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İmproving Stress Adaptation**

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MEVLÜDE TATAR

CHAPTER 0 Selection of Drought-Resilient Cucumber Genotypes Using Multivariate Statistical Approaches

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Introduction

Drought is among the most important environmental constraints limiting agricultural production worldwide, and extreme weather events have been shown to negatively affect global crop production (Lesk et al., 2016). The increasing frequency and intensity of drought events associated with climate change have raised concerns regarding crop productivity and food security, particularly in regions characterized by limited water resources (Lobell & Gourджи, 2012; Ortiz-Bobea et al., 2021; Palmgren & Shabala, 2024). As water availability becomes increasingly unpredictable, the development of drought-resilient crop cultivars has emerged as a major objective of plant breeding programs.

Cucumber (*Cucumis sativus* L.) is one of the most widely cultivated vegetable crops and is highly dependent on adequate water supply throughout its growth cycle. Water deficit conditions may reduce leaf expansion, disrupt photosynthetic activity, impair plant water relations, and ultimately decrease yield. Yield losses caused by drought vary according to stress severity, duration, growth stage, and genotype, indicating the importance of genetic variation for drought adaptation (Farooq et al., 2009; Wahab et al., 2022).

Plant responses to drought involve a range of physiological and biochemical adjustments. Maintenance of relative water content (RWC), accumulation of compatible solutes such as proline, preservation of chlorophyll content, regulation of stomatal

conductance, and limitation of oxidative damage are among the mechanisms commonly associated with drought tolerance (Blum, 2017; Tardieu et al., 2018; Haghpanah et al., 2024). Because these responses are interconnected, evaluating a single trait is often insufficient to characterize drought adaptation accurately.

For this reason, multivariate statistical approaches are increasingly used in plant breeding studies. Methods such as principal component analysis (PCA), correlation analysis, regression analysis, and path coefficient analysis allow the simultaneous evaluation of multiple traits and provide a more comprehensive understanding of genotype performance under stress conditions (Jolliffe & Cadima, 2016; Mohammadi & Prasanna, 2003). These approaches can assist breeders in identifying key selection criteria and distinguishing superior genotypes from less adapted materials.

In recent years, integrating physiological measurements with statistical selection tools has become an effective strategy for identifying drought-resilient germplasm. Such approaches help reveal relationships among traits contributing to stress adaptation and facilitate the selection of breeding materials with improved performance under water-limited environments (Cooper & Messina, 2023; Hammer et al., 2020).

This chapter presents a comparative evaluation of cucumber genotypes subjected to drought stress using yield performance, physiological indicators, and multivariate statistical analyses. Particular emphasis is placed on genotype classification, trait relationships, and the identification of selection criteria that may contribute to the development of climate-resilient cucumber cultivars.

Drought Tolerance as a Breeding Target

The development of drought-tolerant cultivars has become a priority in crop improvement programs as water scarcity increasingly constrains agricultural production worldwide (Ortiz-Bobea et al., 2021; Palmgren & Shabala, 2024). In vegetable crops, drought stress not only reduces biomass accumulation and yield but may also affect product quality and production stability. Consequently, breeding strategies are increasingly

directed toward the identification of genotypes capable of maintaining acceptable performance under limited water availability (Cooper & Messina, 2023).

Drought tolerance is a complex trait controlled by numerous physiological, biochemical, and morphological mechanisms. Unlike traits governed by a small number of genes, drought adaptation results from the interaction of multiple processes that contribute collectively to plant survival and productivity under stress conditions (Farooq et al., 2009; Cattivelli et al., 2008). This complexity makes direct selection for drought tolerance challenging and highlights the need for reliable indirect selection criteria.

Among the physiological responses associated with drought adaptation, maintenance of plant water status is considered particularly important. Relative water content (RWC) is frequently used as an indicator of tissue hydration and reflects the ability of plants to retain water during stress. Genotypes capable of maintaining higher RWC values generally exhibit greater drought resilience because cellular functions can continue despite reduced soil moisture availability (Tardieu et al., 2018).

Photosynthetic stability also plays a central role in drought tolerance. Water deficit often accelerates chlorophyll degradation and restricts gas exchange through stomatal closure, leading to reduced carbon assimilation, impaired photosynthetic regulation, and lower productivity (Chaves et al., 2009). Therefore, chlorophyll concentration and stomatal conductance are commonly evaluated in breeding studies as indicators of plant performance under drought conditions (Blum, 2017; Wahab et al., 2022).

Biochemical responses provide additional information regarding stress adaptation. Proline accumulation is one of the most widely reported responses to drought stress and is associated with osmotic adjustment, cellular protection, and maintenance of metabolic activity. In contrast, malondialdehyde (MDA) is commonly used as an indicator of lipid peroxidation and oxidative damage. Lower MDA accumulation generally reflects more effective protection against stress-induced cellular injury (Haghpanah et al., 2024).

Recent studies in cucumber have demonstrated substantial genetic variation for drought-related physiological traits, indicating that effective selection can be achieved

through the integration of multiple indicators rather than reliance on a single characteristic (Das et al., 2024). Consequently, modern breeding programs increasingly combine physiological evaluation with quantitative selection approaches to improve the identification of drought-resilient germplasm.

The physiological traits examined in this chapter, including proline accumulation, relative water content, chlorophyll concentration, stomatal conductance, and MDA content, represent key components of drought adaptation. Their relationships with yield performance provide valuable information for identifying drought-resilient cucumber genotypes and developing breeding strategies for water-limited environments.

Yield Stability Under Water Deficit

Yield stability under drought conditions is an important consideration in crop improvement programs because the ultimate objective of breeding is to maintain productivity under adverse environmental conditions. Although physiological traits provide valuable information regarding plant responses to stress, yield performance remains one of the most relevant indicators of adaptation from both agronomic and economic perspectives (Blum, 2011; Cooper & Messina, 2023).

The evaluated cucumber genotypes differed in their response to water deficit conditions (Table 1). Yield under well-watered conditions ranged from 490 to 680 g plant⁻¹, whereas drought-stressed plants produced between 430 and 610 g plant⁻¹. Genotype G2 recorded the highest yield under both control and drought conditions, producing 680 and 610 g plant⁻¹, respectively. Similarly, G4 maintained relatively high productivity under stress despite a moderate reduction in yield. In contrast, G5 and G6 exhibited the lowest yield values under drought conditions.

Table 1. Yield and yield reduction under drought stress

Genotype	Yield Control (g/plant)	Yield Stress (g/plant)	Yield Reduction (%)
G1	570	510	10.50
G2	680	610	10.30
G3	600	540	10.00
G4	670	590	11.90

G5	510	460	9.80
G6	490	430	12.20

Note. Reduction was calculated as percentage loss relative to control yield values.

Source: Prepared by the author.

Yield reduction percentages varied from 9.8% to 12.2% among genotypes. The lowest reduction was observed in G5 (9.8%), followed by G3 (10.0%) and G2 (10.3%), whereas G6 showed the greatest reduction (12.2%). These results indicate that the magnitude of yield loss did not always correspond directly with absolute yield performance. For example, although G5 exhibited a relatively small percentage reduction, its yield under drought remained considerably lower than that of G2 and G4. Therefore, both yield retention and absolute productivity should be considered when evaluating drought resilience, as genotype adaptation is commonly assessed by comparing performance across favorable and stress-prone conditions (Finlay & Wilkinson, 1963; Becker & León, 1988).

The distribution of yield reduction values is presented in Figure 3. The relatively narrow range of variation among genotypes suggests that all materials experienced some degree of drought-induced yield limitation; however, differences in productivity under stress conditions indicate variation in adaptation capacity. Genotypes capable of maintaining higher yield levels while minimizing yield reduction are generally regarded as desirable candidates for breeding programs targeting water-limited environments (Eberhart & Russell, 1966; Hammer et al., 2020; Varshney et al., 2021).

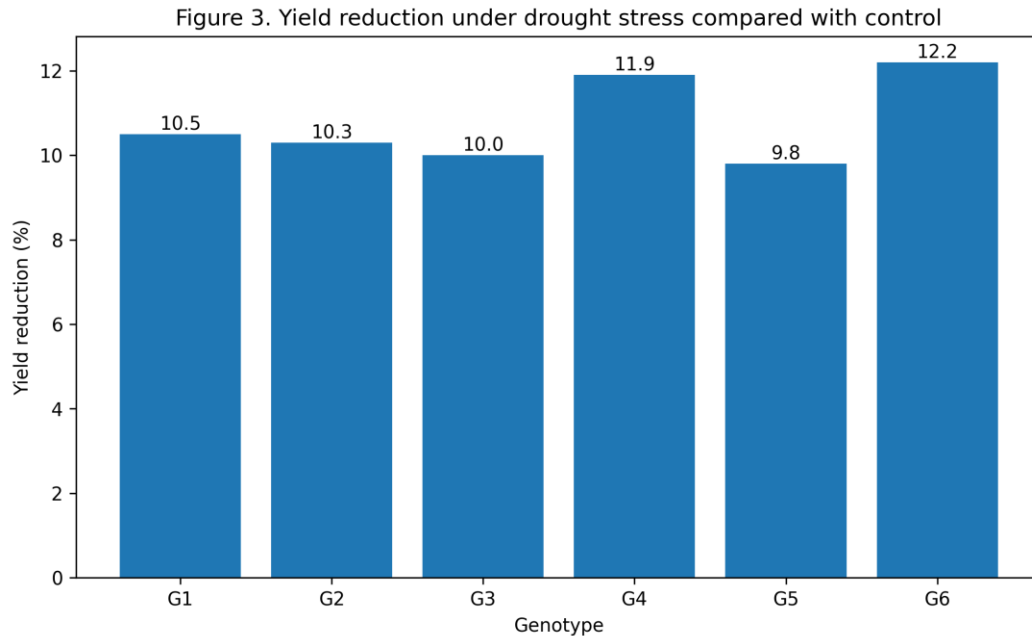


Figure 3. Yield reduction under drought stress compared with control

Source: Prepared by the author.

The results demonstrate that drought resilience cannot be assessed solely on the basis of yield reduction percentages. Instead, the combined evaluation of productivity, yield retention, and physiological performance provides a more reliable basis for genotype selection. This integrated approach is particularly important in vegetable breeding programs where stable production under fluctuating environmental conditions is a primary objective.

Principal Component Analysis for Genotype Classification

Principal component analysis (PCA) is widely used in plant breeding to summarize complex datasets and visualize relationships among genotypes and traits. By reducing multidimensional data into a smaller number of principal components, PCA facilitates the identification of patterns that may not be readily apparent through univariate analyses (Jolliffe, 2002; Jolliffe & Cadima, 2016; Hair et al., 2019; Mohammadi & Prasanna, 2003). Multivariate approaches are particularly useful in genotype evaluation because they allow breeders to interpret several related traits simultaneously rather than considering each trait separately (Crossa et al., 1990).

The PCA biplot presented in Figure 1 illustrates the distribution of cucumber genotypes based on yield and drought-related physiological traits. The first principal component (PC1) explained the majority of the observed variation among genotypes, indicating that differences in drought adaptation were largely associated with the measured physiological responses. Traits contributing positively to drought resilience, including proline accumulation, relative water content (RWC), chlorophyll concentration, stomatal conductance, and yield, were positioned on the positive side of the ordination space. In contrast, MDA was associated with the opposite direction, reflecting its negative relationship with plant performance under drought stress.

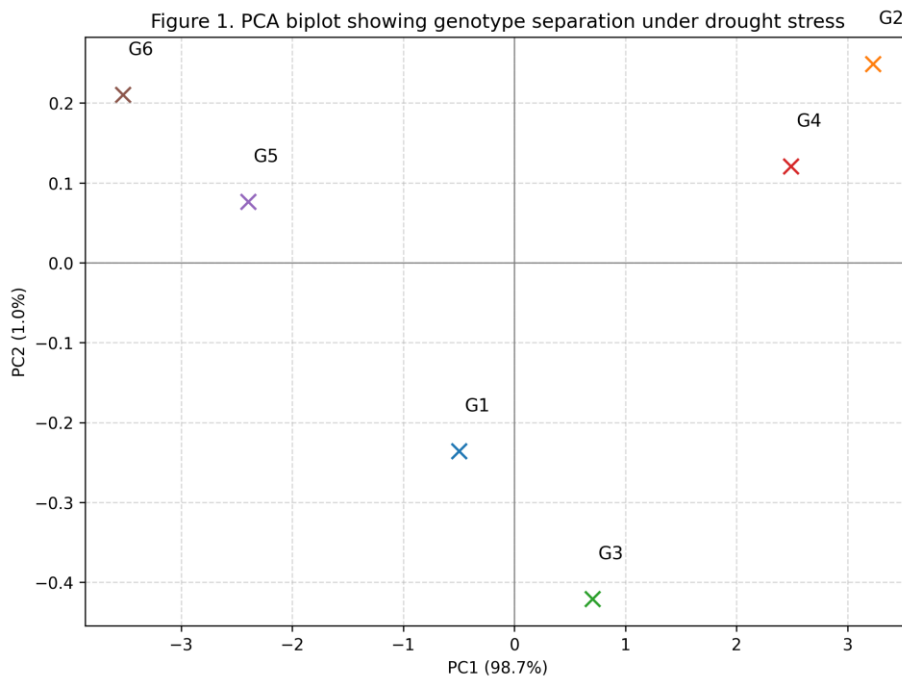


Figure 1. PCA biplot showing genotype separation under drought stress

Source: Prepared by the author.

PC1 explained 98.7% of the total variation, indicating that most differences among genotypes were associated with drought-response traits.

The distribution of genotypes within the PCA space revealed clear differences in adaptation capacity. Genotypes G2 and G4 were located in regions associated with favorable physiological characteristics and higher productivity under drought conditions. Their position in the ordination plot suggests that these genotypes combined effective water status maintenance, photosynthetic stability, and reduced oxidative damage. Such characteristics are commonly associated with improved drought adaptation in breeding populations (Blum, 2017; Tardieu et al., 2018).

Conversely, G5 and G6 were positioned away from the cluster associated with superior physiological performance. Their location indicates weaker association with traits contributing positively to drought resilience and greater susceptibility to stress-related limitations. The separation of these genotypes from G2 and G4 demonstrates the usefulness of PCA for distinguishing contrasting adaptation patterns within breeding materials.

Genotypes G1 and G3 occupied intermediate positions in the PCA plot, suggesting moderate drought tolerance. Although these materials did not exhibit the same level of adaptation as G2 and G4, they were clearly separated from the more sensitive genotypes. This intermediate classification highlights the continuous nature of drought adaptation and emphasizes the importance of evaluating multiple traits simultaneously.

Overall, the PCA results demonstrated substantial variation among cucumber genotypes and provided an effective framework for genotype classification. The clear separation of tolerant, intermediate, and sensitive materials supports the use of multivariate statistical approaches in breeding programs aimed at improving drought resilience. Furthermore, the consistency between genotype distribution in the PCA plot and their observed physiological performance strengthens confidence in the suitability of the evaluated traits as indicators of drought adaptation.

Correlation-Based Selection Criteria

The identification of reliable selection criteria is an essential component of drought-resilience breeding. Because drought tolerance is controlled by multiple interacting physiological mechanisms, the evaluation of relationships among traits can provide

valuable information for indirect selection. Correlation analysis is widely used to identify traits associated with yield performance and to determine which characteristics may serve as useful indicators of stress adaptation (Dewey & Lu, 1959; Singh & Chaudhary, 1979).

The Pearson correlation matrix presented in Table 2 and visualized in Figure 2 revealed several meaningful relationships among physiological traits and yield. Positive correlations were observed among proline accumulation, relative water content (RWC), chlorophyll concentration, stomatal conductance, and yield. These associations suggest that the maintenance of plant water status and photosynthetic activity contributes positively to productivity under drought conditions.

Table 2. Pearson correlation coefficients among physiological traits and yield

Trait	Proline	MDA	RWC	Chlorophyll	Stomatal Conductance	Yield
Proline	1.00	-0.61	0.72	0.55	0.49	0.68
MDA	-0.61	1.00	-0.65	-0.57	-0.51	-0.59
RWC	0.72	-0.65	1.00	0.63	0.61	0.66
Chlorophyll	0.55	-0.57	0.63	1.00	0.68	0.74
Stomatal Conductance	0.49	-0.51	0.61	0.68	1.00	0.79
Yield	0.68	-0.59	0.66	0.74	0.79	1.00

Source: Prepared by the author.

Figure 2. Pearson correlation heatmap among physiological traits and yield

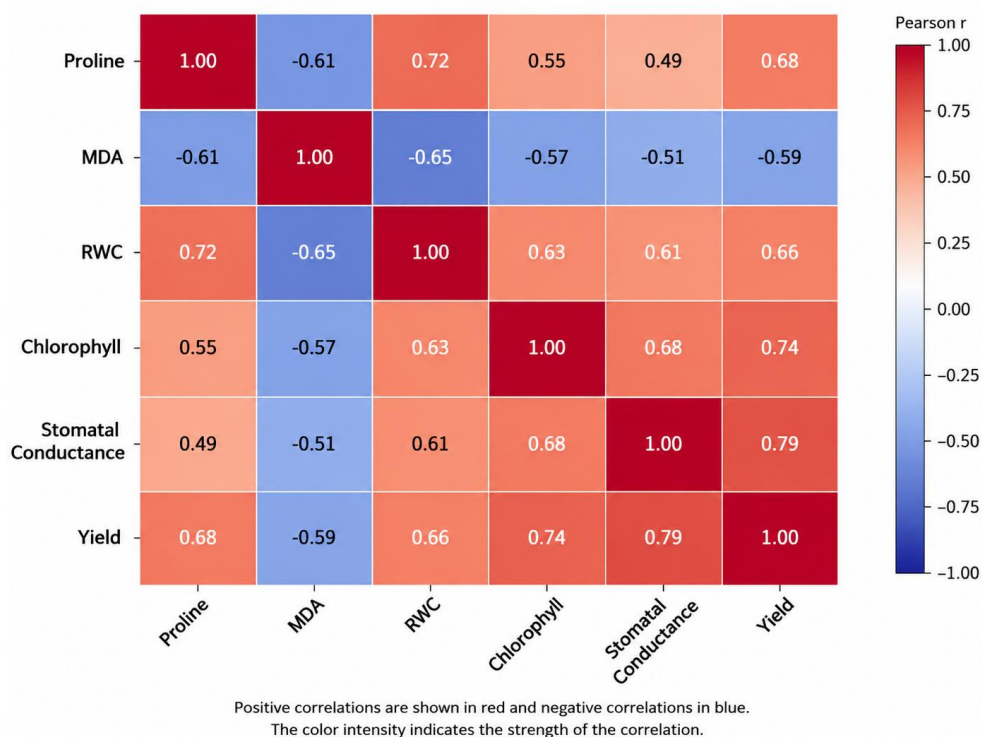


Figure 2. Pearson correlation heatmap among physiological traits and yield

Source: Prepared by the author.

Among the evaluated traits, stomatal conductance exhibited the strongest positive association with yield ($r = 0.79$), followed by chlorophyll concentration ($r = 0.74$). These results indicate that genotypes capable of maintaining gas exchange and preserving photosynthetic pigments under water deficit conditions tend to achieve superior productivity. Similar relationships have been reported in previous drought studies where sustained photosynthetic activity was associated with improved crop performance under stress (Blum, 2017; Wahab et al., 2022).

Proline accumulation also showed a positive relationship with yield ($r = 0.68$). The accumulation of compatible solutes such as proline is commonly regarded as an adaptive response that contributes to osmotic adjustment and cellular protection during drought stress. Although the association was less pronounced than that observed for chlorophyll concentration and stomatal conductance, the results suggest that proline may still represent a useful physiological indicator of drought adaptation.

Relative water content was positively correlated with yield ($r = 0.66$), supporting the importance of tissue hydration in maintaining plant productivity under limited water availability. Genotypes capable of sustaining higher RWC values generally exhibit greater capacity to tolerate drought-induced reductions in soil moisture and maintain normal physiological functions.

In contrast, malondialdehyde (MDA) displayed a negative association with yield ($r = -0.59$). Since MDA is widely used as an indicator of oxidative damage and membrane lipid peroxidation, increased MDA accumulation reflects greater cellular injury under stress conditions. The observed negative relationship suggests that genotypes experiencing lower oxidative damage are more likely to maintain productivity during drought.

The correlation structure observed among traits indicates that drought adaptation in cucumber is governed by the interaction of several physiological processes rather than by a single mechanism. Consequently, selection strategies based on multiple complementary traits are expected to be more effective than those relying exclusively on yield performance. The combined evaluation of stomatal conductance, chlorophyll concentration, proline accumulation, RWC, and MDA may therefore provide a practical framework for identifying drought-resilient breeding materials.

Trait Relationships and Breeding Implications

Regression and path coefficient analyses are widely used in plant breeding to evaluate relationships among traits and to identify characteristics that contribute directly or indirectly to yield performance. These approaches assist breeders in distinguishing primary selection criteria from secondary traits and provide a framework for understanding the relative importance of different components of stress adaptation (Wright, 1921; Dewey & Lu, 1959; Bhatt, 1973). Similar trait-based approaches have been used to interpret yield-related relationships and support selection decisions in crop improvement studies (Samonte et al., 1998).

In practical breeding programs, physiological traits associated with yield under drought conditions are frequently used as indirect selection criteria, particularly when

drought tolerance is controlled by multiple interacting mechanisms. The relative importance of the evaluated physiological traits is summarized in Table 3.

Table 3. Relative importance of physiological traits as indirect selection criteria for drought resilience

Trait	Pearson r with Yield	Strength of Association	Breeding Relevance
Stomatal Conductance	0.79	Strong positive	Primary selection criterion
Chlorophyll	0.74	Strong positive	Important indicator of photosynthetic stability
Proline	0.68	Moderate to strong positive	Indicator of osmotic adjustment capacity
RWC	0.66	Moderate positive	Indicator of plant water status maintenance
MDA	-0.59	Moderate negative	Indicator of oxidative stress damage

Note. Trait importance was determined using Pearson correlation coefficients with yield under drought stress. Positive coefficients indicate favorable contributions to drought adaptation, whereas negative coefficients indicate detrimental effects on plant performance. Stomatal conductance and chlorophyll content exhibited the strongest positive associations with yield and may therefore serve as useful indirect selection criteria in drought-resilience breeding programs.

Source: Prepared by the author.

Among the investigated traits, stomatal conductance exhibited the strongest association with yield ($r = 0.79$), indicating that the maintenance of gas exchange under drought conditions is closely related to plant productivity. This result suggests that stomatal conductance may represent a useful physiological criterion for identifying drought-resilient genotypes during early stages of selection.

Chlorophyll concentration also showed a strong positive relationship with yield ($r = 0.74$). The ability to maintain photosynthetic pigments under water deficit conditions is often associated with sustained carbon assimilation and improved stress adaptation. Consequently, chlorophyll content may serve as a practical indicator of physiological stability under drought stress.

Proline accumulation and relative water content (RWC) displayed moderate positive associations with yield. These traits are commonly linked to osmotic adjustment and maintenance of plant water status, respectively. Their contribution to drought adaptation supports their inclusion in multi-trait selection schemes aimed at improving resilience under water-limited environments.

In contrast, malondialdehyde (MDA) exhibited a negative relationship with yield. Since MDA reflects membrane lipid peroxidation and oxidative damage, increased MDA accumulation is generally associated with reduced physiological performance. Therefore, lower MDA values may be considered favorable when selecting breeding materials for drought-prone environments.

The ranking presented in Table 3 indicates that stomatal conductance and chlorophyll concentration were the most informative traits associated with yield performance under drought stress. However, the observed relationships also demonstrate that drought adaptation cannot be explained by a single physiological parameter. Instead, the combined evaluation of multiple complementary traits provides a more comprehensive basis for genotype selection.

Although formal path coefficient analysis was not conducted, the observed trait–yield relationships provide useful information regarding the relative contribution of physiological characteristics to drought resilience. Such information can support the development of selection strategies that integrate productivity with key adaptive mechanisms in cucumber breeding programs.

Identification of Superior Genotypes

The integration of physiological measurements, yield performance, and multivariate statistical analyses provides a comprehensive basis for identifying drought-resilient breeding materials. Because drought tolerance is a complex trait influenced by multiple interacting mechanisms, genotype selection should rely on the combined evaluation of agronomic and physiological characteristics rather than a single parameter.

The evaluated cucumber genotypes exhibited substantial variation in their responses to drought stress. Differences were observed not only in yield performance and yield reduction but also in physiological indicators associated with drought adaptation. The integration of these variables facilitated the classification of genotypes according to their overall resilience under water-deficit conditions.

To facilitate genotype comparison, an integrated selection index was developed using normalized values of yield under drought stress, yield retention, relative water content (RWC), chlorophyll concentration, stomatal conductance, proline accumulation, and inverse malondialdehyde (MDA) content. Higher index values indicated greater overall drought resilience by combining agronomic and physiological performance into a single comparative measure.

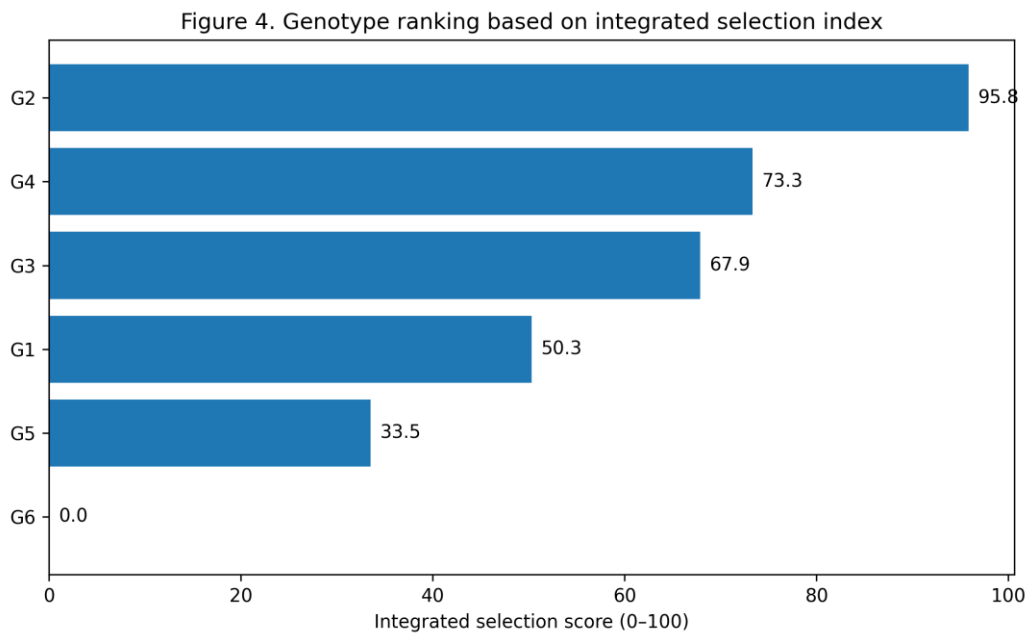


Figure 4. Integrated selection index values and ranking of cucumber genotypes under drought stress.

Source: Prepared by the author.

Integrated selection index was calculated using normalized values of yield, yield retention, stomatal conductance, chlorophyll content, relative water content (RWC), proline accumulation and inverse MDA values. Higher scores indicate greater overall drought resilience.

Among the evaluated materials, genotype G2 consistently demonstrated superior performance across the measured traits. This genotype produced the highest yield under drought conditions while maintaining favorable physiological characteristics associated with stress adaptation. Its position in the PCA ordination space (Figure 1), together with its leading score in the integrated selection index (Figure 4), indicates a high level of drought resilience. The ability of G2 to combine productivity with physiological stability makes it a promising candidate for breeding programs targeting water-limited environments.

Genotype G4 also exhibited strong adaptation to drought stress. Although its yield reduction was slightly greater than that observed in some other materials, its overall physiological profile and PCA classification were consistent with improved drought tolerance. The high ranking of G4 in the integrated selection index further supports its value as a potential breeding parent.

Genotypes G1 and G3 occupied intermediate positions in the selection ranking. These materials maintained acceptable performance under drought conditions and displayed moderate adaptation characteristics. While they did not reach the performance level of G2 and G4, they may still represent useful genetic resources for breeding populations where multiple adaptive traits are desirable.

In contrast, G5 and G6 were classified as the least drought-resilient genotypes. Both materials exhibited lower productivity under stress conditions and achieved the lowest scores in the integrated selection index. Their separation from the more tolerant genotypes in the PCA analysis further supports their classification as drought-sensitive materials.

The consistency observed among yield performance, PCA classification, correlation analysis, and integrated selection index ranking strengthens confidence in the identification of superior genotypes. In particular, the agreement among independent analytical approaches indicates that the evaluated physiological traits effectively captured variation in drought adaptation.

Based on the integrated assessment, G2 and G4 emerged as the most promising drought-resilient genotypes. These materials combined favorable physiological responses with stable productivity under stress conditions and may therefore serve as valuable genetic resources for the development of climate-resilient cucumber cultivars.

Future Perspectives for Climate-Resilient Breeding

The increasing frequency of drought events associated with climate change is expected to intensify challenges for vegetable production systems worldwide. Consequently, the development of climate-resilient cultivars capable of maintaining productivity under water-limited conditions will remain a major objective of future breeding programs (IPCC, 2023; Ortiz-Bobea et al., 2021).

The results presented in this chapter demonstrate that substantial variation exists among cucumber genotypes in their response to drought stress. Such variation provides valuable opportunities for breeding programs seeking to improve adaptation to increasingly unpredictable environmental conditions. The identification of genotypes with favorable physiological characteristics and stable productivity represents an important first step toward the development of drought-resilient cultivars.

Future breeding efforts should increasingly integrate physiological screening with conventional yield evaluation. Traits such as stomatal conductance, chlorophyll concentration, relative water content, proline accumulation, and MDA content provide useful information regarding plant responses to water deficit and may facilitate the early identification of superior breeding materials (Blum, 2017; Haghpanah et al., 2024). The use of these indirect selection criteria may improve breeding efficiency, particularly when large germplasm collections are evaluated.

Advances in phenotyping technologies are expected to further enhance drought-tolerance breeding. High-throughput phenotyping platforms, remote sensing tools, and image-based monitoring systems can provide rapid assessments of physiological traits associated with stress adaptation. The integration of such technologies with traditional breeding approaches may accelerate the identification of promising genotypes and reduce the time required for cultivar development (Tuberosa, 2012; Cooper & Messina, 2023).

The application of multivariate statistical methods will continue to play an important role in genotype evaluation. Techniques such as principal component analysis, correlation analysis, selection indices, GGE biplot analysis, and stability assessment facilitate the

simultaneous interpretation of multiple traits and support more informed breeding decisions (Jolliffe & Cadima, 2016; Mohammadi & Prasanna, 2003; Yan & Kang, 2003; Yan et al., 2007).

The superior performance of G2 and G4 identified in this study suggests that these genotypes may represent valuable genetic resources for future cucumber improvement programs. Their favorable physiological responses and relatively stable productivity under drought conditions indicate potential usefulness as parental materials in breeding populations targeting adaptation to water-limited environments.

Future studies should evaluate these promising materials across multiple environments and growing seasons to determine the stability of their performance under diverse climatic conditions. The incorporation of molecular markers, genomic tools, and genotype-by-environment analyses may further improve the efficiency of selecting drought-resilient germplasm and contribute to the development of cultivars better adapted to future climate scenarios.

Overall, combining physiological evaluation, multivariate statistical analyses, and modern breeding technologies offers a promising pathway for improving drought resilience in cucumber. Such integrated approaches will be increasingly important for sustaining vegetable production under the environmental challenges associated with climate change.

Conclusions

Drought stress remains one of the most important environmental constraints affecting crop productivity and represents a major challenge for sustainable vegetable production under changing climatic conditions. The identification of drought-resilient genotypes is therefore an essential objective of modern breeding programs. Because drought adaptation is governed by multiple interacting physiological mechanisms, the use of integrated evaluation approaches is necessary for effective genotype selection.

The results presented in this chapter demonstrated substantial variation among cucumber genotypes in their responses to drought stress. Differences were observed in

yield performance, yield reduction, physiological characteristics, and overall adaptation capacity. These findings confirm the existence of exploitable genetic variability that can be utilized in breeding programs targeting improved drought resilience.

Yield performance alone did not provide a complete assessment of drought adaptation. The inclusion of physiological traits such as relative water content, chlorophyll concentration, stomatal conductance, proline accumulation, and malondialdehyde content provided a more comprehensive understanding of genotype responses to water deficit conditions. The correlation analysis indicated that stomatal conductance and chlorophyll concentration exhibited the strongest positive associations with yield, whereas MDA showed a negative relationship with productivity under drought stress.

The application of multivariate statistical approaches proved useful for genotype classification and trait evaluation. Principal component analysis successfully separated the genotypes according to their physiological and agronomic performance, while correlation-based assessments helped identify traits with potential value as indirect selection criteria. The integration of these analytical approaches facilitated a more reliable interpretation of drought adaptation than would have been possible using individual traits alone.

The integrated selection index further supported the identification of superior genotypes. Among the evaluated materials, G2 and G4 consistently exhibited favorable physiological characteristics, stable productivity, and high overall performance under drought conditions. These genotypes emerged as the most promising candidates for future breeding efforts aimed at improving drought resilience in cucumber.

Overall, the findings emphasize the importance of combining yield evaluation with physiological measurements and multivariate statistical analyses when assessing drought tolerance. Such integrated approaches improve selection efficiency, enhance the identification of superior breeding materials, and contribute to the development of climate-resilient cucumber cultivars capable of maintaining productivity under increasingly water-limited environments.

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CHAPTER 0

Physiological Responses of Cucumber Genotypes to Drought Stress: Implications for Climate-Resilient Cultivar Development

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Introduction

Climate change has become one of the most serious threats to global agricultural production systems. Rising temperatures, irregular rainfall patterns, prolonged drought periods, and increasing pressure on freshwater resources are substantially affecting crop productivity worldwide (Farooq et al., 2009; Blum, 2017; Flexas et al., 2002). Among the various abiotic stresses associated with climate change, drought is considered one of the most damaging environmental factors because it directly influences plant growth, development, physiology, and yield formation (IPCC, 2023; Lesk et al., 2016). The increasing frequency of drought events, particularly in semi-arid and Mediterranean environments, poses a major challenge to sustainable crop production and food security. The long-term impacts of anthropogenic climate change on agricultural productivity have already been documented at the global scale, highlighting the urgent need for developing crop cultivars capable of maintaining productivity under increasingly adverse environmental conditions (Ortiz-Bobea et al., 2021).

Vegetable crops are generally more sensitive to water deficits than many field crops due to their relatively shallow root systems, high transpiration rates, and continuous demand for water throughout the growing season. Water scarcity can adversely affect germination, vegetative growth, flowering, fruit development, and marketable yield (Farooq et al., 2009; Flexas et al., 2002). In addition to reducing productivity, drought stress often decreases product quality and economic value, making the development of drought-resilient vegetable cultivars an important objective for modern breeding programs.

Cucumber (*Cucumis sativus* L.) is one of the most widely cultivated vegetable crops in both open-field and protected cultivation systems. The crop is characterized by rapid vegetative growth, a high leaf area index, and substantial water requirements, making it particularly vulnerable to drought stress. Water limitation during critical developmental stages can reduce photosynthetic activity, impair nutrient uptake, accelerate leaf senescence, decrease fruit set, and ultimately result in significant yield losses (Wang et al., 2012; Wang et al., 2024). As climate change continues to intensify water scarcity in many cucumber-growing regions, understanding the physiological mechanisms underlying drought tolerance has become increasingly important.

Plants employ a range of adaptive responses to mitigate the adverse effects of drought stress. These responses include morphological adjustments, physiological regulation, biochemical protection, and molecular signaling pathways (Wahab et al., 2022). Among physiological responses, osmotic adjustment, maintenance of cellular water status, preservation of photosynthetic capacity, and reduction of oxidative damage are considered essential components of drought tolerance. The efficiency of these mechanisms often varies among genotypes, providing opportunities for the identification and selection of superior germplasm for breeding purposes (Das et al., 2024).

Proline accumulation is one of the most widely studied physiological responses to drought stress. As an important osmoprotectant, proline contributes to osmotic adjustment, membrane stabilization, enzyme protection, and reactive oxygen species detoxification under water-limited conditions (Bates et al., 1973; Blum, 2017). Numerous studies have demonstrated that drought-tolerant genotypes generally accumulate higher levels of proline, allowing them to maintain cellular integrity and metabolic activity during stress periods. Recent reviews have also highlighted the central role of proline in coordinating osmotic adjustment, antioxidant defense, and stress signaling pathways under drought conditions (Haghpanah et al., 2024).

In contrast, drought stress frequently induces oxidative damage through excessive production of reactive oxygen species (ROS). One of the most commonly used indicators of oxidative injury is malondialdehyde (MDA), a by-product of membrane lipid peroxidation (Heath & Packer, 1968). Elevated MDA concentrations are generally

associated with increased cellular damage and reduced stress tolerance, whereas lower MDA accumulation may indicate more efficient antioxidant defense mechanisms.

Relative water content (RWC) is another important physiological indicator widely used in drought studies. RWC reflects the ability of plant tissues to maintain hydration under water-deficit conditions and is closely associated with drought adaptation (Barrs & Weatherley, 1962; Blum, 2011). Genotypes capable of maintaining higher RWC values generally exhibit improved water retention capacity and enhanced physiological stability under stress conditions. Similarly, chlorophyll content provides valuable information regarding the integrity of the photosynthetic apparatus during drought. The maintenance of chlorophyll concentration under stress conditions is often associated with improved photosynthetic efficiency and greater tolerance to environmental constraints.

Stomatal conductance represents a key physiological trait linking plant water relations with carbon assimilation. Through stomatal regulation, plants attempt to balance water conservation and photosynthetic activity under drought conditions. Efficient stomatal control can reduce excessive water loss while maintaining sufficient carbon uptake for growth and physiological functioning. Consequently, stomatal conductance has been widely recognized as an important selection criterion in drought tolerance studies involving vegetable crops.

Recent advances in physiological phenotyping and statistical analysis have enhanced the ability of researchers to identify drought-tolerant genotypes using multiple complementary traits. Integrative approaches combining physiological measurements with multivariate analyses, such as principal component analysis (PCA), correlation analysis, and regression models, provide valuable insights into genotype performance under stress conditions. These approaches facilitate the identification of key selection criteria and support the development of climate-resilient breeding strategies.

Given the increasing challenges imposed by climate change and water scarcity, the identification of reliable physiological indicators for drought tolerance in cucumber has become an important research priority. This chapter examines the physiological responses of cucumber genotypes to drought stress, with particular emphasis on proline accumulation, malondialdehyde content, relative water content, chlorophyll concentration, and stomatal conductance. The potential application of these physiological traits as

practical selection criteria for drought-tolerant and climate-resilient cucumber breeding is also discussed.

Drought Stress and Plant Water Relations

Water is essential for virtually all physiological and biochemical processes in plants. It functions as a solvent, a transport medium for nutrients, a substrate for metabolic reactions, and a key component in maintaining cell turgor and structural integrity. Consequently, water deficiency disrupts numerous physiological processes and often represents one of the most severe environmental constraints limiting agricultural productivity worldwide (Farooq et al., 2009).

Drought stress occurs when water availability becomes insufficient to meet the transpiration demand of plants. Under such conditions, the balance between water uptake by roots and water loss through transpiration is disturbed, resulting in reduced tissue hydration and altered cellular metabolism. The severity of drought effects depends on several factors, including stress duration, intensity, environmental conditions, plant developmental stage, and genotype-specific tolerance mechanisms. Recent studies further emphasize that drought tolerance is a complex trait governed by dynamic interactions among water availability, plant developmental stage, and environmental conditions, making physiological responses highly context dependent (Tardieu et al., 2018).

One of the earliest responses of plants to drought stress is a reduction in leaf water potential and cellular turgor pressure. Loss of turgor limits cell expansion, reduces leaf growth, and ultimately restricts overall plant development. As drought progresses, plants initiate a series of adaptive responses designed to conserve water and maintain essential physiological functions. These responses include stomatal closure, osmotic adjustment, modification of root architecture, accumulation of compatible solutes, and activation of antioxidant defense systems (Blum, 2017; Farooq et al., 2009).

In cucumber, drought stress rapidly affects water relations due to the species' relatively shallow root system and high transpiration demand. Water limitation reduces root water uptake capacity, resulting in decreased leaf hydration and impaired physiological performance. Previous studies have demonstrated that cucumber plants exposed to drought exhibit reductions in leaf area, biomass accumulation, chlorophyll

concentration, photosynthetic activity, and fruit yield (Wang et al., 2012; Wang et al., 2024). These effects become particularly pronounced during flowering and fruit development stages, when water demand is highest.

Relative water content (RWC) is widely regarded as one of the most reliable indicators of plant water status under drought conditions. Unlike soil moisture measurements alone, RWC directly reflects the hydration status of plant tissues and provides valuable information regarding the ability of plants to maintain cellular water balance. Genotypes capable of maintaining higher RWC values generally exhibit improved drought tolerance because they can sustain physiological activity despite limited water availability. Consequently, RWC has become one of the most frequently used screening criteria in drought tolerance studies involving vegetable crops.

Stomatal regulation represents another critical mechanism governing plant water relations under drought stress. Stomata are microscopic pores located on the leaf surface that regulate both carbon dioxide uptake and water vapor loss. Under drought conditions, plants often reduce stomatal aperture to minimize transpiration and conserve water. Although this response improves water-use efficiency, it simultaneously restricts carbon dioxide assimilation and photosynthetic activity. Therefore, drought tolerance often depends on a plant's ability to optimize the balance between water conservation and carbon fixation (Flexas et al., 2002).

Photosynthesis is particularly sensitive to water deficit (Chaves et al., 2009; Ashraf & Harris, 2013). Reduced stomatal conductance limits carbon dioxide availability within leaf tissues, while dehydration may directly affect chloroplast structure and function. These changes frequently result in chlorophyll degradation, reduced photosynthetic efficiency, and lower biomass production. In severe cases, prolonged drought may induce irreversible damage to photosynthetic machinery, substantially reducing plant productivity (Chaves et al., 2009).

To counteract the detrimental effects of dehydration, plants accumulate various osmoprotective compounds that contribute to osmotic adjustment. These compounds help maintain cellular hydration, stabilize proteins and membranes, and support metabolic activity under stress conditions. Among them, proline is one of the most extensively studied compatible solutes associated with drought tolerance. Increased proline accumulation has

been reported in numerous crop species, including cucumber, and is generally considered an important adaptive response to water deficit stress (Bates et al., 1973; Verbruggen & Hermans, 2008).

Drought stress is also closely associated with oxidative stress (Mittler, 2002; Gill & Tuteja, 2010). Reduced water availability frequently disrupts electron transport processes in chloroplasts and mitochondria, leading to excessive production of reactive oxygen species (ROS). When ROS accumulation exceeds the capacity of antioxidant defense systems, oxidative damage occurs in cellular membranes, proteins, and nucleic acids. Lipid peroxidation products such as malondialdehyde (MDA) are therefore commonly used as indicators of cellular damage and stress severity (Heath & Packer, 1968; Ayala et al., 2014).

The complexity of plant water relations under drought highlights the importance of integrating multiple physiological parameters when evaluating stress tolerance. Individual traits may provide only limited information, whereas combined assessment of water status, osmotic adjustment, stomatal behavior, photosynthetic integrity, and oxidative damage offers a more comprehensive understanding of plant adaptation mechanisms. Such integrative approaches have become increasingly important in modern breeding programs aimed at developing climate-resilient cucumber cultivars capable of maintaining productivity under water-limited environments.

Proline Accumulation as an Osmotic Adjustment Mechanism

Among the numerous physiological responses induced by drought stress, proline accumulation is considered one of the most important adaptive mechanisms contributing to plant survival under water-deficit conditions. Proline is a multifunctional amino acid that accumulates in plant tissues in response to various abiotic stresses, including drought, salinity, heat, and oxidative stress. Its accumulation is generally associated with enhanced stress tolerance and improved maintenance of cellular homeostasis under adverse environmental conditions (Bates et al., 1973; Blum, 2017; Verbruggen & Hermans, 2008; Hayat et al., 2012).

Under drought stress, declining water availability reduces cellular water potential and disrupts normal metabolic processes. To counteract these effects, plants synthesize and

accumulate compatible solutes, commonly referred to as osmolytes. These compounds facilitate osmotic adjustment by lowering cellular osmotic potential without interfering with normal biochemical reactions. Proline is among the most effective osmolytes because it contributes to water retention, stabilization of proteins and membranes, protection of cellular structures, and maintenance of enzyme activity during dehydration (Blum, 2017; Hosseinifard et al., 2022).

In addition to its osmotic functions, proline plays an important role in mitigating oxidative stress. Drought-induced water deficits frequently stimulate the production of reactive oxygen species (ROS), which can damage membranes, proteins, and nucleic acids. Proline contributes to cellular protection by scavenging free radicals and supporting antioxidant defense mechanisms. Furthermore, proline serves as a temporary reservoir of carbon and nitrogen that can be utilized during recovery following stress alleviation (Hayat et al., 2012; Hosseinifard et al., 2022).

Numerous studies have reported a positive association between proline accumulation and drought tolerance in vegetable crops. Drought-tolerant genotypes often exhibit greater proline accumulation than sensitive genotypes, suggesting that this trait may serve as a useful physiological marker for stress adaptation. In cucumber, increased proline concentrations have been associated with improved maintenance of leaf water status, reduced cellular damage, and enhanced recovery following drought exposure (Wang et al., 2024; Das et al., 2024; Cui et al., 2019).

The results obtained in the present study support these observations. Considerable genotypic variation was detected for proline accumulation under drought stress conditions. Among the evaluated genotypes, G2 and G4 exhibited the highest proline concentrations, reaching 42.1 and 39.7 $\mu\text{mol g}^{-1}$ fresh weight, respectively. In contrast, the lowest proline levels were observed in G6 (22.5 $\mu\text{mol g}^{-1}$ fresh weight) and G5 (24.8 $\mu\text{mol g}^{-1}$ fresh weight). Intermediate values were recorded for G1 and G3. These findings indicate substantial differences among genotypes in their capacity for osmotic adjustment under water-limited conditions. These values are summarized in Table 1.

Table 1. Mean physiological responses of cucumber genotypes under drought stress

Genotype	Proline ($\mu\text{mol/g}$ FW)	MDA (nmol/g FW)	RWC (%)	Chlorophyll (mg/g)	Stomatal Conductance ($\text{mmol/m}^2/\text{s}$)
G1	28.50	8.90	65.00	1.45	185
G2	42.10	6.10	74.30	1.67	220
G3	31.00	7.50	69.20	1.51	195
G4	39.70	6.30	73.10	1.60	210
G5	24.80	10.40	58.60	1.35	160
G6	22.50	11.20	55.20	1.28	145

Note. Values are means of three replications under drought conditions.

Source: Prepared by the author.

Analysis of variance further confirmed the significance of genotypic differences in proline accumulation. Proline content was significantly affected by genotype ($F = 18.42$, $p < 0.001$), and Tukey's multiple comparison test classified G2 and G4 within the highest statistical group, while G5 and G6 formed the lowest group. These results demonstrate that drought-induced proline accumulation is strongly genotype dependent and may provide a useful criterion for distinguishing tolerant and sensitive cucumber genotypes. The statistical significance of these differences is presented in Table 2.

Table 2. ANOVA and Tukey HSD results for physiological traits under drought stress

Trait	F-value	p-value	Tukey Groups (a–c)
Proline	18.42	<0.001	G2a G4a G3b G1b G5c G6c
MDA	12.76	<0.01	G6a G5a G1b G3b G4c G2c
RWC	21.55	<0.001	G2a G4a G3b G1b G5c G6c
Chlorophyll	10.33	<0.01	G2a G4a G3b G1b G5c G6c
Stomatal Conductance	15.90	<0.001	G2a G4a G3b G1b G5c G6c

Note. Different letters indicate statistically significant differences at $p < 0.05$ (Tukey HSD). Yield row was removed to keep Chapter 1 focused on physiological traits.

Source: Prepared by the author.

Interestingly, proline accumulation was positively associated with several favorable physiological traits. Correlation analysis revealed positive relationships between proline and relative water content ($r = 0.72$), chlorophyll concentration ($r = 0.55$), and stomatal conductance ($r = 0.49$). Conversely, proline exhibited a negative association with malondialdehyde content ($r = -0.61$), suggesting that increased osmotic adjustment may contribute to reduced oxidative damage under drought stress. These relationships support the hypothesis that proline accumulation is closely linked to overall stress tolerance and improved physiological performance.

The superior performance of G2 and G4 suggests that these genotypes possess more efficient drought-adaptation mechanisms than the remaining genotypes. Their ability to accumulate higher levels of proline was accompanied by higher relative water content and lower oxidative damage under drought conditions. Such characteristics are particularly valuable in breeding programs targeting climate-resilient cucumber cultivars adapted to water-limited environments.

From a practical breeding perspective, proline represents an attractive physiological selection criterion because it can be measured relatively rapidly and provides direct information regarding plant responses to water deficit. Although drought tolerance is a complex trait governed by multiple physiological and genetic factors, proline accumulation may serve as an effective component of integrated screening systems when evaluated together with water status and oxidative stress indicators.

Overall, the findings presented in this chapter reinforce the importance of proline as a key physiological marker associated with osmotic adjustment and drought adaptation in cucumber. The observed variation among genotypes further highlights the potential of utilizing proline accumulation as a selection tool for identifying superior germplasm suitable for future climate-resilient breeding programs (Blum, 2005; Blum, 2011).

Malondialdehyde (MDA) as an Indicator of Oxidative Damage

Drought stress not only limits plant water availability but also disrupts cellular metabolic processes, frequently leading to the excessive generation of reactive oxygen species (ROS). These highly reactive molecules, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, can damage proteins, nucleic acids, pigments, and

membrane lipids when their production exceeds the detoxification capacity of antioxidant defense systems. Consequently, oxidative stress has become recognized as one of the most important secondary effects of drought stress in plants (Farooq et al., 2009; Ayala et al., 2014).

One of the most widely used indicators of oxidative damage is malondialdehyde (MDA), a by-product of membrane lipid peroxidation. Lipid peroxidation occurs when reactive oxygen species attack polyunsaturated fatty acids within cellular membranes, resulting in membrane destabilization, increased permeability, and impairment of cellular functions. Because MDA accumulation reflects the degree of membrane damage, it is frequently employed as a biochemical marker for evaluating drought-induced oxidative stress (Heath & Packer, 1968; Ayala et al., 2014).

Numerous studies have demonstrated that drought-sensitive genotypes generally exhibit higher MDA concentrations than tolerant genotypes. Elevated MDA levels indicate increased membrane degradation and reduced capacity to protect cellular structures against oxidative injury. In contrast, drought-tolerant plants often maintain lower MDA concentrations through more effective antioxidant defense systems (Mittler, 2002; Gill & Tuteja, 2010). Including enzymatic antioxidants such as superoxide dismutase, catalase, and peroxidases, as well as non-enzymatic antioxidants that scavenge reactive oxygen species before significant cellular damage occurs (Ayala et al., 2014; Farooq et al., 2009).

In cucumber, drought-induced increases in MDA content have been reported consistently across a wide range of experimental conditions. Water deficit frequently causes membrane instability, chlorophyll degradation, and reductions in photosynthetic efficiency, all of which are associated with enhanced lipid peroxidation. Consequently, MDA has become one of the most commonly measured biochemical indicators in cucumber drought tolerance studies and is widely utilized in physiological screening programs (Wang et al., 2024; Das et al., 2024).

The results of the present study clearly demonstrate substantial genotypic variation in MDA accumulation under drought stress conditions. Among the evaluated cucumber genotypes, G6 and G5 exhibited the highest MDA concentrations, reaching 11.2 and 10.4 nmol g⁻¹ fresh weight, respectively. In contrast, the lowest MDA values were recorded in G2 (6.1 nmol g⁻¹ fresh weight) and G4 (6.3 nmol g⁻¹ fresh weight). Intermediate levels

were observed in G1 and G3. These findings indicate that G2 and G4 experienced considerably lower oxidative damage under drought stress than the more sensitive genotypes.

Analysis of variance further confirmed the significance of these differences. MDA content differed significantly among genotypes ($F = 12.76$, $p < 0.01$), and Tukey's HSD test separated G6 and G5 into the highest statistical group, while G2 and G4 were classified within the lowest group. These results suggest that oxidative stress tolerance varies considerably among cucumber genotypes and may contribute substantially to differences in drought adaptation.

Correlation analysis provided additional insight into the physiological significance of MDA accumulation. MDA exhibited negative correlations with relative water content ($r = -0.65$), chlorophyll concentration ($r = -0.57$), and stomatal conductance ($r = -0.51$). Furthermore, a strong negative association was observed between MDA and proline accumulation ($r = -0.61$). These relationships indicate that increased oxidative damage is generally accompanied by reduced physiological performance under drought stress conditions.

The contrasting responses observed between G2–G4 and G5–G6 provide a clear illustration of the importance of oxidative stress regulation in drought tolerance. Genotypes characterized by lower MDA accumulation maintained superior water status, higher chlorophyll concentrations, and greater stomatal conductance. Conversely, elevated MDA levels in sensitive genotypes were associated with greater physiological deterioration.

From a breeding perspective, MDA represents a valuable physiological indicator because it provides direct information regarding cellular damage caused by drought stress. MDA measurements can facilitate early-stage screening by identifying genotypes capable of maintaining membrane integrity under adverse environmental conditions. The integration of MDA assessment with other physiological traits, including proline accumulation, relative water content, and stomatal conductance, may substantially improve the efficiency of drought tolerance screening programs.

Overall, the findings presented in this study confirm that MDA is a reliable indicator of oxidative damage and drought stress severity in cucumber. The substantial genotypic variation observed among the evaluated materials highlights the importance of oxidative

stress regulation as a component of drought adaptation and supports the use of MDA as a practical physiological marker in climate-resilient cucumber breeding programs (Gill & Tuteja, 2010).

Relative Water Content (RWC) and Water Retention Capacity

Relative water content (RWC) is widely recognized as one of the most informative physiological indicators used to evaluate plant water status under drought conditions. Unlike soil moisture measurements, which only provide information about water availability in the growing medium, RWC directly reflects the hydration level of plant tissues and their ability to maintain cellular water balance. Consequently, RWC has become one of the most frequently used parameters in drought physiology studies and is often considered a reliable indicator of plant tolerance to water deficit (Barrs & Weatherley, 1962; Farooq et al., 2009; Blum, 2011; Blum, 2017).

Water availability is essential for maintaining cellular turgor, metabolic activity, nutrient transport, and photosynthetic processes. Under drought stress, reduced soil water availability limits root water uptake, resulting in declining leaf water content and loss of turgor pressure. Such changes directly affect cell expansion, leaf growth, stomatal behavior, and ultimately plant productivity. Therefore, the capacity of a genotype to maintain tissue hydration under drought conditions is often closely associated with its ability to tolerate water stress.

The maintenance of higher RWC values under drought reflects the effectiveness of several adaptive mechanisms. These include improved root water acquisition, enhanced osmotic adjustment through compatible solute accumulation, reduced transpirational water loss, and more efficient stomatal regulation. Genotypes capable of sustaining higher RWC levels generally exhibit greater physiological stability and are better able to preserve metabolic processes during periods of limited water availability (Farooq et al., 2009; Blum, 2017).

In cucumber, water deficit commonly causes substantial reductions in leaf water content due to the crop's relatively shallow root system and high transpiration demand. Previous studies have shown that reductions in RWC are frequently accompanied by decreases in chlorophyll concentration, photosynthetic efficiency, and stomatal

conductance. Consequently, RWC has become an important criterion in physiological screening programs aimed at identifying drought-tolerant cucumber genotypes (Wang et al., 2024; Wang et al., 2012).

The results obtained in the present study revealed significant variation among cucumber genotypes in their ability to maintain water status under drought stress conditions. The highest RWC values were recorded in G2 (74.3%) and G4 (73.1%), indicating superior water retention capacity. In contrast, the lowest values were observed in G6 (55.2%) and G5 (58.6%), suggesting greater susceptibility to dehydration. Intermediate RWC levels were detected in G1 and G3. These findings indicate that substantial genotypic differences exist in the capacity to maintain tissue hydration under water-limited conditions.

Analysis of variance confirmed the significance of these differences. RWC varied significantly among genotypes ($F = 21.55$, $p < 0.001$), and Tukey's HSD test separated G2 and G4 into the highest statistical group, while G5 and G6 were classified within the lowest group. The magnitude of these differences highlights the importance of water retention capacity as a distinguishing feature of drought tolerance in cucumber.

Correlation analysis further emphasized the physiological significance of RWC. Relative water content exhibited strong positive associations with proline accumulation ($r = 0.72$), chlorophyll concentration ($r = 0.63$), and stomatal conductance ($r = 0.61$). Conversely, RWC was negatively correlated with malondialdehyde content ($r = -0.65$), indicating that plants maintaining higher tissue hydration experienced lower levels of oxidative damage. These relationships demonstrate the central role of water status in determining overall plant performance under drought stress.

The superior performance of G2 and G4 suggests that these genotypes possess more efficient drought adaptation mechanisms than the remaining materials evaluated in this study. Their ability to maintain higher RWC values was accompanied by enhanced osmotic adjustment, reduced oxidative stress, and improved physiological performance under drought conditions. Such integrated responses are characteristic of drought-tolerant genotypes and are highly desirable in breeding programs targeting climate resilience.

From a practical perspective, RWC offers several advantages as a physiological screening parameter. The measurement is relatively simple, inexpensive, and reproducible,

allowing the evaluation of large numbers of genotypes in breeding populations. Furthermore, because RWC integrates multiple physiological processes related to water acquisition, retention, and utilization, it provides a comprehensive assessment of plant responses to drought stress.

Overall, the results of this study confirm that relative water content is a highly informative indicator of drought tolerance in cucumber. The substantial genotypic variation observed among the evaluated materials highlights the importance of water retention capacity as a key component of stress adaptation. The strong relationships between RWC and other physiological traits further support its application as a valuable selection criterion in cucumber improvement programs aimed at developing climate-resilient cultivars for water-limited environments (Das et al., 2024).

Chlorophyll Stability Under Drought Stress

Chlorophyll is the primary photosynthetic pigment responsible for capturing light energy and converting it into chemical energy during photosynthesis. The maintenance of chlorophyll content is therefore essential for sustaining plant growth, biomass accumulation, and yield formation. Under drought conditions, chlorophyll degradation is one of the most common physiological responses observed in plants under drought stress (Chaves et al., 2009; Ashraf & Harris, 2013) and is frequently associated with reductions in photosynthetic capacity and overall plant productivity (Flexas et al., 2002; Farooq et al., 2009).

Drought stress affects chlorophyll metabolism through several mechanisms. Water deficit often limits nutrient uptake, disrupts chloroplast structure, accelerates leaf senescence, and promotes the accumulation of reactive oxygen species (ROS). These processes collectively contribute to pigment degradation and impairment of the photosynthetic apparatus (Chaves et al., 2009). As chlorophyll concentration declines, carbon assimilation is reduced, leading to decreased biomass production and lower yield potential (Farooq et al., 2009; Wang et al., 2024).

The ability to maintain chlorophyll stability under drought stress is widely regarded as an important indicator of stress tolerance. Genotypes capable of preserving chlorophyll concentration generally sustain higher photosynthetic efficiency and exhibit improved

growth under water-limited conditions. Consequently, chlorophyll content has become one of the most frequently used physiological traits in drought tolerance screening programs (Flexas et al., 2002; Das et al., 2024).

In cucumber, drought-induced reductions in chlorophyll content have been reported in numerous studies. Water deficit frequently leads to chloroplast damage, reduced photosynthetic activity, and impaired carbohydrate production. However, considerable genotypic variation exists in the degree of chlorophyll degradation under stress conditions. Drought-tolerant genotypes often exhibit smaller reductions in chlorophyll concentration than sensitive genotypes, indicating a greater capacity to protect photosynthetic structures during dehydration (Wang et al., 2024; Das et al., 2024).

The results of the present study clearly demonstrated significant differences among cucumber genotypes in chlorophyll stability under drought stress. The highest chlorophyll concentrations were observed in G2 (1.67 mg g⁻¹) and G4 (1.60 mg g⁻¹), whereas the lowest values were recorded in G6 (1.28 mg g⁻¹) and G5 (1.35 mg g⁻¹). Intermediate values were detected in G1 and G3. These results suggest that G2 and G4 were more effective in preserving photosynthetic pigments under water-deficit conditions.

Analysis of variance further confirmed the significance of genotypic variation in chlorophyll concentration. Chlorophyll content differed significantly among genotypes ($F = 10.33$, $p < 0.01$), and Tukey's HSD test separated G2 and G4 into the highest statistical group while G5 and G6 were classified within the lowest group. These findings indicate that chlorophyll stability may serve as a useful physiological indicator for distinguishing drought-tolerant and drought-sensitive cucumber genotypes.

Correlation analysis revealed that chlorophyll concentration was positively associated with several favorable physiological traits. Significant positive correlations were observed between chlorophyll concentration and relative water content ($r = 0.63$) and stomatal conductance ($r = 0.68$). Conversely, chlorophyll content exhibited a negative correlation with malondialdehyde accumulation ($r = -0.57$), suggesting that genotypes capable of maintaining higher chlorophyll levels experienced lower oxidative damage. These relationships highlight the close connection between photosynthetic stability and drought adaptation.

Plants that preserve chlorophyll content are better able to sustain carbon assimilation and biomass production despite water limitations. This observation is consistent with previous studies demonstrating that chlorophyll stability is closely linked to drought tolerance and yield maintenance in vegetable crops (Farooq et al., 2009; Wang et al., 2024).

The superior performance of G2 and G4 suggests that these genotypes possess enhanced mechanisms for protecting the photosynthetic apparatus under stress conditions. Their higher chlorophyll concentrations were accompanied by greater relative water content, lower MDA accumulation, higher stomatal conductance, and improved physiological adaptation under drought conditions. Such coordinated physiological responses contribute substantially to drought adaptation and represent desirable characteristics for breeding programs.

From a practical breeding perspective, chlorophyll content offers several advantages as a selection criterion. Measurements can be performed rapidly using portable chlorophyll meters, allowing large numbers of genotypes to be evaluated efficiently. Furthermore, chlorophyll concentration reflects the combined effects of water status, oxidative stress, and photosynthetic performance, making it a valuable integrative indicator of plant health under drought conditions.

Overall, the results presented in this study demonstrate that chlorophyll stability is closely associated with drought tolerance in cucumber. The substantial variation observed among genotypes and the strong positive relationship between chlorophyll content and physiological performance support its use as an effective physiological marker in climate-resilient cucumber breeding programs.

Stomatal Conductance Responses to Drought Stress

Stomatal conductance is one of the most important physiological parameters governing plant responses to drought stress. Stomata are specialized epidermal structures that regulate the exchange of gases between plant tissues and the atmosphere. Through the opening and closing of stomatal pores, plants control carbon dioxide uptake for photosynthesis while simultaneously regulating water loss through transpiration. Consequently, stomatal conductance serves as a critical link between plant water relations, photosynthetic performance, and productivity (Flexas et al., 2002; Chaves et al., 2009).

Under drought conditions, plants typically respond by reducing stomatal aperture in order to minimize transpirational water loss. This response is primarily mediated by drought-induced hormonal signals, particularly abscisic acid (ABA), which triggers stomatal closure when water availability declines. While stomatal closure contributes to water conservation, it also restricts carbon dioxide diffusion into leaf tissues, thereby reducing photosynthetic activity and biomass accumulation. As a result, drought tolerance depends largely on a plant's ability to balance water conservation with carbon assimilation (Flexas et al., 2002; Farooq et al., 2009 ; Chaves et al., 2009).

The degree of stomatal regulation varies considerably among plant species and genotypes. Drought-tolerant genotypes often exhibit more efficient stomatal control, allowing them to reduce excessive water loss while maintaining sufficient photosynthetic activity. In contrast, drought-sensitive genotypes may experience severe reductions in stomatal conductance that ultimately compromise growth and physiological functioning. Therefore, stomatal conductance is frequently used as a physiological indicator for evaluating drought adaptation and identifying superior genotypes in breeding programs (Blum, 2017; Das et al., 2024).

In cucumber, stomatal conductance is particularly important because of the crop's large leaf area and relatively high transpiration rate. Water deficit rapidly affects stomatal behavior, leading to reduced gas exchange and photosynthetic efficiency. Previous studies have demonstrated that maintenance of higher stomatal conductance under moderate drought stress is often associated with improved water-use efficiency, greater photosynthetic capacity, and enhanced physiological stability (Wang et al., 2024; Wang et al., 2012).

The results of the present study revealed significant variation among cucumber genotypes in stomatal conductance under drought conditions. The highest values were observed in G2 ($220 \text{ mmol m}^{-2} \text{ s}^{-1}$) and G4 ($210 \text{ mmol m}^{-2} \text{ s}^{-1}$), while the lowest values were recorded in G6 ($145 \text{ mmol m}^{-2} \text{ s}^{-1}$) and G5 ($160 \text{ mmol m}^{-2} \text{ s}^{-1}$). Intermediate levels were measured in G1 and G3. These findings indicate that G2 and G4 maintained more favorable gas exchange characteristics under water-deficit conditions than the sensitive genotypes.

Analysis of variance confirmed the significance of genotypic differences in stomatal conductance. The observed variation was highly significant ($F = 15.90$, $p < 0.001$), and Tukey's HSD test classified G2 and G4 within the highest statistical group, whereas G5 and G6 were placed in the lowest group. These results suggest that stomatal conductance is a highly discriminative trait for evaluating drought tolerance in cucumber genotypes.

Correlation analysis further emphasized the importance of stomatal regulation under drought stress. Stomatal conductance exhibited positive relationships with proline accumulation ($r = 0.49$), relative water content ($r = 0.61$), and chlorophyll concentration ($r = 0.68$). In contrast, stomatal conductance was negatively correlated with MDA content ($r = -0.51$), suggesting that reduced oxidative damage contributes to the preservation of stomatal function during drought stress.

Among all measured physiological traits, stomatal conductance exhibited strong associations with the other physiological parameters evaluated. This finding highlights the central role of stomatal regulation in determining drought tolerance. Plants capable of maintaining moderate stomatal opening under drought conditions are better able to sustain photosynthesis and carbon assimilation. Consequently, stomatal conductance may serve as one of the most reliable physiological indicators for predicting genotype performance under water deficit (Flexas et al., 2002; Das et al., 2024).

The superior performance of G2 and G4 demonstrates the importance of efficient stomatal regulation as part of an integrated drought adaptation strategy. These genotypes not only maintained higher stomatal conductance but also exhibited higher proline accumulation, greater relative water content, lower MDA concentrations, and improved chlorophyll stability. Such coordinated physiological responses contribute to superior stress tolerance and enhanced physiological resilience.

From a breeding perspective, stomatal conductance represents a valuable selection criterion because it integrates multiple physiological processes associated with drought adaptation. Although measurements require specialized equipment, advances in portable porometers and high-throughput phenotyping technologies have facilitated large-scale screening of breeding populations. When used in combination with traits such as RWC, proline accumulation, chlorophyll content, and oxidative stress indicators, stomatal conductance can significantly improve the accuracy of drought tolerance evaluation.

Overall, the findings presented in this study demonstrate that stomatal conductance plays a fundamental role in drought adaptation in cucumber. The substantial variation among genotypes and the strong associations between stomatal conductance and other physiological indicators underscore its value as a physiological marker for identifying drought-tolerant germplasm and supporting the development of climate-resilient cucumber cultivars.

Comparative Evaluation of Cucumber Genotypes Under Drought Stress

The evaluation of drought tolerance requires a comprehensive assessment of multiple physiological and agronomic traits because plant responses to water deficit are inherently complex and multifactorial. Individual physiological parameters may provide valuable information regarding specific aspects of stress adaptation; however, no single trait can fully explain genotype performance under drought conditions. Consequently, integrative evaluation of physiological, biochemical, and productivity-related traits is essential for accurately identifying drought-tolerant genotypes and supporting breeding decisions (Ludlow & Muchow, 1990; Blum, 2005).

The results obtained in the present study revealed substantial variation among the six cucumber genotypes evaluated under drought stress. Differences were consistently observed across all measured physiological parameters, including proline accumulation, malondialdehyde content, relative water content, chlorophyll concentration, and stomatal conductance. These findings indicate that drought adaptation in cucumber is governed by multiple interacting physiological mechanisms rather than by a single stress-response pathway.

Among the evaluated materials, genotypes G2 and G4 demonstrated the highest level of drought tolerance. These genotypes consistently exhibited superior performance across nearly all measured physiological parameters. Higher proline accumulation, greater relative water content, enhanced chlorophyll stability, higher stomatal conductance, and lower MDA concentrations collectively indicate more effective drought adaptation mechanisms and improved physiological resilience under water-deficit conditions. The integrated physiological performance of the genotypes is illustrated in Figure 1.

Figure 1. Radar chart of normalized physiological performance under drought stress

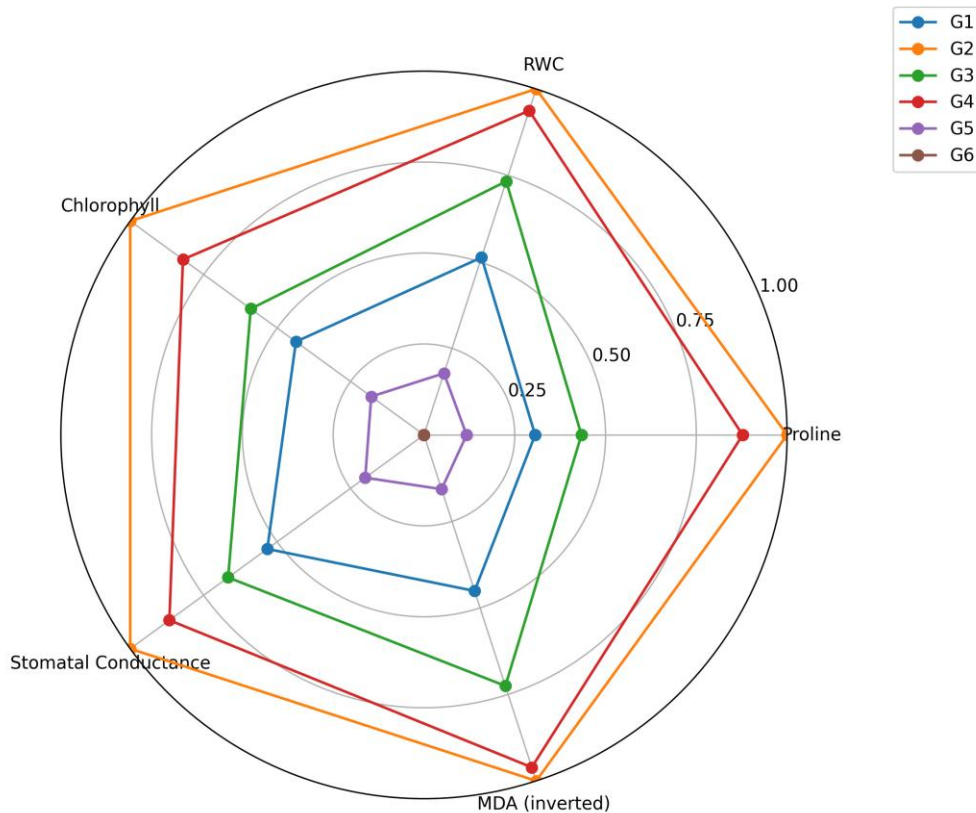


Figure 1. Integrated physiological performance of cucumber genotypes under drought stress based on proline accumulation, MDA content, relative water content, chlorophyll concentration, and stomatal conductance.

Source: Prepared by the author.

The comparative evaluation revealed clear physiological differentiation among cucumber genotypes exposed to drought stress. Positive associations among proline accumulation, relative water content, chlorophyll concentration, and stomatal conductance indicate coordinated drought adaptation mechanisms. Conversely, increased MDA accumulation was associated with impaired physiological performance. These findings demonstrate that drought tolerance in cucumber is governed by the integration of multiple physiological processes rather than by a single adaptive trait.

The observed relationships among physiological traits further support the distinction between tolerant and sensitive genotypes. Conversely, the negative relationships observed between MDA and the remaining traits suggest that oxidative damage represents a major factor contributing to drought sensitivity. These findings emphasize the importance of

evaluating multiple physiological indicators simultaneously rather than relying on individual traits in isolation.

The comparative evaluation of genotypes also demonstrated the usefulness of statistical analyses in drought tolerance assessment. Analysis of variance confirmed significant genotypic differences for all measured traits, highlighting the presence of exploitable genetic variation within the evaluated germplasm. Such variation is essential for breeding programs because it provides opportunities for selecting superior parental materials and developing improved cultivars adapted to water-limited environments.

From a physiological perspective, drought tolerance in cucumber appears to depend on the integration of several complementary mechanisms. Osmotic adjustment through proline accumulation, maintenance of tissue hydration, preservation of photosynthetic pigments, regulation of stomatal behavior, and reduction of oxidative damage collectively contribute to improved stress adaptation. Genotypes possessing favorable performance across all these parameters are more likely to maintain growth and productivity under drought conditions than those relying on only one or two mechanisms. Such integrated physiological responses are increasingly recognized as essential targets for breeding crops adapted to future drought- and heat-prone environments (Hammer et al., 2020).

The findings of this study have important implications for cucumber breeding. Traditional selection based solely on yield performance may overlook physiological traits that contribute to stress adaptation. In contrast, the integration of physiological indicators into breeding programs can improve selection efficiency by identifying superior genotypes before yield differences become fully apparent. Traits such as proline accumulation, MDA concentration, relative water content, chlorophyll stability, and stomatal conductance may therefore serve as valuable complementary criteria for drought tolerance screening.

Overall, the comparative evaluation conducted in this study clearly identified G2 and G4 as the most promising drought-tolerant genotypes among the evaluated materials. Their superior physiological performance and greater physiological resilience indicate substantial potential for utilization in breeding programs targeting climate-resilient cucumber production. Conversely, the sensitivity of G5 and G6 highlights the importance of physiological screening in eliminating unsuitable materials from breeding populations. The integration of multiple physiological indicators provides a robust framework for

identifying drought-tolerant germplasm and accelerating the development of cucumber cultivars adapted to increasingly water-limited agricultural environments.

Conclusions and Future Perspectives

Drought stress remains one of the most significant environmental constraints limiting cucumber production worldwide, particularly in regions increasingly affected by climate change and water scarcity (IPCC, 2023; Lesk et al., 2016). The complex physiological responses triggered by water deficit influence multiple aspects of plant growth, development, and productivity. Understanding these responses is therefore essential for the development of effective strategies aimed at improving drought tolerance and ensuring sustainable cucumber production under future climatic conditions.

The findings presented in this chapter demonstrate that drought tolerance in cucumber is governed by the coordinated interaction of several physiological and biochemical mechanisms, consistent with previous reports on plant physio-biochemical responses to drought stress (Wahab et al., 2022). Osmotic adjustment through proline accumulation, maintenance of tissue hydration, preservation of chlorophyll stability, regulation of stomatal conductance, and mitigation of oxidative damage collectively contribute to plant adaptation under water-limited conditions. No single physiological trait alone was sufficient to explain drought tolerance; rather, superior performance resulted from the integration of multiple complementary responses.

Substantial genotypic variation was observed among the evaluated cucumber materials. Genotypes G2 and G4 consistently exhibited superior physiological performance under drought stress. These genotypes maintained higher proline accumulation, greater relative water content, enhanced chlorophyll stability, and higher stomatal conductance while simultaneously exhibiting lower levels of malondialdehyde accumulation. Such responses indicate more effective osmotic adjustment, improved water retention, better preservation of photosynthetic capacity, and stronger protection against oxidative damage.

In contrast, genotypes G5 and G6 exhibited characteristics commonly associated with drought sensitivity, including reduced proline accumulation, lower tissue hydration, higher oxidative damage, and greater reductions in physiological activity. Their reduced physiological performance under drought conditions further highlights the importance of

integrated physiological adaptation mechanisms in determining stress tolerance. The clear distinction observed between tolerant and sensitive genotypes emphasizes the value of physiological screening in cucumber improvement programs.

The strong relationships identified among physiological traits further support their application as practical selection criteria. Positive associations among proline accumulation, relative water content, chlorophyll concentration, and stomatal conductance indicate that these traits can provide valuable information regarding genotype performance under water-limited conditions. Conversely, the negative association between malondialdehyde content and physiological performance highlights the importance of minimizing oxidative damage as part of drought adaptation strategies.

From a breeding perspective, the results suggest that physiological indicators can substantially enhance the efficiency of drought tolerance evaluation. Traditional selection approaches based solely on yield performance may overlook important adaptive traits expressed during early developmental stages. Integrating physiological parameters into breeding programs may therefore facilitate earlier and more accurate identification of drought-tolerant germplasm. Traits such as proline accumulation, relative water content, chlorophyll stability, stomatal conductance, and malondialdehyde concentration appear particularly promising as complementary screening tools.

Future research should focus on integrating physiological assessments with molecular, genomic, and high-throughput phenotyping approaches (Ji et al., 2018; Blum, 2011). Advances in transcriptomics, metabolomics, genome-wide association studies, and marker-assisted selection offer significant opportunities for improving the understanding of drought tolerance mechanisms in cucumber. The identification of genetic loci associated with key physiological traits could accelerate the development of climate-resilient cultivars capable of maintaining productivity under increasingly challenging environmental conditions. Recent advances in crop adaptation research also emphasize the importance of restoring and utilizing abiotic stress tolerance traits that may have been lost during intensive breeding processes (Palmgren et al., 2024). Integrating physiological trait-based screening with modern breeding approaches may substantially improve the development of climate-resilient cultivars capable of maintaining productivity under drought-prone environments (Cooper & Messina, 2023).

The application of emerging technologies, including hyperspectral imaging, thermal sensing, chlorophyll fluorescence analysis, and machine learning-based phenotyping platforms, may further improve the precision and efficiency of drought tolerance screening. Such approaches would allow large breeding populations to be evaluated rapidly and non-destructively while capturing complex physiological responses that are difficult to measure using conventional methods.

In conclusion, this chapter highlights the importance of physiological trait-based approaches for understanding and improving drought tolerance in cucumber. The superior performance of genotypes G2 and G4 demonstrates the potential value of combining osmotic adjustment, water retention, photosynthetic stability, and oxidative stress mitigation as integrated indicators of drought adaptation. The incorporation of these physiological criteria into future breeding programs may contribute significantly to the development of climate-resilient cucumber cultivars capable of sustaining productivity under increasingly water-limited agricultural environments.

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