation strategies rather than allocating them to rigid groups, therefore offering a more continuous picture of variation than the hierarchical clustering technique. PCA showed the degree of response variations among every genotype, whereas cluster analysis obviously split the genotypic treatments into two main groups: control against salinity-stressed. Especially, 'Pop-1-Salinity' stayed closer to its control in PCA space, implying a steadier phenotype under stress, whereas 'Gohar-19-Salinity' showed a clearer change, so confirming the theory that 'Gohar-19' experiences more physiological changes under stress. The great agreement between PCA and cluster analysis validates the resilience of the observed patterns and emphasizes that trait-specific adaptation mechanisms mostly control genotypic variations in salinity response, hence should be taken into account in breeding programs aiming at stress tolerance improvement.

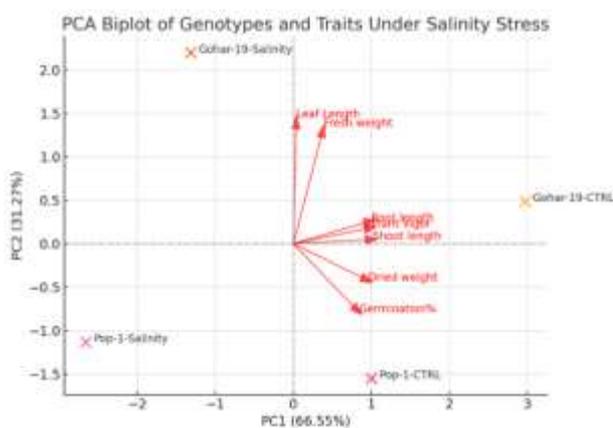


Fig. 6: PCA analysis of studied traits and treatments under normal and salinity stress conditions.

Comprehensive Mind Map of Genotypic Responses to Drought and Salinity Stress

The whole mind map shows the genotypic reactions of maize ('Gohar-19' and "Pop-1") to salinity stress and drought (Fig. 7), so stressing different adaptation strategies depending on the examined features. While "Pop-1" showed a more marked drop

(88%→82.7%), suggesting greater sensitivity, "Gohar-19" showed more stability in germination percentage (78.5%→77%), suggesting better seed viability under water deficit. Comparatively to "Pop-1" (8.96 cm→6.68 cm), "Gohar-19" (14.24 cm→12.6 cm) showed much better retention of root elongation, implying increased water absorption capacity under low moisture. Comparatively, "Gohar-19" maintained greater shoot length (10.5 cm→9.16 cm) and plant vigor (1929.72→1719.04) than "Pop-1," which shown more declines, so confirming that "Gohar-19" uses a growth maintenance strategy under drought. Fascinatingly, leaf length in "Pop-1" rose (3.98 cm→4.58 cm), while it dropped in "Gohar-19" (4.42 cm→3.9 cm), so indicating different drought adaptation strategies, whereby "Gohar-19" reduces water loss while "Pop-1" maximizes light capture. With dried weight: 2.05 g→1.67 g and fresh weight: 3.29 g→3.09 g, "Pop-1" displayed better biomass retention, so stressing its resilience in preserving structural integrity despite water stress. Both genotypes suffered decreases in germination percentage (~5%), under salinity stress; "Pop-1" showed rather better retention. Root length dropped more in "Pop-1," (6.22 cm→3.64 cm) than in "Gohar-19," (8.52 cm→5.22 cm), suggesting that under saline conditions "Gohar-19" had more root adaptability. Likewise, shoot length was more lowered in "Pop-1" (5.99 cm→4.2 cm) than in "Gohar-19" (6.88 cm→4.96 cm), indicating that under salinity "Gohar-19" had better shoot growth maintenance. Unlike drought stress, leaf length in "Gohar-19" slightly increased (3.74 cm→3.96 cm), while "Pop-1" showed a minor decrease (3.6 cm→3.56 cm), implying different salt tolerance strategies, with "Gohar-19" maybe maintaining photosynthetic efficiency while "Pop-1" conserves resources. Reflecting its ability to more successfully control osmotic balance and ion toxicity, "Pop-1" showed better biomass retention under salinity (dried weight: 2.5 g→2.1 g, fresh weight: 2.89 g constant). While "Pop-1" showed greater resilience under salinity by maintaining biomass and controlling physiological responses, "Gohar-19" performed better overall under drought by maintaining root elongation, shoot growth, and plant vigor. While salinity tolerance is linked with biomass conservation (dried and fresh weight, leaf adaptation), suggesting valuable insights for breeding stress-resilient maize varieties, the clustering of traits suggests that drought tolerance in maize is mostly linked to growth maintenance traits (root and shoot length, plant vigor).

The mind map convincingly combines the observed clustering and PCA findings to show that stress tolerance is not a single attribute but rather a set of interconnected physiological reactions varying between genotypes. Whereas 'Gohar-19' shows a dynamic growth-maintenance strategy that enables active development even under stress, 'Pop-1' adopts a more conservative biomass-preservation strategy that increases survival by reducing metabolic expenditure. These discoveries are absolutely vital for breeding

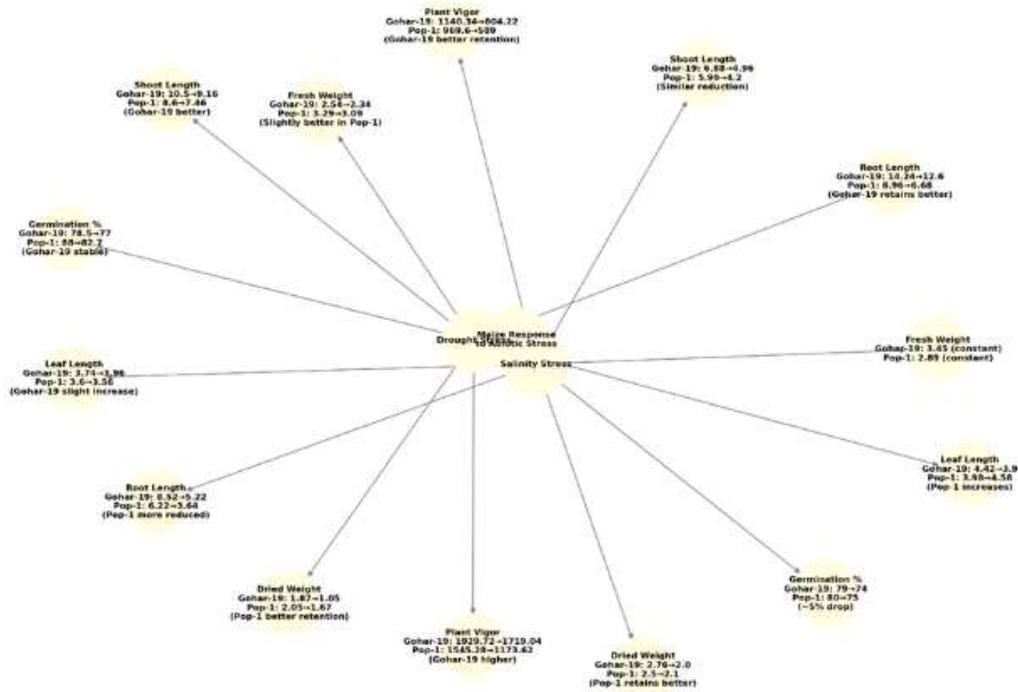


Fig. 7: Comprehensive mind map of studied traits under drought and salinity stress.

projects trying to create stress-resistant crop varieties since they imply that alternative genotypes can be chosen depending on the particular environmental circumstances they are predicted to face. 'Gohar-19' may be a better choice in areas where water supply varies because of its continuous root and shoot development; whereas, 'Pop-1' could be more appropriate for extended saline circumstances where osmotic adjustments and biomass conservation are vital for survival. The mind map offers a useful conceptual framework for choosing genotypes with ideal adaptation methods catered to various abiotic stress environments, therefore helping to build more robust crop systems.

DISCUSSION

Drought and salinity are two major abiotic stresses that severely hinder plant growth and productivity. Both stresses trigger extensive morphological, physiological, and biochemical changes in plants, ultimately reducing growth and yield (Seleiman et al. 2021; Islam et al. 2024). In our observations, the maize genotype 'Gohar-19' exhibited better growth maintenance under drought stress, while 'Pop-1' showed greater biomass retention under salinity stress. These differential responses suggest that each genotype leverages distinct tolerance mechanisms suited to the specific stress. Under drought, water deficit causes symptoms like reduced leaf expansion, leaf rolling, stunting, and wilting in maize (Jain et al. 2019). In our case, 'Gohar-19' maintained higher plant height and leaf area under drought compared to 'Pop-1'. This indicates Gohar-19 experienced less severe growth inhibition, likely due to effective drought

tolerance traits. Pop-1, in contrast, showed more pronounced growth reduction (e.g. smaller leaves, slower growth), reflecting a stronger drought impact. However, Pop-1 still survived by conserving resources, as evidenced by leaf rolling and reduced transpiration – classic drought avoidance responses (Seleiman et al. 2021).

Saline conditions impose both osmotic stress (water deficit due to high salt in soil) and ionic stress (toxic buildup of Na^+ and Cl^- in tissues). This commonly leads to chlorosis, leaf burn, and stunted growth in maize (Islam et al. 2024; Turan et al. 2009). 'Gohar-19' under salinity stress showed a notable reduction in new growth and some leaf chlorosis, suggesting salt-induced growth suppression. In contrast, 'Pop-1' retained a higher proportion of its biomass (fresh and dry weight) under salinity. For example, Pop-1 shed fewer leaves and maintained thicker, greener stems relative to Gohar-19. This biomass retention in Pop-1 implies it mitigated salt damage to tissues more effectively – possibly by excluding toxic ions and maintaining cell turgor. As a result, Pop-1 plants looked relatively healthy (though growth was slow), whereas Gohar-19 showed more obvious salt injury and biomass loss (e.g. leaf dry-down). Notably, each genotype's advantage was stress-specific: Gohar-19 excelled at keeping growth going in water-limited conditions, whereas Pop-1 excelled at preserving tissue integrity in salt-affected conditions. These findings align with general observations that different maize varieties cope with drought and salinity via different strategies (Eskikoy and Kutlu 2024).

Gohar-19 likely maintains growth under drought by sustaining water uptake. Drought-tolerant genotypes often develop deeper or more extensive roots to

extract water from dry soil, and adjust their root-to-shoot ratio in favor of roots (Birami et al. 2018; Miranda et al. 2021). By investing in root growth while limiting excessive shoot expansion, plants can continue acquiring water to support critical growth. Indeed, drought-stressed plants commonly show relatively greater root growth compared to shoots as an adaptive strategy (Miranda et al. 2021). Gohar-19 may exhibit this trait, allowing it to keep cells hydrated and turgid, thereby sustaining cell enlargement and plant growth even as soil moisture drops. Additionally, stomatal regulation plays a role: drought causes partial stomatal closure to reduce water loss. Gohar-19 appears to balance this well – partially closing stomata to conserve water, but not so much that photosynthesis halts completely. This balance helps maintain an adequate carbon supply for growth while avoiding desiccation. Many plants achieve this via increased abscisic acid (ABA) signaling that prompts timely stomatal closure and other water-saving responses (Seleiman et al. 2021).

Another key mechanism is osmotic adjustment – the accumulation of compatible solutes to retain water in cells. Gohar-19 likely accumulates higher levels of osmolytes (such as proline, sugars, and glycine betaine) under drought. These small molecules lower cell osmotic potential, which helps draw water into cells and maintain turgor pressure for growth (Hasegawa et al. 2000; Kaya et al., 2010; Saxena et al. 2013). For example, proline is a well-known Osmo protectant that increases under drought stress in tolerant plants, allowing them to continue absorbing water and keep cells hydrated (Yang et al. 2021). By maintaining turgor, Gohar-19's cells can still expand and divide, supporting continued growth (leaf expansion, stem elongation) when a sensitive plant would wilt. Increased soluble sugar content in tissues is also part of osmotic adjustment; sugars help retain water and can serve as carbon reserves for metabolism (Birami et al. 2018; Miranda et al. 2021). Effective osmotic adjustment in Gohar-19 is evidenced by its higher relative water content and less wilting under drought compared to Pop-1. This mechanism directly contributes to its growth maintenance.

Drought stress often leads to oxidative stress due to excess light energy (from stomatal closure limiting CO₂) and metabolic disruption. A distinguishing feature of Gohar-19 could be a strong antioxidant defense system that protects its growing tissues from reactive oxygen species (ROS). Tolerant genotypes tend to show elevated activities of enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidases under stress (Asada 1999; Gupta et al., 2005). These antioxidants detoxify ROS (e.g. converting superoxide and hydrogen peroxide into harmless molecules) and thereby prevent cellular damage. In drought, this means less membrane lipid peroxidation and less protein/nucleic acid damage in Gohar-19's cells, allowing its shoots to continue functioning and

growing. Indeed, research shows drought-tolerant dent maize maintains lower accumulation of harmful H₂O₂ and malondialdehyde (an indicator of lipid peroxidation) by keeping antioxidant enzymes active (Eskikoy and Kutlu 2024). Gohar-19 likely mirrors this pattern – it “stays green” and avoids premature leaf senescence due to an efficient ROS-scavenging system. By contrast, a less tolerant plant would suffer oxidative damage leading to cell death or growth arrest. Thus, Gohar-19's antioxidant capacity helps safeguard its growth processes under drought. Gohar-19 may also possess morphological traits that support growth under water stress. For instance, moderate leaf rolling (to reduce surface area and water loss) and a thicker leaf cuticle help conserve water without completely halting photosynthesis (Seleiman et al. 2021).

Plant physiology is known to be significantly impacted by salinity stress on both osmotic and ionic level. This is especially true for water uptake and nutrient transport. Salinity stress in this study resulted in shorter roots, shorter shoots, and lower fresh biomass, but these variations were not statistically significant. The absence of a significant effect ($p = 0.5427$ for leaf length) may suggest that the used maize varieties have built-in defenses against stress in the early germination. This may be due to increased osmotic control or selective ion uptake, as proposed by (Farooq et al. 2015), who observed comparable patterns in maize exposed to moderate salinity. However, considering how little stress was applied throughout the course of this investigation, longer exposure times could result in more noticeable impacts.

These results are consistent with other studies, including (Wang et al. 2019), which show that plants' response to salinity and drought stress are complicated and regulated by a variety of components at the physiological, biochemical, and genetic levels.

Conclusion

This work offered a thorough assessment of the genotypic responses of maize (“Gohar-19” and “Pop-1”) to salinity and drought stress, so exposing different adaptation mechanisms that could guide future breeding initiatives for stress-resilient cultivars. Under drought, “Gohar-19” displayed a growth maintenance strategy marked by stable germination rates, improved root elongation, continuous shoot development, and higher plant vigor, so indicating its capacity to maximize water intake and minimize drought-induced growth inhibition. Resilient in saline conditions, “Pop-1” showed a biomass retention strategy under salinity, preserving greater fresh and dried weight, controlling ion homeostasis, and adjusting leaf shape. The different stress-response mechanisms among these genotypes highlight the complexity of maize adaptation, in which salinity tolerance is linked to biomass conservation and osmotic control while drought tolerance is mostly related with maintaining

growth traits (root length, shoot elongation, and vigor). The PCA and clustering studies underlined even more how trait-specific rather than generally adaptive genotypic responses to stress support the need of multi-trait selection in breeding programs. The results of this work provide insightful analysis considering the growing influence of climate change on crop output for creating maize varieties with more resilience to saline and water-deficit environments. The molecular and biochemical pathways behind these stress reactions should be the main emphasis of future studies so that focused genetic enhancements may maximize maize performance in demanding agro-ecological conditions.

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