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**PHYSIOLOGICAL RESILIENCE OF LEGUMES REGARDING ABIOTIC
STRESS CONDITIONS**

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1. INTRODUCTION

1.1 Justification of the study

Climate change has emerged as one of the greatest threats to global agriculture, with increasing temperatures, erratic precipitation patterns, and deteriorating soil and water quality placing severe pressure on food systems. Among the most significant threats to agricultural systems posed by climate change are water scarcity and soil salinization. These critical abiotic stresses severely limit crop development, yield, and long-term agricultural viability. Diminished precipitation and the excessive depletion of groundwater sources are driving irrigation water shortages, resulting in declining agricultural output. Concurrently, the accumulation of salt in soil, especially in irrigated regions, is becoming a primary limitation by degrading soil health and crop production. According to a recent Food and Agriculture Organization report (FAO, 2024), approximately 1.4 billion hectares of land are already affected by salinity, with another 1 billion hectares at risk due to the combined effects of climate change and anthropogenic activities. This salinization diminishes soil fertility and can result in yield reductions of up to 70%, particularly in irrigated areas. The FAO underscores the immediate necessity for adopting sustainable farming techniques, such as breeding salt-resilient crop varieties, enhancing irrigation systems, and implementing restorative agricultural practices, to address these issues and ensure worldwide food supply. In areas already facing acute water shortages, like the Middle East and North Africa, rising soil salinity further endangers farming output and food stability. This demands integrated strategies for water management, soil preservation, and climate adaptation (Choukr-Allah et al., 2023). Such stresses impair a plant's ability to absorb water and transport nutrients, disrupting key physiological functions and ultimately leading to stunted development and substantial reductions in harvests. Key grain legumes, including soybean (*Glycine max* L.) and chickpea (*Cicer arietinum* L.), are especially susceptible to these conditions. These crops serve as vital sources of vegetable protein, oil, and essential micronutrients, while also contributing to sustainable farming via biological nitrogen fixation (Ben Gaied et al., 2024). Their nutritional, economic, and environmental importance renders them crucial for nations at all development stages. Nevertheless, their high sensitivity to abiotic stresses remains a major constraint, particularly under rainfed or low fertility growing environments (Dasgupta et al., 2025). Chickpea, mainly grown in semi-arid zones, is

prone to late-season drought stress, which critically affects the flowering and pod-formation phases and causes considerable decline in yield (Sachdeva et al., 2022).

Soybean, although widely cultivated worldwide, is sensitive to both drought and salinity during critical growth phases. Reports suggest that salinity can reduce chickpea yield by up to 10%, while drought can cause over 50% loss in soybean productivity globally (Arriagada et al., 2022). These environmental stresses disturb key functions such as photosynthesis, stomatal regulation, osmotic balance, and antioxidant enzyme activity, thereby compromising plant performance and survival (Cao et al., 2025).

These global challenges are also very important in Hungary, where climate change is already affecting local agriculture. Located in Central Europe, Hungary has experienced a noticeable rise in the occurrence and intensity of droughts, particularly in the summer months. This trend has had a direct impact on crop production, particularly in the Great Hungarian Plain, the country's key agricultural zone. As noted by Gaál and Tornay (2023), drought frequency is increasing in Hungary, with projections indicating further intensification due to climate change. Moreover, moisture deficits in arable soils are increasingly affecting crop stability and yield reliability. In parallel, rising soil salinity-particularly in irrigated and poorly drained areas-is emerging as an additional threat, further compounding the vulnerability of crops to environmental stressors (Sahbeni, 2021). As a result, soybean production has increased in Hungary due to its rising significance as a source of protein. Nevertheless, the yield of soybeans in the region is still heavily influenced by water availability and susceptible to abiotic stresses (Anda et al., 2020). While chickpea cultivation is not common in Hungary at present, it is considered a promising crop for Central European agriculture, particularly in crop rotation schemes (Ben Gaied et al., 2024). To promote its adoption, there is a requirement for a more thorough comprehension of its stress tolerance mechanisms in the specific agro-climatic conditions.

Building upon this foundation, the current research aims to assess the morpho-physiological and biochemical responses of selected soybean (YAKARTA and POCA) and chickpea (ELMO and ORION) genotypes to salinity (NaCl-induced) and drought (PEG-induced) stresses within a controlled hydroponic environment. By simulating these stresses during the early vegetative phases (V1–V4 for soybean and

E–F for chickpea), the work intends to uncover genotype-specific tolerance mechanisms, including alterations in growth, physiology, and biochemistry. This investigation is especially pertinent for advancing climate-resilient agriculture and aligns with national initiatives in Hungary to support legume-based farming systems. Through its focus on stress-adaptive traits and genetic variation, the study will aid in establishing efficient early-stage screening methods and selection benchmarks for breeding. The anticipated results are expected to inform the development of more resilient soybean and chickpea varieties suited for water-scarce and saline conditions. To sum up, this research fills a crucial gap in understanding legume stress physiology and seeks to offer useful resources for breeding and enhancing crops under abiotic stress conditions. The results have significant implications for improving agricultural sustainability, ensuring food security in Hungary and other regions, and reducing the negative impacts of climate change on crop productivity.

1.2 Objectives

- To assess genetic variability in tolerance to abiotic stresses, the response of two soybean genotypes (YAKARTA and POCA) and two chickpea genotypes (ELMO and ORION) were evaluated. Individual hydroponic experiments were conducted during the early vegetative stage, exposing the plants to NaCl-induced salinity and PEG-induced water deficit. A comprehensive analysis of morphological, physiological, and biochemical characteristics was employed to identify the most resilient genotypes.
- To identify key morphological, physiological, and biochemical traits associated with salinity and drought tolerance in the selected soybean and chickpea genotypes. These traits will serve as potential selection criteria for breeding programs focused on developing high-yielding varieties for stress-prone environments.
- Performing Pearson correlation analysis among the studied parameters to validate the relationships between interrelated traits.
- The anticipated outcomes will aid in the development of effective screening methods for salinity and water deficit tolerance and will support breeding efforts to enhance chickpea and soybean performance in salt-affected soils and water-limited environments.
- Through this integrated approach, the study aims to enhance our understanding of how stress-inducing factors and genotypes interact to impact plant performance in hydroponic conditions, ultimately contributing to the advancement of more resilient crop systems.

2. LITERATURE REVIEW

2.1 Importance of legumes in global and sustainable agriculture

Soybean (*Glycine max* (L.) Merrill) and chickpea (*Cicer arietinum* L.) hold a pivotal position in global agriculture due to their multifaceted contributions to nutrition, sustainability, and farm economics. These leguminous crops are increasingly recognized as essential components in strategies to enhance food security, build climate resilience, and improve soil health (Yanni et al., 2024). Soybean is a cornerstone crop, prized as a primary source of high-quality plant protein, essential amino acids, and vegetable oil for both human consumption and livestock feed (Gai et al., 2025). Chickpea, similarly, valued, provides substantial protein (20–25%), carbohydrates (49–55%), and vital micronutrients like iron and zinc, underpinning its importance in nutritious and plant-based diets (Kumar et al., 2025). Agronomically, their deep root systems contribute to soil structure and confer adaptability to semi-arid and marginal environments (Jukanti et al., 2012). A key ecological benefit of these legumes is their capacity for biological nitrogen fixation through symbiotic relationships with rhizobia. This natural process decreases reliance on synthetic fertilizers, thereby reducing greenhouse gas emissions and input costs. Additionally, incorporating legumes into cropping systems promotes biodiversity, enhances soil fertility, and boosts the productivity of subsequent crops (Yang et al., 2023).

Globally, soybean dominates legume production, with the United States, Brazil, and Argentina accounting for the majority of global output. In contrast, chickpea production is concentrated in South Asia, particularly India, which alone contributes approximately 65% of global chickpea yield, followed by Pakistan (10%), Turkey (7%), and Iran (3%). Despite being cultivated in over 50 countries, chickpea production remains limited in scale in most regions. Only 22 countries allocate more than 20,000 hectares to chickpea cultivation, and just 19 others plant between 10,000 and 20,000 hectares annually (FAO, 2020).

In the European context, legumes are gaining strategic importance due to their environmental services and protein content. Hungary, while not a major global producer, has increasingly prioritized legume cultivation as part of its sustainable agriculture policies and Common Agricultural Policy (CAP) Strategic Plan (Balázs et al., 2021). According to Eurostat data, the cultivation area of dried pulses in Hungary has shown a gradual increase in recent years, reflecting growing interest in pulse production and food system diversification across Europe (Eurostat, 2023). Chickpea is also used in animal

feed and human diets, making them highly versatile. Their ability to biologically fix nitrogen contributes to enhanced soil fertility, further supporting sustainable farming systems (Nahusenay et al., 2024). Similarly, soybean is the most widely harvested legume worldwide, offering 36–40% protein and 18–24% vegetable oil per seed, and is central to human nutrition, livestock feed, and biofuel production (Jin et al., 2023; Kumar et al., 2021). Roughly 85% of global soybean output is processed for these sectors. Additionally, soybean functions effectively as an intercrop, improving both soil health and system sustainability (Sun et al., 2024). Generally, as climate change intensifies challenges to global agriculture, the resilience and multifunctionality of legumes position them as key crops for building more adaptive and climate-smart food systems.

2.2 Impact of climate change on crop production

Climate change is a major concern for global agriculture due to the rising occurrence and severity of abiotic stresses like drought, salinity, and extreme heat. These environmental factors have a detrimental impact on plant growth, development, and yield potential. This challenge is particularly acute in already vulnerable regions. Both drought and salinity stress, either alone or, more deleteriously, in combination, disrupt essential plant functions. They impair photosynthesis, hinder nutrient uptake, and reduce water-use efficiency, collectively leading to diminished productivity and an elevated risk of crop failure (Zandalinas et al., 2021).

Drought stress, the most pervasive abiotic threat, reduces soil moisture availability, impairs stomatal function, and limits carbon assimilation in plants. Salinity stress, often resulting from poor irrigation practices or rising sea levels, leads to osmotic imbalances and ion toxicity, which can inhibit seed germination and impair shoot and root development (Shrivastava and Kumar, 2015). When both stresses occur simultaneously, their impact is often synergistically harmful, causing more severe metabolic and morphological damage than either stress alone. This poses a particular challenge for rain-fed agricultural systems, which are less equipped to buffer such fluctuations (Mittler and Blumwald, 2010).

The escalating effects of climate change are reshaping precipitation regimes, prolonging dry periods, and increasing evapotranspiration, collectively intensifying pressure on vital food crops worldwide. Regions such as Sub-Saharan Africa, South Asia, and Latin America are already experiencing reduced agricultural productivity due to extended droughts and increased climatic unpredictability. According to the Intergovernmental

Panel on Climate Change (IPCC, 2023), without robust adaptation strategies, global yields of staple crops, including wheat, maize, and legumes, could decline by up to 25% by mid-century in vulnerable areas.

In Central and Eastern Europe, including Hungary, climate change has manifested through rising average temperatures, reduced summer rainfall, and increased frequency of heatwaves and soil moisture deficits. These conditions are especially harmful to traditional field crops like wheat, maize, and sunflower (Pinke et al., 2024). Legumes, such as soybean and chickpea, are relatively more stress-tolerant but are still affected. Prolonged droughts during crucial growth stages, like flowering and pod filling, can lead to substantial yield reductions and lower seed quality (Kumar et al., 2021). Additionally, the region's reliance on rain-fed systems increases susceptibility to seasonal fluctuations. The combined impacts of drought and salinity stress, worsened by climate change, underscore the pressing requirement for adaptive approaches in crop management. These measures encompass the creation of stress-tolerant crop varieties, the enhancement of irrigation effectiveness, and the expansion of cropping systems with resilient legumes to better withstand climate challenges (FAO, 2022). As climate challenges intensify, integrating such practices will be critical to sustaining agricultural productivity and food security in both global and regional contexts.

2.3 Water deficit stress and its impact on plant functionality

The global agricultural sector is under mounting pressure to satisfy rising food demands driven by rapid 21st-century population growth. This pressure is significantly amplified by climate change, as abiotic stresses, notably drought and soil salinity, increasingly impedes plant development and reduces agricultural productivity (Kaushal and Wani, 2016). Climate-induced shifts, including altered precipitation regimes, elevated temperatures, and more intense weather extremes, are contributing to the greater frequency and severity of drought conditions (IPCC, 2021). It is predicted that around one-third of the world's arable land will be affected by severe drought conditions, further limiting agricultural production (Dai, 2013). Water is essential in plant physiology, as it is involved in important processes like photosynthesis, nutrient and metabolite transport, maintaining cellular turgor, and regulating temperature through transpiration (Joshi et al., 2022). A deficit in water availability disrupts these vital functions and imposes severe constraints on plant growth and yield. Water deficit stress, which occurs when water loss through transpiration exceeds absorption due to insufficient soil moisture, is among the

most detrimental abiotic stressors for crop plants (Haghpanah et al., 2024). It hinders photosynthetic efficiency, decreases turgor pressure, changes stomatal conductance, and triggers oxidative stress by generating an excess of reactive oxygen species (ROS) (Qiao et al., 2024).

The onset and development of water deficit stress in plants are primarily driven by environmental and physiological factors. Drought can result from prolonged periods of low precipitation, elevated evapotranspiration rates due to high temperatures, and reduced water availability for irrigation. These conditions cause a progressive decline in soil moisture content, leading to a water potential gradient that limits water uptake by plant roots (Qing et al., 2023). Consequently, cellular dehydration ensues, adversely affecting membrane integrity, enzyme activity, and overall metabolic homeostasis (Kapoor et al., 2020).

Plant susceptibility to water deficit is influenced by factors such as species, genotype, developmental stage, and the intensity and duration of the stress (Nour et al., 2024). Drought exposure, especially during sensitive growth periods like flowering and pod formation, can result in permanent damage to plant morphology, anatomy, and physiology. This includes reduced leaf expansion due to inhibited cell enlargement and leaf area development (Abd El-Mageed et al., 2022), suppression of root growth under progressive soil moisture decline (Maan et al., 2023), and impaired reproductive development, such as decreased pollen viability, reduced pod and seed set, and lower seed weight, ultimately resulting in yield reduction and compromised food security (Qiao et al., 2024). Additionally, socioeconomic consequences such as water scarcity and economic losses among farming communities are common in drought-prone regions, exacerbating food insecurity and livelihood vulnerability (Sisinyize and Tjiueza, 2025). Plants use various adaptive mechanisms to reduce the impact of water deficit. Strategies for drought avoidance involve morphological changes like decreased leaf area (Naikwade, 2022), increased epicuticular wax deposition (He et al., 2022), stomatal closure (Muhammad Aslam et al., 2022), leaf rolling (Wang et al., 2023), and higher root-to-shoot ratios to improve water absorption and reduce loss (Kooyers, 2015). On the other hand, drought tolerance mechanisms involve cellular and biochemical adjustments such as osmotic adjustment (He et al., 2024), activation of antioxidant defense systems (e.g., SOD, CAT, POD) (Hasanuzzaman et al., 2020), and maintenance of membrane stability to counteract oxidative stress and preserve cellular functions under water-limited conditions (Mittler, 2017).

Legume crops like soybean and chickpea are especially susceptible to water shortage, which can significantly impact their growth and yield, yet their improvement under drought conditions holds substantial promise for ensuring food security. Research efforts have focused on identifying drought-resilient genotypes through characterization of morphological, physiological, and biochemical traits associated with water deficit tolerance (Fazeli-Nasab et al., 2025; Sanusi et al., 2025). Key traits include maintenance of relative water content (Kumar et al., 2024), efficient stomatal regulation (McAdam et al., 2023), high photosynthetic capacity (Singh-Bakala et al., 2025), and robust antioxidant responses (Dalvi et al., 2017). A comprehensive assessment of genotypic responses under drought stress requires integration of growth indices, physiological measurements (e.g., chlorophyll fluorescence, gas exchange parameters), biochemical markers (e.g., proline accumulation, ROS scavenging), and molecular profiling. Such analyses are essential for identifying drought-responsive traits and incorporating them into breeding programs aimed at developing high-yielding, water-efficient cultivars. Enhancing drought resilience in legume crops through such approaches offers a sustainable strategy for stabilizing seed production in water-limited environments (Hasanuzzaman et al., 2020). In summary, the challenge of water deficit stress poses a complex threat to worldwide agriculture, highlighting the importance of comprehending plant reactions and adaptive strategies. Acknowledging this variability is essential for developing robust crop varieties capable of sustaining yield under increasingly unpredictable water availability.

2.3.1 Effect of water deficit stress on morphological traits

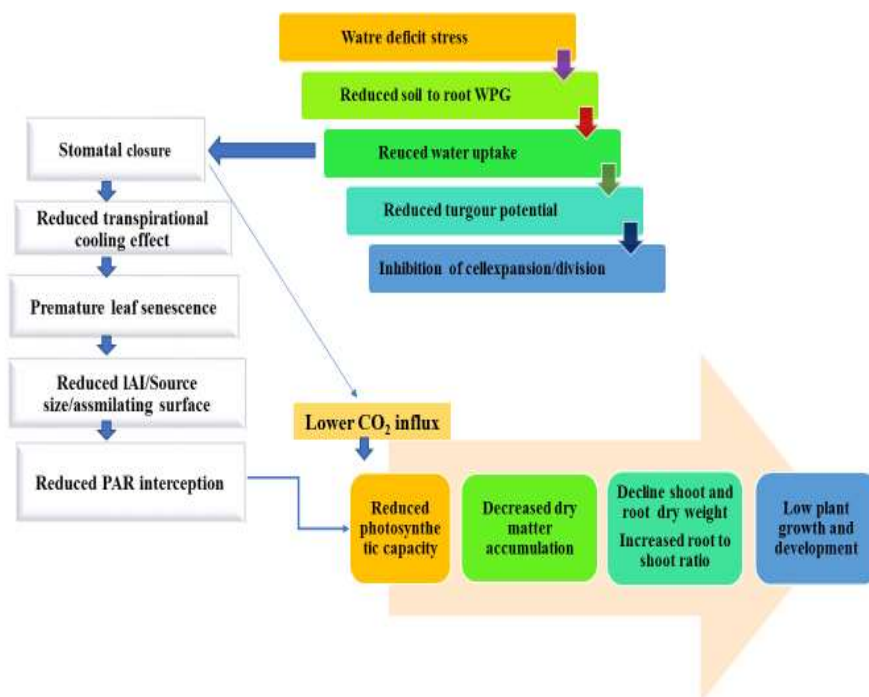
Morphological studies consistently indicate that the growth and development of leguminous crops such as chickpea and soybean are highly susceptible to water deficit stress (Ben Gaied et al., 2024). Under water-limited conditions, these species display substantial alterations in shoot and root architecture compared to non-stressed environments (Fig. 1). Severe drought impedes water transport from the xylem to expanding cells, disrupting cell elongation, division, and tissue development (Nonami, 1998). Water-deficient plants often exhibit reduced nitrogen uptake, which limits chlorophyll synthesis and radiation use efficiency (Ullah et al., 2019), thereby diminishing leaf area, accelerating senescence and reducing the leaf area index—the principal determinant of source size and photosynthetic capacity (Ben Gaied et al., 2024).

In controlled greenhouse trials, Petcu et al. (2024) assessed soybean responses to drought stress, revealing that sensitive genotypes showed up to an 82.56% reduction in leaf area and 66.84% reduction in biomass under stress, while tolerant genotypes displayed significantly lower reductions (25.55% and 11.82%, respectively), demonstrating genotypic variability in drought resilience. Similarly, Narayana et al. (2024) reported that drought-tolerant soybean genotypes during early vegetative growth maintained superior performance under moderate water stress (~50% field capacity), exhibiting enhanced plant height, greater root-to-shoot ratios, and biomass accumulation, indicating adaptive water-foraging strategies.

Morsy et al. (2018) demonstrated that water deficit reduced dry matter accumulation, crop growth rate, and leaf area index across 12 soybean genotypes. In chickpea, studies by Fazeli-Nasab et al. (2025) show that moderate to severe water stress significantly impairs key morphological traits, including plant height, branching, leaf initiation, lamina expansion, and trifoliate leaf formation. When prolonged, especially during critical growth phases, such stress can cause irreversible damage to aboveground biomass. The impact on belowground structures is equally consequential. Water stress can restrict root growth and distribution, which in turn diminishes shoot biomass by limiting the plant's capacity for water uptake (Nosalewicz and Lipiec, 2014). In legumes, the adaptation to drought is heavily influenced by root system architecture (RSA). Research by Ramamoorthy et al. (2016) on chickpea revealed that while roots are typically concentrated in upper soil layers under well-watered conditions, drought prompts deeper root penetration and enhances water extraction from depths below 30 cm. This observation is consistent with the findings of Kashiwagi et al. (2006), who identified a positive correlation between chickpea seed yield under drought and both root depth and density. Comparative analyses highlight species-specific strategies. Benjamin and Nielsen (2006) noted that soybean roots remain predominantly shallow (approximately 97% within 0.23 m) irrespective of soil moisture. In contrast, chickpea exhibits a more plastic RSA, developing deeper roots under dry conditions to optimize access to subsoil water reserves.

Recent advances in root phenotyping and controlled-environment experimentation have enabled the precise evaluation of morphological responses to water deficit in hydroponic and soilless systems. For instance, Misal et al. (2024) used polyethylene glycol (PEG-6000) to simulate osmotic stress in chickpea and observed significant reductions in germination percentage, shoot and root length, and shoot-to-root ratio. These changes

closely resembled those induced by soil-based drought conditions. Among the tested genotypes, ICC 4958 and PDKV Kanak exhibited relatively higher resilience, highlighting the genotypic variability in drought tolerance mechanisms. Semi-hydroponic systems have also been employed to investigate root traits in grain legumes, revealing that drought-tolerant genotypes exhibit increased root length density, finer lateral roots, and more extensive root systems under water stress conditions (Afonso et al., 2025). Moreover, advanced aeroponic and sensor-based phenotyping methods have been applied to legumes like soybean to detect morphological stress responses under controlled water-deficit environments. Advanced phenotyping techniques are enhancing our understanding of these morphological responses. For instance, Panigrahi et al. (2025) effectively employed UAV-based LiDAR and multispectral imaging to estimate key parameters like plant height, lodging, and seed yield, demonstrating the utility of such systems for characterizing growth under stress. In summary, water deficit stress profoundly impacts legume morphology, significantly altering critical traits including plant height, leaf area, root architecture, and the partitioning of biomass. Integration of conventional soil-based observations with modern hydroponic, aeroponic, and phenomics tools strengthens our understanding of drought-induced morphological plasticity and offers a strategic advantage in breeding programs targeting water-use efficiency and yield stability under climate-induced water scarcity.



LAI: Leaf Area Index, WPG: Water Potential Gradient, PAR: Photosynthetically Active Radiation.
Figure 1. Detrimental effects of water deficit stress on morphological parameters.

2.3.2 Phenotypic mechanisms associated with water deficit tolerance

Plants display various phenotypic adaptations when faced with water deficit stress to reduce water loss and regulate internal water levels. A common early response is a decrease in leaf number and size, which aids in reducing transpiration (Yavas et al., 2024). In parallel, root system architecture undergoes significant modification, as roots are the primary organs responsible for water uptake from the soil (Kou et al., 2022). Under drought conditions, plants typically increase root elongation rates, root length density, lateral root proliferation, and overall root system spread to enhance water acquisition from deeper and wider soil profiles (Wahab et al., 2022). The development of a deep, well-branched, and spatially expansive root system has long been recognized as a critical trait for drought avoidance (Comas et al., 2013). In legumes, particularly, the diameter and spatial positioning of metaxylem vessels have been linked to improved water transport efficiency and drought resilience (Zahedi et al., 2024). For instance, in soybean, an increased number of metaxylem vessels has been associated with enhanced water conductivity under stress conditions, thereby supporting better plant hydration and physiological performance (Prince et al., 2017).

Additionally, modifications in root anatomy, such as the formation of aerenchyma tissues in soybean, can facilitate deeper soil penetration and reduce metabolic costs of root maintenance in dry soils. In chickpea, genotypes with greater root length density have consistently shown better drought tolerance and yield stability under water-limited conditions compared to genotypes with sparse or shallow root systems (Fazeli-Nasab et al., 2025). Water deficit also promotes the formation of fibrous roots, increases total root biomass, and enhances the root-to-shoot ratio (Seidel et al., 2024), all of which contribute to a more efficient water uptake capacity relative to transpiring leaf area. These morphological adaptations collectively play a pivotal role in conferring drought tolerance by optimizing water extraction while minimizing losses (Fig. 2).



Characters of the susceptible genotypes: Lower plant height, lower number and size of leaves, less fresh and dry shoot and root weight, thinner shallow less extensive roots and lower root to shoot ratio.



Characters of the tolerant genotypes: Higher plant height, Higher number and size of leaves, greater fresh and dry shoot and root weight, thicker, deeper, well-branched roots and higher root to shoot ratio.

Figure 2. Morphological responses of susceptible and tolerant soybean genotypes (left: POCA and YAKARTA) and chickpea (right: ELMO and ORION) to water deficit stress under hydroponic conditions. Photographs were taken by the authors during controlled experiments conducted at the Plant Biology Institute, University of Debrecen, Hungary, in 2025.

2.3.3 Deleterious effects of water deficit on physiological traits

Water deficit exerts profound effects on the physiological functioning of leguminous crops, impacting fundamental processes that determine plant growth, productivity, and survival. These interconnected physiological responses form a cascade, initiated by shifts in plant water status and progressing to significant alterations in gas exchange, photosynthetic efficiency, and overall biomass production (Khatun et al., 2021). To

counter water deficit, plants activate adaptive strategies like osmotic adjustment and enhanced antioxidative defenses. However, these survival mechanisms frequently incur a metabolic cost, leading to diminished growth rates and overall performance (Anjum et al., 2011). In legumes such as chickpea and soybean, water deficit-induced physiological impairments are typically observed in the form of reduced relative water content (RWC), disrupted photosynthetic activity, and limited dry matter production (Karim et al., 2022). Furthermore, water deficit significantly affects plant water relations, including transpiration and stomatal conductance (g_s), which are crucial for maintaining water balance and regulating carbon assimilation (Fatema et al., 2023). Understanding how these physiological traits respond under water-limited conditions is essential for evaluating drought tolerance and for guiding breeding programs aimed at improving legume resilience in water-scarce environments.

2.3.3.1 Relative water content (RWC%)

Relative water content (RWC) is a crucial physiological indicator of plant water status and is a dependable measure of cellular hydration during drought conditions. Prolonged exposure to water deficit stress worsens cellular water loss, causing irreversible tissue damage and ultimately leading to plant mortality (Shen et al., 2017). To counteract these harmful effects, plants have developed various adaptive responses, including structural, physiological, and biochemical changes, to survive and complete their life cycle under stress (Yang et al., 2007). One of the initial responses to drought is an increase in resistance to water flow in the soil-plant continuum, primarily due to decreased soil matric potential (Homaee et al., 2002).

In soybean, water deficit-induced reductions in RWC are often accompanied by visible symptoms such as leaf drooping, wilting, and impaired water balance due to reduced turgor pressure and decreased cell water potential (Zhang et al., 2020). Leaf survival and overall metabolic activity are closely correlated with RWC, making it a vital parameter for evaluating drought tolerance. Chowdhury et al. (2017) reported a marked decrease in RWC across various soybean genotypes under water deficit, with susceptible genotypes showing greater reductions compared to tolerant ones. In their study, the most vulnerable genotype exhibited a leaf water potential of -1.2 MPa, while the relatively tolerant genotype reached -1.58 MPa, indicating a stronger capacity for osmotic adjustment in the latter.

Recent research has shown that there is a notable variation in relative water content responses among different soybean cultivars when exposed to drought stress. For example, a study conducted by Nguyen et al. (2024) examined how wild soybean accessions responded to drought at various growth stages. The results demonstrated that water deficit significantly decreased relative water content (RWC) across all evaluated genotypes. Notably, specific accessions exhibited enhanced capacity for water retention and more favorable physiological adjustments, indicating a greater inherent tolerance to drought stress. This diversity highlights the significance of choosing and developing soybean varieties with improved drought tolerance characteristics, such as the capacity to sustain higher RWC levels during periods of water scarcity.

In chickpea, large-scale screening of 78 desi genotypes revealed that RWC and canopy temperature depression (CTD) declined under increasing water deficit. Genotypes such as SAGL152210, SAGL152252, SAGL152347, SAGL22-115, and JG11 were identified as tolerant based on their superior physiological performance under stress conditions (Asati et al., 2024). Further supporting this, a comprehensive screening of over 500 chickpea germplasm lines under rainfed stress conditions identified ICC 181, ICC 5929, and ICC 6363 as key tolerant genotypes. These lines consistently maintained higher relative water content (RWC) and membrane stability index (MSI) values, signaling superior drought adaptation (Kohakade et al., 2023). The authors recommend these accessions for advanced molecular characterization and integration into breeding programs aimed at developing resilient varieties. Collectively, these findings confirm that RWC is a fundamental trait for screening water deficit tolerance in legumes. When combined with other physiological and morphological indicators, it significantly improves the precision of drought tolerance evaluations in chickpea and soybean breeding initiatives.

2.3.3.2 Photosynthesis and dry matter accumulation

Photosynthesis is a vital physiological process closely associated with plant growth, development, and the final economic yield. It is very responsive to abiotic stress, especially water deficit, which has a detrimental impact on various aspects of the photosynthetic system (Andrianasolo et al., 2016). Under water deficit, plants experience reduced stomatal conductance, decreased intercellular CO₂ concentration, impaired electron transport, and limited enzymatic activity in the Calvin–Benson cycle (Awasthi

et al., 2014; Lu et al., 2021). These effects collectively result in lower net photosynthetic rates and diminished dry matter accumulation.

In soybean, water deficit reduces leaf area, chlorophyll content, and mesophyll thickness, which compromise light interception and CO₂ assimilation capacity (Lawson et al., 2003; Wang et al., 2018). Moreover, water deficit impairs the functioning of key photosynthetic enzymes, including Rubisco, PEPCase, PPDK, and FBPase, leading to reduced carbon fixation, ATP production, and NADPH synthesis (Farooq et al., 2009; Medrano et al., 2002). Stomatal closure under water stress, while conserving water, also restricts CO₂ influx and increases photorespiration due to enhanced oxygenase activity of Rubisco (Lu et al., 2021). These physiological disruptions ultimately lower biomass production and yield potential. Similarly, water deficit significantly affects photosynthetic efficiency and dry matter partitioning in chickpeas. Common responses to limited water availability include leaf wilting, decreased relative water content, and early senescence (Zaman-Allah et al., 2011). Research confirms that chickpea varieties with higher stress tolerance maintain superior physiological function under adverse conditions. These resilient genotypes consistently demonstrate greater chlorophyll content, higher photosynthetic rates, and improved water-use efficiency when compared to susceptible varieties under both drought and salinity stress (Kashiwagi et al., 2006; Pang et al., 2017). Tolerant genotypes often maintain higher stomatal conductance and intercellular CO₂ concentration, supporting ongoing carbon assimilation even in suboptimal conditions. For instance, Tiwari et al. (2023) found that tolerant chickpea genotypes like JG 11 maintained higher photosynthetic rates and chlorophyll fluorescence parameters (Fv/Fm), leading to improved biomass production and grain yield under water-limited conditions. Negussu et al. (2023) further demonstrated that genotype-specific responses in chickpea affect the preservation of photosynthetic function. The Desi genotype PI5980808 showed substantial reductions in chlorophyll content and photosynthetic activity, whereas the Kabuli genotype Flip07-318C was relatively unaffected by drought stress, maintaining photosynthetic efficiency and chlorophyll integrity. This highlights the inherent genetic variation in drought tolerance mechanisms across chickpea germplasm. Collectively, these studies illustrate that soybean and chickpea employ a spectrum of morphological and physiological adaptations to mitigate water deficit, among which the maintenance of photosynthetic capacity is paramount. The significant genotypic variation observed in preserving photosynthesis and dry matter accumulation

under drought conditions represents a vital target for breeding programs focused on developing climate-resilient legume varieties.

2.3.3.3 Plant water relations, transpiration, and stomatal conductance (g_s)

Plant water status is regulated by a network of interdependent physiological traits, such as relative water content (RWC), leaf water potential (Ψ_{leaf}), transpiration rate (E), canopy temperature, and stomatal conductance (g_s). Stomatal regulation is particularly crucial, as it manages the fundamental trade-off between carbon uptake and water loss during periods of water scarcity (Flexas et al., 2015). In water-limited settings, declining soil moisture weakens the water potential gradient from soil to root, hindering water uptake. This reduction lowers leaf turgor pressure, triggering stomatal closure—a defensive response that curtails gas exchange. Consequently, this limits both transpirational water loss and the influx of CO_2 , leading to a decline in photosynthesis and overall growth (Chaves et al., 2009; Osakabe et al., 2014).

Research on soybeans confirms that water stress induces a substantial decline in key gas-exchange parameters, including photosynthetic rate, transpiration rate, and stomatal conductance. Atti et al. (2004) found that under drought conditions, these parameters were reduced by 78.4%, 85.4%, and 92% respectively. This suggests that stomatal closure is more strongly linked to decreases in transpiration than in photosynthesis. Similarly, Mak et al. (2014) observed a 57% decline in transpiration in soybean due to reduced abscisic acid (ABA)-regulated stomatal conductance under drought. ABA accumulation, which increases up to 50-fold during water stress, serves as a primary signal mediating stomatal closure (Aslam et al., 2022).

Makbul et al. (2011) also showed that soybean genotypes experienced a significant decrease in leaf water potential under water-deficit conditions, dropping from -0.88 MPa in well-watered conditions to -1.18 MPa in water deficit situations. In chickpea, Mafakheri et al. (2010) observed that water deficit significantly reduced stomatal conductance and transpiration rate across different genotypes, highlighting the genotypic variability in stomatal sensitivity to water stress.

Recent studies further emphasize that the regulation of stomatal aperture in legumes is a highly adaptive trait under water deficit. Khatun et al. (2021) have highlighted that legumes like soybean and chickpea effectively modulate g_s in response to declining water availability, balancing water conservation with continued photosynthetic function to varying extents depending on genotype and developmental stage.

2.3.4 Biochemical attributes and their affiliation with water deficit tolerance

Water deficit stress triggers a complex network of biochemical adaptations essential for plant survival. Key responses include the synthesis of osmoprotectants (e.g., proline, glycine betaine, soluble sugars), the upregulation of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and the activation of specific stress-signaling pathways. Collectively, these mechanisms work to preserve cellular homeostasis, safeguard membranes and proteins, and mitigate oxidative injury from reactive oxygen species (ROS) generated under drought conditions (Muhammad Aslam et al., 2022). In legumes like soybean and chickpea, the capacity to efficiently regulate these biochemical markers correlates strongly with water deficit tolerance. Water deficit also alters hormone levels—such as increased abscisic acid (ABA), which further modulates gene expression and biochemical pathways critical for stress resilience (Khatun et al., 2021). Therefore, understanding the water deficit-induced biochemical changes provides key insights into physiological resilience mechanisms and supports the identification of tolerant genotypes.

2.3.4.1 Alteration in photosynthetic pigments

Water deficit stress critically impairs the photosynthetic apparatus, primarily through the structural disruption of chloroplasts and the degradation of essential photosynthetic pigments. Chlorophyll-a, chlorophyll-b, and carotenoids, integral components of the thylakoid membranes in mesophyll chloroplasts, which are notably susceptible to breakdown under drought conditions (Evert & Eichhorn, 2013). These pigments play critical roles in light harvesting and energy transfer during photosynthesis, and their reduction leads to a marked decline in photosynthetic efficiency and plant productivity. Under conditions of water deficit, both the vegetative and reproductive stages experience a significant decrease in chlorophyll a, chlorophyll b, and total chlorophyll content. These changes are often linked to pigment photo-oxidation and impaired chlorophyll biosynthesis, leading to reduced light absorption and decreased CO₂ fixation (Anjum et al., 2011; Fathi and Tari, 2016). For example, in soybean, water deficit can result in a reduction of chlorophyll content by up to 31% compared to non-stressed conditions (Hao et al., 2013). In a study comparing three soybean genotypes under drought conditions (50% field capacity), the cultivar Umut 2002 exhibited greater tolerance, maintaining higher SPAD chlorophyll values and biomass accumulation under stress (Yavas et al., 2024).

A greenhouse evaluation of eight soybean genotypes revealed genotype-specific responses in chlorophyll degradation under water deficit. After 14 days of water deficit, chlorophyll content decreased by only 2.72% in the tolerant genotype *Steara*, while the susceptible genotype *2:14007 SI-3* exhibited a 21.69% reduction. Notably, chlorophyll levels showed a rapid recovery upon rehydration, indicating a reversible loss of pigment in tolerant genotypes (Anjum et al., 2011). In chickpea, water stress induces comparable physiological impairment, marked by significant reductions in chlorophyll content, chlorophyll fluorescence (Fv/Fm), and net photosynthetic rate, the key metrics of both damage and resilience (Mafakheri et al., 2010).

Carotenoids, especially β -carotene, serve a vital protective function by scavenging reactive oxygen species (ROS) produced under stress. Nonetheless, severe water deficit can overwhelm these defenses, leading to ROS accumulation that degrades the photosystem I (PSI) and photosystem II (PSII) core complexes within thylakoid membranes. This structural damage further diminishes photosynthetic efficiency (Tomer and Singh, 2020). Furthermore, β -carotene and other isoprenoid pigments serve critical antioxidant roles within photosystem I and II; however, their oxidative degradation under stress reduces photoprotection and destabilizes light-harvesting complexes (Tomer and Singh, 2020). Controlled-environment studies in soybean have shown that exogenous treatments (e.g., amino acids or silicon) can partially preserve carotenoid levels and counteract water deficit-induced pigment loss (Makbul et al., 2011). These findings highlight the significance of chlorophyll and carotenoid stability as indicators of water deficit tolerance in legumes, such as soybeans and chickpeas.

2.3.4.2 Membrane integrity and malondialdehyde (MDA) accumulation

Water scarcity induces physiological dysfunction, notably through the peroxidation of lipids in cell membranes, which impairs their integrity (Thankamani et al., 2003). Malondialdehyde, a byproduct of this peroxidation, is a widely used indicator for measuring the extent of oxidative membrane damage caused by such stress (Hasanuzzaman et al., 2020). This oxidative stress arises from a physiological imbalance, wherein the generation of reactive oxygen species (ROS) in chloroplasts and mitochondria exceeds the plant's capacity to neutralize them via its antioxidant systems (Simova-Stoilova et al., 2009). If the antioxidant defenses are overpowered, the accumulating ROS—superoxide radicals, hydrogen peroxide, and hydroxyl radicals,

aggressively oxidize vital cellular constituents, including membranes, proteins, and genetic material, thereby worsening the stress injury (Gill and Tuteja, 2010).

The accumulation of malondialdehyde (MDA) serves as a dependable indicator of oxidative membrane damage under various abiotic stresses, including drought, salinity, and temperature extremes (Guo et al., 2018; Rui et al., 2018; Hasanuzzaman et al., 2020). Consequently, the capacity to minimize this accumulation is a key trait of stress-tolerant genotypes, as those resilient to water deficit generally maintain lower MDA levels, indicative of more effective antioxidant defenses and superior cellular integrity (Ma et al., 2015; Ahmad et al., 2023). This relationship is exemplified in soybean research, where all cultivars under water stress exhibit elevated MDA. However, the degree of increase which is directly proportional to the extent of oxidative injury, varies markedly depending on genetic constitution and the severity of the stress applied (Dong et al., 2019; Katam et al., 2020; Farooq et al., 2021).

Recent investigations by Wang et al. (2022) demonstrated a progressive increase in MDA content in soybean genotypes HN44 (drought-tolerant) and HN65 (drought-sensitive) as soil moisture declined during the flowering stage. MDA peaked after seven days of drought treatment, with significantly lower accumulation in HN44, correlating with enhanced drought tolerance. In chickpea, lipid peroxidation and MDA content have also been widely used to differentiate water deficit tolerance among cultivars. A controlled glasshouse study assessing 11 chickpea genotypes revealed significant genotypic variation in membrane stability under water deficit, tolerant cultivars exhibited markedly lower membrane permeability and MDA levels, coupled with elevated relative water content, proline accumulation, and antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Fazeli-Nasab et al., 2025). These physiological and biochemical adaptations contribute to enhanced membrane preservation and ROS mitigation, critical for sustaining cellular functions during water deficit.

The dynamic response of membrane integrity in legumes like chickpea and soybean to water deficit and combined stresses is being clarified by recent hydroponic research under controlled conditions. A notable example is the evaluation of 78 desi chickpea genotypes under terminal drought, which tracked physiological and biochemical changes during pod filling. While drought universally reduced physiological metrics (RWC, MSI, CTD, chlorophyll) and increased oxidative markers (H_2O_2 , lipid peroxidation), tolerant genotypes displayed a distinct protective response. Cultivars such as SAGL152252,

ICC4958, and JG11 maintained cellular homeostasis by boosting their antioxidant enzyme systems (CAT, SOD, POD, APX) and accumulating protective osmolytes (proline, soluble sugars), thereby mitigating membrane damage (Asati et al., 2024). Similarly, Sanusi et al. (2025) demonstrated that water deficit induced by polyethylene glycol (PEG 6000) led to significant morphological, physiological, and biochemical changes in soybean (TGX-1835-10E) under controlled conditions. Moderate stress (8% PEG) improved root development and seedling fresh weight, while severe stress (16% PEG) resulted in reduced chlorophyll content and elevated malondialdehyde (MDA) and proline levels, indicating increased oxidative damage. Antioxidant responses were also stress-level dependent, with ascorbate peroxidase activity increasing at moderate stress but declining under severe conditions, and catalase showing limited responsiveness. These results highlight the cultivar's partial drought adaptability through modulation of antioxidant defense and osmotic adjustment mechanisms.

Collectively, this evidence confirms that robust oxidative stress management and membrane stability are central to drought tolerance in legumes. Progress in this field is increasingly driven by the integration of physiological data with biochemical indicators like MDA and modern molecular techniques. Such a comprehensive approach is essential for accelerating the development of climate-resilient cultivars through advanced breeding and biotechnology.

2.3.4.3 Accumulation of organic compatible solutes

Under water deficit conditions, plants accumulate compatible solutes—also known as osmolytes—as a key adaptive mechanism for osmotic adjustment and osmoprotection, thereby maintaining cellular turgor and overall physiological stability (Lu et al., 2021; Tomer and Singh, 2020). Osmotic adjustment involves the intracellular accumulation of inorganic ions such as Na^+ , K^+ , Ca^{2+} , and Cl^- within the vacuole, as well as organic solutes including sugars (e.g., glucose, fructose), polyols (e.g., glycerol), amino acids, and other nitrogenous compounds in the cytoplasm (Hasanuzzaman et al., 2020). These osmolytes contribute to lowering the cellular osmotic potential, thereby facilitating water uptake and retention during water deficit (Fathi and Tari, 2016). Osmolytes, including oligosaccharides, polyols, proline, polyamines, and specific amino acids, confer multifaceted stress protection by stabilizing enzymes and membranes while scavenging reactive oxygen species (ROS) (Slama et al., 2015). Among these, proline serves particularly diverse functions. It acts not only as a compatible solute that safeguards

membranes from peroxidation and detoxifies ROS to maintain redox balance but also as a signaling molecule that modulates stress-responsive gene expression to facilitate recovery from water deficit (Solanki and Sarangi, 2015; Shinde et al., 2016).

In chickpea, water deficit-induced osmolyte accumulation has been extensively studied as a determinant of drought resilience. Amede et al. (2003) demonstrated that increased solute accumulation correlated positively with improved water deficit tolerance. More recently, Negussu et al. (2023) reported genotype-specific responses to water deficit in chickpea, where the Kabuli genotype Flip07 318C exhibited significant proline accumulation under water stress, contrasting with the Desi genotype PI5980808 which showed negligible changes. These differences highlight the genetic variability in osmotic adjustment mechanisms among chickpea cultivars.

In soybean, osmolyte accumulation under water deficit has also been documented. Basal et al. (2024) evaluated two soybean genotypes, Speeda and Coraline, under water-limited conditions combined with exogenous hydrogen peroxide (H₂O₂) treatments. Both genotypes exhibited significant increases in leaf proline content after three days of stress, indicating a conserved osmoprotective response across genotypes. Parallel hydroponic investigations have confirmed that soybean plants accumulate higher concentrations of osmolytes, including proline and soluble sugars, in response to polyethylene glycol (PEG)-induced osmotic stress. In these studies, genotypes identified as tolerant exhibited a more pronounced capacity for osmotic adjustment and a more efficient reactive oxygen species (ROS) scavenging system (Sanusi et al., 2025). Collectively, these studies emphasize the critical role of compatible solute accumulation in maintaining cellular homeostasis and enhancing water-deficit tolerance in chickpea and soybean, underscoring their importance as biochemical markers in breeding programs targeting water-deficit resilience.

2.3.4.4 Non-enzymatic antioxidants: ascorbate and carotenoids

To counteract oxidative damage from reactive oxygen species (ROS) overproduction during water deficit, plants activate a complex antioxidant defense network. This system relies on essential non-enzymatic antioxidants like ascorbic acid (AsA) and carotenoids, which are vital for maintaining cellular homeostasis, safeguarding membrane integrity, and preventing oxidative injury (Hasanuzzaman et al., 2020). These molecules function as direct ROS scavengers and as critical cofactors for enzymatic processes in redox regulation.

A key component of the antioxidant system, ascorbic acid (AsA), is crucial for neutralizing ROS, recycling other antioxidants like tocopherols, and protecting photosynthetic efficiency during stress. In soybeans, this pathway is genetically regulated; Seminario et al. (2017) observed that stress led to suppressed expression of the VTC1 gene and a decline in GalLDH enzyme activity, directly impacting the biosynthesis of AsA. Exogenous application of AsA significantly improved water deficit resilience by enhancing growth, photosynthetic pigments, and antioxidant capacity in soybean (Basal et al., 2024). Similarly, chickpea plants responded positively to foliar AsA treatments under water deficit, exhibiting increased proline levels, photosynthetic pigment retention, and improved redox balance (El-Beltagi et al., 2022). Moreover, the external application of ascorbic acid (AsA) has been demonstrated to enhance critical physiological metrics, such as biomass, relative water content, and membrane stability, in chickpea genotypes under drought stress (Raut et al., 2025). Simultaneously, the lipid-soluble carotenoid pigments in chloroplasts serve as a vital dual function during water deficit. They protect the photosynthetic system by quenching singlet oxygen and dissipating surplus light energy via the xanthophyll cycle (Havaux, 2013). Their antioxidant function helps prevent chlorophyll degradation and lipid peroxidation under stress conditions. Studies have shown that in soybean, water deficit can lead to a decrease in carotenoid content, resulting in reduced photosynthetic efficiency (Anik et al., 2023). However, the application of antioxidants such as AsA and α -tocopherol has been found to stabilize carotenoid levels in water deficit-stressed soybean and chickpea, helping to maintain chloroplast integrity and reduce oxidative damage (El-Beltagi et al., 2022). Research by Farjam et al. (2014) in chickpea demonstrated that foliar application of AsA can mitigate the decrease in carotenoids and chlorophylls induced by water deficit, enhancing stress resilience. Additionally, in controlled hydroponic water deficit experiments, carotenoid accumulation has been used as a biomarker to assess stress tolerance in different chickpea genotypes (Kumar et al., 2024). Together, ascorbate and carotenoids constitute key components of the non-enzymatic antioxidant network in legumes. Their accumulation not only enhances tolerance to oxidative damage but also contributes to improved physiological performance under water deficit, offering promising targets for stress-adaptive management and breeding programs.

2.3.4.5 Role of hydrogen peroxide (H₂O₂) in water deficit tolerance

Hydrogen peroxide (H₂O₂) is a pivotal molecule in plants with a dual nature: it orchestrates stress defense signaling at low concentrations but induces oxidative harm and cell death when it accumulates (Quan et al., 2008). Its regulatory influence extends to fundamental processes like stomatal movement, photorespiration, and cell division (Noctor and Foyer, 1998; Bright et al., 2006). Furthermore, H₂O₂ functions as a central hub in drought signaling, integrating inputs from phytohormones and calcium to modulate both gene transcription and physiological outcomes.

Recent studies on chickpea and soybean emphasize the significant role of hydrogen peroxide (H₂O₂) in the response to water deficit. An investigation by Asati et al. (2024) involving 78 desi chickpea genotypes under drought stress recorded substantial increases in H₂O₂, malondialdehyde (MDA), total soluble sugars, and proline. Concurrent decreases were observed in relative water content (RWC), membrane stability index (MSI), and canopy temperature depression (CTD). These findings point to H₂O₂'s function as a signaling molecule that triggers osmotic adjustment, especially in genotypes with differing stress tolerance. Furthermore, a decline in chlorophyll content across all genotypes suggests that oxidative damage to photosynthetic pigments occurred during the stress period. In soybean, the external application of H₂O₂ has demonstrated potential in enhancing tolerance to water deficits. Okunlolaa et al. (2022) demonstrated that foliar treatment with H₂O₂ enhanced biomass accumulation, photosynthetic pigment stability, and ion homeostasis, particularly increasing potassium (K⁺) levels in drought-stressed genotypes NGBO 03515 and NGBO 03524. This ionic adjustment contributed to higher cell turgor pressure, promoting growth under limited water availability. Notably, the drought-sensitive genotype NGBO 03519 exhibited poor response without H₂O₂ supplementation, underscoring its regulatory role in cell expansion and survival under stress conditions. Further supporting these findings, Basal et al. (2024) investigated two soybean genotypes (*Speeda* and *Coraline*) exposed to water-limited conditions with exogenous H₂O₂ spray. Both genotypes showed increased levels of proline and antioxidant enzyme activity, suggesting that H₂O₂ acted as a priming agent that enhanced water deficit tolerance through activation of antioxidant pathways. Similarly, Waqas et al. (2019) reported that H₂O₂ treatment in chickpea improved root biomass, photosynthetic rate, and leaf relative water content, indicating a systemic physiological response to H₂O₂ signaling under water deficit. Taken together, these findings confirm that hydrogen peroxide serves as a critical regulator of water deficit responses in legumes

by enhancing antioxidant defenses, maintaining ionic balance, and improving osmotic regulation. Its application, particularly under controlled conditions such as hydroponics, may serve as a promising approach to improve water deficit tolerance in sensitive genotypes (Fig. 3).

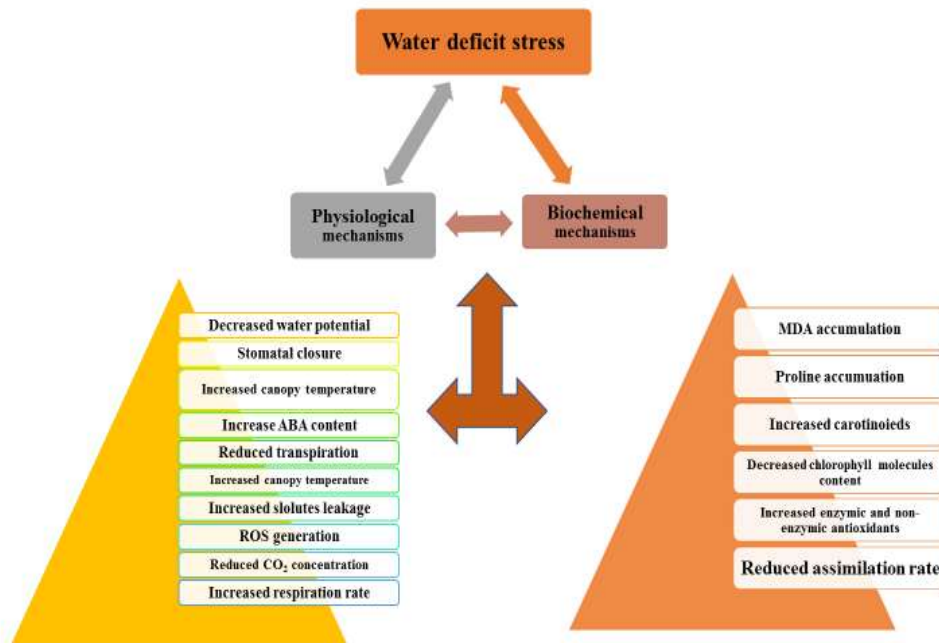


Figure 3. Effects of water deficit stress on physiological and biochemical parameters.

2.3.5 Water deficit: impacts on legumes during vegetative stages

Water deficit during the vegetative growth stage critically affects leguminous crops by disrupting physiological, morphological, and biochemical processes essential for plant development. During early vegetative growth, water stress reduces cell expansion, leaf area development, photosynthetic capacity, and root proliferation, ultimately leading to reduced biomass accumulation (Farooq et al., 2009). Legumes such as chickpea and soybean are particularly sensitive at this stage, where water availability plays a pivotal role in establishing root-shoot balance and canopy architecture (Nguyen et al., 2024). Early-stage water deficit limits stomatal conductance, carbon assimilation, and nitrogen fixation, significantly impairing vegetative vigor and setting the stage for yield penalties at later reproductive phases (Fenta et al., 2011).

In chickpea, water deficit during vegetative growth leads to significant reductions in relative water content (RWC), chlorophyll concentration, and leaf expansion rate, with genotypic differences observed in the degree of sensitivity. Water deficit-tolerant genotypes tend to maintain higher RWC, root-to-shoot ratios, and antioxidant capacity

(Asati et al., 2024). Recent studies have shown that even under controlled environments, moderate water stress reduces leaf area and photosynthetic pigment concentration, particularly in Desi-type genotypes (Khatun et al., 2021). Similarly, soybean genotypes exhibit reductions in plant height, leaf number, and shoot fresh weight under vegetative-stage water deficit, accompanied by increased accumulation of stress markers such as proline, malondialdehyde (MDA), and reactive oxygen species (ROS) (Basal et al., 2024).

The degree of impact from water deficit is frequently contingent upon the genetic background of the plant. This is illustrated by a study in soybean where a tolerant genotype maintained a more effective root architecture, resulting in superior water acquisition and reduced oxidative stress during drought, unlike its sensitive counterpart (Wang et al., 2022). Parallel mechanisms are observed in chickpea, where stress during early development alters the expression of genes key to osmolyte and antioxidant pathways (Negussu et al., 2023).

2.4 Salinity stress as a major abiotic constraint to legume production

Current United Nations projections indicate a global population of 9.3 billion by 2050, rising from 7.93 billion in 2022. To meet the food demands of this growth, global agricultural output must increase by approximately 70% without major expansion of cultivated areas, while also improving input efficiency and preventing ecosystem degradation. A primary barrier to this objective is soil salinity, a widespread abiotic stress that severely constrains crop production. Of the world's estimated 4.8 billion hectares of agricultural land, roughly 0.9 billion hectares are adversely affected by saline (0.34 billion ha) or sodic (0.56 billion ha) conditions (Kopittke et al., 2019). This stress represents a critical threat to global food security, substantially impairing plant growth and causing extensive yield reductions (Varshney et al., 2013; Qadir et al., 2014; Zulfikar and Ashraf, 2021; Ashraf and Munns, 2022).

Its global scale is considerable: salinity affects over 6% of the world's land area, including 20% of all irrigated land, leading to estimated annual economic losses of US\$27.3 billion (Munns et al., 2020a). The issue is especially severe in irrigated systems, where secondary salinization has degraded approximately 25-30% of the 275 million hectares under irrigation (FAO, 2023). Alarming, the area of salt-affected soil continues to grow due to unsustainable farming methods and the use of poor-quality irrigation water (Rengasamy, 2016).

Rising demands for domestic and industrial water, the depletion of freshwater aquifers, and climate change are driving agriculture toward the use of marginal-quality water. Climate change intensifies this challenge by elevating evapotranspiration rates, which concentrate soluble salts in the root zone and render soils unsuitable for salt-sensitive crops such as chickpea and soybean (Samineni et al., 2011; Rani et al., 2020). As a result, the utilization of saline water for irrigation is becoming an increasingly unavoidable reality for sustaining future agricultural output (Munns & Tester, 2008).

Salinity stress impacts plants in sequential phases. Initially, an osmotic phase reduces water availability, inducing a physiological drought. This is followed by ionic stress, as the accumulation of toxic sodium (Na^+) and chloride (Cl^-) ions disrupts critical nutrient ratios (e.g., K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$), inhibits enzyme function, and compromises cellular membrane stability (Munns et al., 2020a; Rahnama et al., 2010). These primary effects trigger secondary oxidative stress, marked by excessive production of reactive oxygen species (ROS). This leads to lipid peroxidation, protein denaturation, and DNA damage, ultimately suppressing photosynthesis, stunting growth, and causing substantial yield losses (Zulfiqar & Ashraf, 2021).

The global challenge of soil salinization, impacting over 1.38 billion hectares and 20% of irrigated land (Buttar et al., 2021), is now affecting agricultural areas in Hungary. Here, secondary salinization threatens the cultivation of critical legume crops. Chickpea and soybean, despite their nutritional and agronomic benefits, are notably salt-sensitive (James et al., 2011; Flowers et al., 2019). Even moderate salt stress (25 – 40 mM NaCl) during key growth stages can severely compromise their biomass accumulation, photosynthetic capacity, and final yield (Samineni et al., 2011; Shin et al., 2020).

To tackle this issue, the identification and development of salt-tolerant genotypes offer a viable and environmentally friendly solution. Salt-tolerant legume varieties are more adept at regulating ion levels, preserving photosynthetic function, storing compatible solutes like proline and glycine betaine, and bolstering antioxidant mechanisms when faced with salinity stress (Varshney et al., 2021). Introducing these genotypes into agricultural systems affected by salinity not only boosts yield but also promotes sustainable soil practices and strengthens resilience to climate challenges.

2.4.1 Mechanisms of ion toxicity, osmotic stress, and nutrient imbalances in salinity-stressed plants

Soil salinity impedes plant growth through a combination of ion toxicity, osmotic stress, and nutrient imbalances, which collectively disrupt water uptake, metabolism, and productivity. The primary toxic ions, sodium (Na^+) and chloride (Cl^-), accumulate in plant tissues after being absorbed by roots. Excess Na^+ disrupts cellular function by competing with essential potassium (K^+) for uptake, leading to K^+ deficiency that impairs enzyme activity, stomatal function, and photosynthesis (Munns and Tester, 2008). Similarly, high Cl^- concentrations cause chlorosis and necrosis by damaging cellular membranes and photosynthetic pigments (Ceritoğlu et al., 2020).

Osmotic stress arises because the increased salt concentration in the soil solution lowers the osmotic potential, making water less available to plants despite adequate soil moisture. This leads to reduced water absorption by roots, decreased cell turgor, stomatal closure, and limited cell expansion, which visibly manifest as stunted growth and reduced leaf area (Farooq et al., 2009; Buckley, 2019). Osmotic stress triggers immediate physiological adjustments, most notably the rapid closure of stomata driven by abscisic acid (ABA). Although this response minimizes transpirational water loss, it comes at the cost of reduced carbon assimilation due to limited CO_2 diffusion (Muller et al., 2011). This salinity-induced drought condition also disrupts cellular water status and ion transport, creating metabolic imbalances as the plant strives to sustain essential functions (Annunziata et al., 2017).

Nutrient imbalances further exacerbate salt stress effects. High Na^+ levels disrupt the uptake of essential minerals such as calcium (Ca^{2+}), magnesium (Mg^{2+}), nitrate (NO_3^-), and phosphate (PO_4^{3-}), resulting in deficiencies that impair critical physiological functions like photosynthesis, protein synthesis, and energy metabolism (Ashraf and Munns, 2022; Kumar et al., 2019). The displacement of Ca^{2+} by Na^+ can destabilize plasma membranes, increasing permeability and further damaging cells (Soni et al., 2021).

Despite their agronomic value as protein-rich, nitrogen-fixing species, chickpea and soybean are particularly vulnerable to salinity. The combined stresses of ion toxicity, osmotic pressure, and nutrient disruption lead to severe growth and yield penalties in chickpea, even under relatively low salt stress (Pujol et al., 2000; Samineni et al., 2011). Soybean, a globally important protein and oilseed crop, is also highly sensitive to salinity, with reductions in germination, biomass, and physiological performance occurring at

moderate salt levels (Chen et al., 2024). These legumes suffer from impaired water uptake due to osmotic stress, as well as disruptions in ionic homeostasis that limit potassium and other nutrient availability, which are essential for their growth and nitrogen fixation processes (Ashraf and Munns, 2022).

Ion toxicity, osmotic stress, and nutrient imbalances collectively pose a significant challenge for plants, leading to a variety of symptoms including leaf chlorosis, necrosis, and decreased biomass and yield. These stresses are most detrimental during critical growth phases such as seedling establishment, vegetative growth, and flowering, particularly for crops like chickpea and soybean that are sensitive to salt.

2.4.2 Plant defense mechanisms: Na^+ exclusion and ion compartmentalization

Plants exposed to salinity stress activate several key defense mechanisms to mitigate the toxic effects of excess sodium ions (Na^+) and maintain cellular homeostasis. One of the primary strategies is Na^+ exclusion, where plants restrict the entry of Na^+ into the roots or actively pump it out to the soil solution to prevent its accumulation in sensitive tissues. This exclusion is primarily regulated by membrane-bound transporters such as the SOS1 (Salt Overly Sensitive 1) Na^+/H^+ antiporter located in the plasma membrane of root cells, which extrudes Na^+ ions back into the soil, thereby protecting the cytoplasm from toxic sodium levels (Wang et al., 2023). Additionally, selective uptake mechanisms allow plants to preferentially absorb essential ions like potassium (K^+) over Na^+ , maintaining an optimal Na^+/K^+ ratio that is critical for enzyme function and cellular processes (Shabala and Pottosin, 2014).

When exclusion alone is insufficient, plants employ ion compartmentalization, which involves sequestering Na^+ into vacuoles or other intracellular compartments to reduce its concentration in the cytosol and prevent interference with vital metabolic activities. This compartmentalization is facilitated by tonoplast-localized Na^+/H^+ antiporters, such as the NHX family transporters, which use proton gradients to drive the active transport of Na^+ into the vacuole (Apse et al., 1999). By isolating toxic ions, plants can maintain cytosolic ion homeostasis, osmotic balance, and turgor pressure, which are essential for growth under saline conditions (Blumwald, 2000). Ion compartmentalization also contributes to osmotic adjustment by increasing the osmotic potential inside vacuoles, helping plants retain water during osmotic stress caused by high external salt concentrations.

Together, Na^+ exclusion and ion compartmentalization are complementary defense mechanisms that enable plants to tolerate saline environments by reducing ion toxicity,

preserving nutrient balance, and maintaining cellular functions. Consequently, a central objective of modern breeding is to enhance salinity resilience by deciphering and fortifying these innate defense pathways. Evidence from salt-tolerant soybean and chickpea varieties, which exhibit up-regulated expression and function of key transporter proteins, validates this approach and provides specific targets for genetic improvement (Balasubramaniam et al., 2023).

Chickpea, studies have shown that genotypes with enhanced salinity tolerance exhibit more efficient Na^+ exclusion from the shoots, limiting toxic ion accumulation in photosynthetic tissues. For example, Kotula et al. (2019) reported that salt-tolerant chickpea lines maintain lower Na^+ content in leaves while sustaining higher K^+ levels, thus preserving ionic balance and osmotic potential. Moreover, the expression of ion transporter genes related to Na^+ exclusion has been linked to improved salt tolerance in chickpea under hydroponic and soil-based salinity stress (Kumar et al., 2019). Similarly, soybean utilizes both Na^+ exclusion and ion compartmentalization to manage ionic stress. Soybean cultivars tolerant to salinity have shown increased activity of NHX-type Na^+/H^+ antiporters, which sequester Na^+ into vacuoles, reducing cytoplasmic ion toxicity (Amirjani, 2010). Controlled-environment soybean experiments indicate that salt-tolerant genotypes maintain lower cytosolic Na^+ and a higher K^+/Na^+ ratio—often via enhanced activity of Golgi-localized Na^+/H^+ antiporters such as GmNHX5—which supports osmotic adjustment and preserves photosynthetic efficiency (Sun et al., 2021). This ion sequestration is critical for preventing the disruption of metabolic processes, especially during early vegetative growth stages when plants are most vulnerable.

Together, Na^+ exclusion and ion compartmentalization represent fundamental defense strategies in chickpea and soybean, enabling these legumes to maintain cellular ion homeostasis and mitigate salt-induced damages. The identification and manipulation of these mechanisms through molecular breeding or biotechnological approaches offer promising avenues for enhancing salinity tolerance in these economically important crops (Toker et al., 2021).

2.4.3 Salinity tolerance-associated traits

Alkaline soils with high salt content contain various ions that form salt-alkalization complexes (Läuchli and Lüttge, 2002). The resulting low osmotic potential of these soils severely restricts plant-available water, limiting the plant's capacity to meet its metabolic and turgor requirements. Consequently, plants exhibit reduced growth, especially during

early vegetative and reproductive stages. Salinity triggers a cascade of physiological and biochemical mechanisms aimed at maintaining water status, reducing ion toxicity, and stabilizing metabolism, such as ion compartmentalization, osmolyte biosynthesis, activation of antioxidant defenses, hormonal signaling, and others (Muller et al., 2011). In early responses, stomatal closure—mediated by abscisic acid, aims to reduce transpiration losses. However, primary sinks like young leaves and root tips are more susceptible than photosynthetic sources, leading to reduced carbon assimilation and biomass accumulation (Li et al., 2018). Under longer stress durations, secondary responses such as reactive oxygen species (ROS) detoxification, compatible solute accumulation, cell wall modification, and metabolic reprogramming help plants cope with salt-induced damage (Dichala et al., 2022; Liu et al., 2020).

2.4.3.1 Effect of salinity stress on morphological traits

Legumes, despite their ecological and nutritional importance, are particularly vulnerable to salinity. The vulnerability of chickpea and soybean to salinity is most acute during early growth and flowering, leading to severe yield reductions (Samineni et al., 2011; Hadi and Karimi, 2012). The physiological manifestation in chickpea involves disrupted water absorption and inhibited elongation, ultimately resulting in decreased biomass (Kaur et al., 2021). This damage stems from the sequential stress of osmotic imbalance, which immediately limits water availability, and subsequent ion-specific toxicity from Na^+ and Cl^- accumulation, which damages cellular membranes and plant vigor (Ceritoğlu et al., 2020; Soni et al., 2021). Genotypes with enhanced osmotic adjustment and root hydraulic conductivity exhibit greater survival under stress, highlighting the critical role of these traits in conferring salinity tolerance (Kaur et al., 2021). Under saline conditions, declining plant water content causes cell shrinkage and cell wall relaxation, leading to a loss of turgor pressure. This results in a significant reduction in leaf water potential (ψ_w) and inhibits cell expansion (Farooq et al., 2009). The salinity stress response unfolds in distinct phases that severely inhibit plant development. The initial osmotic phase rapidly limits water uptake, suppressing cell expansion and reducing leaf area, which is evident as stunted root and shoot growth (Farooq et al., 2012). This is consistently shown in chickpea studies by significant reductions in root and shoot length, as well as in fresh and dry biomass across genotypes (Kaur et al., 2021). The ensuing ionic phase involves the toxic accumulation of Na^+ and Cl^- ions, which further lowers tissue water potential (ψ_w), disrupts nutrient homeostasis, and produces visible damage such as chlorosis and necrosis

(Soni et al., 2021; Ceritoğlu et al., 2020). A key adaptive mechanism is a plant's ability to osmotically adjust by actively lowering its osmotic potential (ψ_s), which is essential for maintaining turgor and withstanding this physiological drought.

Recent hydroponic research has been pivotal in elucidating the mechanisms of salinity tolerance. For instance, studies on soybean have demonstrated that tolerant genotypes can maintain key morphological traits, such as shoot length, root mass, and leaf area, despite exposure to elevated NaCl levels, highlighting their capacity for sustained growth under stress (Wu et al., 2014; Essa, 2002). Similarly, in chickpea, salt-tolerant accessions exhibited larger root volumes and surfaces that facilitated better ion exclusion, whereas hydroponic assessments revealed that salt-sensitive lines consistently showed early chlorosis, reduced root proliferation, and leaf senescence, particularly under 50–100 mM NaCl (Kaur et al., 2021).

2.4.3.2 Effect of salinity stress on physiological traits

Salinity stress leads to reduced relative water content (RWC), photosynthetic efficiency, and stomatal conductance in both species. In chickpea, Kumar et al. (2019) and Soni et al. (2021) noted significant declines in RWC and water potential under salt stress, indicating impaired water balance and cell expansion. Samineni et al. (2011) reported reductions in stomatal conductance and chlorophyll fluorescence in chickpea seedlings under saline conditions, indicating compromised photosystem II performance and water status. Similarly, in soybean, Mannan et al. (2013) showed that tolerant lines had higher stomatal conductance, chlorophyll content, and membrane stability compared to sensitive ones in hydroponic systems, making these physiological markers reliable indicators of salinity response. Kao et al. (2006) further demonstrated that salt stress in soybean decreases photosynthesis and transpiration rates, linked to sodium accumulation and stomatal closure. Additionally, Hniličková et al. (2019) found that salt stress impaired root hydraulic conductivity and increased electrolyte leakage in soybean, correlating with inhibited growth and water uptake.

2.4.3.3 Effect of salinity stress on biochemical traits

A prevalent physiological adaptation in salt-tolerant genotypes is the synthesis of osmoprotectants such as proline, glycine betaine, and soluble sugars. In chickpea, tolerant lines under hydroponic salinity stress showed increased proline accumulation and heightened activity of antioxidant enzymes (SOD, CAT, APX), which was associated

with lower malondialdehyde (MDA) content, indicating less oxidative damage to membranes (Shahid et al., 2018). Corresponding research in soybean reveals that resilient genotypes sustain higher levels of ascorbic acid and phenolic compounds, coupled with a more effective ROS-detoxifying system (Noreen et al., 2021; Razmi et al., 2017). This integrated response, characterized by proline-driven osmotic adjustment and reduced lipid peroxidation was validated in soybean exposed to 100 mM NaCl stress, highlighting its function in preserving cellular equilibrium (Per et al., 2017).

Despite the progress, integrated studies combining morphological, physiological, and biochemical traits under hydroponic systems remain limited, especially in chickpea. Most studies have focused on field performance or molecular markers in isolation. A holistic approach that evaluates multiple tolerance traits in controlled hydroponic conditions could offer robust screening tools and accelerate the breeding of salt-resilient legume varieties. As legumes are essential for sustainable cropping systems, improving their salt tolerance is not only agronomically important but also critical for maintaining soil fertility in saline-prone regions.

2.5 Genotypic variability and stress tolerance in soybean and chickpea

Salinity stress is a significant abiotic factor that restricts crop productivity globally, particularly in legumes such as soybean and chickpea, which are moderately to highly susceptible to salty conditions. However, the impact of salinity and plant reactions varies among different genotypes. Both soybean and chickpea show considerable genetic diversity, with specific varieties demonstrating natural tolerance mechanisms that enable them to sustain growth, photosynthesis, and ionic equilibrium in saline environments. This genetic diversity serves as a crucial basis for breeding and selection initiatives focused on enhancing salt tolerance. It is essential to comprehend these genotype-specific reactions, not just to pinpoint salt-tolerant varieties but also to understand the physiological, morphological, and biochemical characteristics that provide resilience. Comparing tolerant and sensitive genotypes under salinity stress, especially in hydroponic systems, has uncovered significant variations in Na⁺ exclusion, root structure, stomatal control, and antioxidant function. These results underscore the significance of utilizing the genetic diversity present in legume germplasm collections to improve salt tolerance and maintain productivity in less fertile areas. Salinity tolerance varies widely among legumes such as chickpea and soybean due to genetic differences. This variability is evident in various morphological, physiological, and biochemical traits when exposed

to salt stress. In chickpea, genotypes like JG 11, ICC 4958, ICCV 10, and Pusa 1105 exhibit tolerance through mechanisms such as reduced Na⁺ accumulation, higher relative water content (RWC), and improved chlorophyll retention. On the other hand, sensitive genotypes like ICCV 92318, Pusa 256, and ICCV 2 show decreased shoot/root biomass, increased electrolyte leakage, and disrupted ion balance under saline conditions (Kaur et al., 2021; Samineni et al., 2011; Toker et al., 2021). Similarly, in soybean, tolerant genotypes such as Clark 63, Lee 68, Tanjin-6, and DT2008 maintain higher photosynthetic efficiency, membrane stability, and K⁺/Na⁺ selectivity under stress, while sensitive genotypes like Jack, Bragg, and Williams 82 often experience growth reduction, Na⁺ toxicity, and oxidative stress (Amirjani, 2010; Mannan et al., 2013; Essa, 2002). These genetic variations are crucial for breeding programs and screening methods aimed at identifying salt-tolerant cultivars for sustainable agriculture in salt-affected areas (Table 1).

Table 1. Genotypic variability in salinity tolerance among soybean and chickpea genotypes.

Crop	Tolerant Genotypes	Sensitive Genotypes	Key Traits Under Salinity Tolerance	References
Chickpea	JG 11, ICC 4958, ICCV 10, Pusa 1105	ICCV 2, Pusa 256, ICCV 92318	Low Na ⁺ accumulation, higher RWC, chlorophyll stability, biomass retention	Kaur et al. (2021); Samineni et al. (2011); Toker et al. (2021)
Soybean	Clark 63, Lee 68, Tanjin-6, DT2008	Jack, Bragg, Williams 82	Higher stomatal conductance, Na ⁺ exclusion, membrane stability, K ⁺ /Na ⁺ ratio	Mannan et al. (2021); Amirjani (2010); Essa (2002)

2.5.1 Screening and selection criteria for salinity tolerance in legumes

Evaluating salt-tolerant genotypes of soybean and chickpea involves a thorough examination of morphological, physiological, and biochemical traits that are closely linked to stress tolerance. Key morphological indicators include root length, shoot length, and biomass accumulation. Salt-tolerant genotypes generally display superior root and shoot elongation under saline conditions, a result of their capacity to sustain cell expansion and water absorption (Kaur et al., 2021; Kumar et al., 2019). Quantifying the plant's response to salinity relies on key physiological indicators. Critical among these are traits like relative water content (RWC) and water potential, which directly measure the plant's effectiveness in regulating its internal water balance against osmotic stress (Mannan et al., 2013).

Chlorophyll content and fluorescence (Fv/Fm) are key indicators of photosynthetic integrity, with higher values distinguishing salt-tolerant varieties (Soni et al., 2021). Biochemical markers of tolerance include elevated proline for osmotic adjustment, lower malondialdehyde (MDA) for membrane stability, and increased activity of antioxidant enzymes (SOD, CAT, POD) for ROS detoxification (Amirjani, 2010; Sadak et al., 2020; Wu et al., 2014; Shahid et al., 2018). Advanced screening now also prioritizes homeostasis, particularly the maintenance of a low Na⁺/K⁺ ratio through regulated Na⁺ exclusion or compartmentalization, which is critical for preserving metabolic function (Mannan et al., 2013; Toker et al., 2021). This integrated set of parameters provides a robust framework for selecting salt-tolerant genotypes in legume breeding programs.

Table 2. Key traits used for screening and selection of salt tolerance in chickpea and soybean.

Trait Type	Trait	Significance	Supporting Studies
Morphological	Root and shoot length	Indicates salt-induced growth inhibition; sensitive genotypes show greater reductions	Kaur et al. (2021); Buttar et al. (2021)
Morphological	Seedling vigor index	Used to evaluate early-stage stress responses in seedlings	(Himaja et al., 2023)
Physiological	Relative Water Content (RWC)	Reflects plant water status and osmotic adjustment capacity	Kumar et al. (2019); Soni et al. (2021)
Physiological	Stomatal conductance	Assesses gas exchange and stress-induced stomatal regulation	Amirjani (2010); Mannan et al. (2013)
Physiological	Chlorophyll fluorescence (Fv/Fm)	Used to measure PSII efficiency under salt stress	Wu et al. (2014)
Biochemical	Proline content	Important osmoprotectant accumulated under stress	Sadak et al. (2020); Shahid et al. (2018)
Biochemical	Malondialdehyde (MDA) content	Lipid peroxidation marker; indicates oxidative damage	Soni et al. (2021); Razmi et al. (2017)
Biochemical	Antioxidant enzyme activity (SOD, CAT, POD)	Key defense against ROS accumulation under salinity	Noreen et al. (2021)
Molecular	Expression of salt-responsive genes (e.g., <i>GmSALT3</i> , <i>CaNHX1</i>)	Indicates genetic regulation and activation of defense mechanisms	Do et al. (2016); Liu et al. (2020)

2.5.2 Experimental simulation of salinity and water deficit using NaCl and PEG

In plant stress physiology research, polyethylene glycol (PEG) and sodium chloride (NaCl) are commonly used to induce osmotic and salinity stress, respectively, in controlled experimental settings. These compounds enable researchers to replicate important aspects of soil salinity and water deficit stress in hydroponic or soilless systems, providing valuable insights into plant responses at various levels, including morphology, physiology, and biochemistry.

NaCl treatment mimics saline conditions by introducing ionic stress through excess sodium (Na^+) and chloride (Cl^-) ions, leading to ion toxicity, osmotic imbalance, and nutrient deficiency. Studies on chickpea and soybean often utilize NaCl concentrations ranging from 25 to 100 mM to simulate mild to moderate salinity stress. For instance, Kumar et al. (2019) applied NaCl concentrations of 25, 50, 75, and 100 mM in hydroponic systems to investigate salinity tolerance in chickpea genotypes, observing gradual reductions in growth and physiological parameters with increasing salt levels. Similarly, Mannan et al. (2013) employed NaCl at 50 and 100 mM to assess soybean genotypes, linking ion accumulation and photosynthetic efficiency to tolerance. These concentrations are commonly used as NaCl levels above 100 mM can cause severe toxicity not reflective of typical agricultural saline environments, while levels below 25 mM may only elicit minimal stress responses (Shin et al., 2020).

In contrast, PEG is a high molecular weight polymer that creates osmotic stress by reducing water availability without causing ionic toxicity, isolating the effects of water deficit from ion-specific stress. PEG solutions are typically measured in terms of osmotic potential (MPa). Previous studies on chickpea and soybean have utilized PEG at osmotic potentials of -0.2, -0.4, -0.6, and -0.8 MPa to mimic varying levels of drought-like stress (Soni et al., 2021; Amirjani, 2010). For example, Soni et al. (2021) subjected chickpea seedlings to PEG-induced osmotic stress ranging from -0.2 to -0.8 MPa in hydroponic culture, observing reductions in relative water content and biomass corresponding to the concentration of PEG. Similarly, Amirjani (2010) imposed PEG stress at -0.3 and -0.6 MPa in soybean to investigate stomatal conductance and antioxidant enzyme activities. These osmotic potentials represent moderate to severe water deficit conditions similar to those experienced in actual drought situations (Munns and Tester, 2008).

By utilizing NaCl and PEG treatments at these specific concentrations supported by existing literature, researchers can effectively distinguish between the ionic and osmotic aspects of salinity stress, leading to a more accurate understanding of tolerance

mechanisms in soybean and chickpea. This method also ensures applicability to real-world conditions, guiding breeding efforts towards practical stress adaptation strategies.

2.6 Screening techniques under controlled conditions

Screening for salinity and water deficit tolerance in crops like soybean and chickpea is crucial for identifying and developing stress-resistant varieties. Evaluating these traits under controlled conditions enables researchers to control environmental factors accurately, ensuring reliable and reproducible outcomes. Hydroponic systems have become popular for early-stage screening of tolerance due to their effectiveness. These systems offer a valuable tool for studying plant responses to salinity and water deficit without the interference of variable soil conditions.

2.6.1 Hydroponic techniques for early-stage evaluation of salinity tolerance in plants

Screening for salinity tolerance is essential for pinpointing genotypes capable of maintaining productivity under salt stress. Conducting evaluations at the early growth stages enables the detection of key physiological and biochemical responses, thereby facilitating efficient selection for breeding programs before plants reach maturity. Among various screening methods, hydroponic systems have gained significant attention due to their ability to provide precise control over environmental conditions and nutrient supply. This controlled setting ensures uniform exposure to salinity stress, minimizing the variability often observed in soil-based experiments.

Hydroponic techniques offer numerous advantages for the early-stage screening of salinity tolerance. Firstly, they allow for precise control over the concentration and composition of salts in the nutrient solution, ensuring consistent stress application across all tested genotypes (Loudari et al., 2020; Kumar et al., 2019). This precision enables comparative studies between tolerant and sensitive lines under uniform conditions. Secondly, the absence of soil eliminates potential confounding variables such as uneven salt distribution and microbial interactions, which can mask the direct impacts of salinity on plants (Soni et al., 2021). Thirdly, hydroponics enables continuous monitoring of plant responses, including root growth dynamics, ion uptake, and physiological parameters, which are challenging to assess in soil (Amirjani, 2010). In summary, hydroponic techniques offer a dependable, effective, and scalable approach for early-stage screening, aiding in the identification of salt-tolerant soybean, chickpea, and other crop genotypes.

2.6.2 Comparison of hydroponic and soil-based screening methods for evaluating salinity and water deficit tolerance in legumes

In legume stress physiology, hydroponic screening is a key complement to field trials due to its high level of experimental control. Researchers can precisely regulate salt levels and water potential to impose uniform salinity or osmotic stress (Kumar et al., 2019; Mannan et al., 2013). This capability supports high-throughput early screening and the discovery of tolerance-related traits. Perhaps most importantly, hydroponics provides an unparalleled view of the root zone, enabling direct study of root behavior and ion transport essential for understanding stress adaptation (Shavrukov et al., 2012).

On the other hand, soil-based or field screening mimics the intricate, multifaceted conditions of natural growth environments, where plants are subjected to a combination of stresses such as varying salinity levels, fluctuating water availability, soil diversity, and interactions with other organisms (Farooq et al., 2012). While this realism offers valuable insights into how crops perform in actual environmental settings, it can result in significant variability that obscures the specific impacts of salinity or drought stress on plant growth. Field trials also require substantial resources, time, and manpower, often necessitating evaluations across multiple locations and seasons to accommodate environmental fluctuations (Soni et al., 2021).

Furthermore, the uneven drying patterns and unpredictable water availability in soil under water deficit stress make it challenging to assess plant responses. The spatial and temporal variations in salinity effects in soil, caused by salt leaching and accumulation, add to the complexity of the situation. These factors can hinder the screening process and make it harder to interpret the performance of different genotypes. A combination of hydroponic screening and soil-based trials offers an effective approach for a thorough evaluation of salinity and water deficit tolerance. Hydroponics provides a fast and efficient way to identify potential genotypes and understand the mechanisms of tolerance under controlled stress conditions. Subsequent validation in soil or field settings ensures that the selected genotypes consistently exhibit tolerance under realistic agricultural conditions, thereby expediting breeding programs for stress-resistant legumes like chickpea and soybean.

2.6.3 Empirical studies of hydroponics in legume stress physiology research

Hydroponic systems are a standard methodology in plant science for examining the response of crops, including key legumes such as soybean and chickpea, to abiotic stresses like salinity and drought. This approach provides precise control over the root environment, including nutrient availability, salt concentration, and osmotic potential, thereby ensuring experimental consistency and reproducibility. Many studies have used hydroponics to study how different plant varieties respond to abiotic stress, looking at factors like genetic differences, physiological changes, and biochemical adaptations. For example, Mannan et al. (2013) used hydroponic setups to test different soybean genotypes under varying levels of NaCl-induced salinity, ranging from 25 to 100 mM. They found significant variations in traits such as ion accumulation, membrane stability, and proline content among the genotypes. Similarly, in a study by Amirjani (2010), sensitive soybean lines showed decreases in photosynthetic pigments and increased electrolyte leakage when exposed to similar hydroponic NaCl treatments.

In chickpea, Kumar et al. (2019) and Soni et al. (2021) studied salinity responses under controlled environmental conditions and found that tolerant genotypes maintained higher relative water content and lower Na⁺ accumulation. Their findings identified physiological markers such as stomatal conductance and chlorophyll fluorescence as effective indicators for screening salt tolerance at early growth stages.

Hydroponic setups have also been employed in drought simulation using polyethylene glycol (PEG) to induce osmotic stress. For instance, Kalefetoğlu Macar et al. (2009) investigated chickpea responses to PEG- and NaCl-induced stress at the seedling stage. They found significant reductions in growth and relative water content, with tolerant genotypes maintaining better water status and biomass, highlighting their potential for stress-tolerant breeding. Similar experimental approaches were taken by Tripathi et al. (2015), confirming the usefulness of hydroponics for studying drought-related physiological traits in legumes.

These studies emphasize the importance of hydroponics in not only minimizing environmental variability but also facilitating early-stage, high-throughput screening of stress tolerance traits in various genotypes. The reliability and precision provided by these systems have made them essential in functional genomics and breeding research focused on enhancing resilience to abiotic stress.

2.7 Research gaps and rationale for the present study

Despite extensive research on salinity and drought stress in crops, most studies have focused on major cereals, neglecting legumes such as chickpea and soybean, particularly in terms of early-stage physiological screening. While different genotypes of these legumes have shown varying levels of tolerance under stress, there is a lack of controlled hydroponic studies that assess multiple physiological and morphological traits across diverse genotypes. Although chickpea genotypes have displayed variability in response to NaCl and PEG-induced stress, these findings are often limited to field conditions or specific trait evaluations. Research in this area from Hungary and Eastern Europe is particularly lacking, making it challenging to identify locally adapted, stress-tolerant chickpea varieties. Additionally, there is a scarcity of comparative hydroponic studies on chickpea and soybean genotypes under standardized salinity and water-deficit conditions, which hinders the development of broadly applicable screening protocols.

In addition, despite significant progress in understanding plant responses to salinity and drought stresses, there are still notable gaps, particularly in the early-stage screening of legumes such as chickpea and soybean using hydroponic systems. Much of the current research either focuses on field-based evaluations, which can be influenced by environmental factors, or on molecular analyses without accompanying physiological or biochemical validation. Furthermore, there is a lack of studies that simultaneously assess multiple traits—morphological, physiological, and biochemical, for a comprehensive screening approach. Research on genotypic variability under standardized PEG and NaCl concentrations, particularly using controlled hydroponic setups, is limited and fragmented. It is also important to establish correlations between observable phenotypic traits and biochemical stress markers to develop effective early-stage screening protocols. Therefore, the current study aims to address these crucial gaps by:

- Assessing genotypic variability, systematically evaluating a diverse range of chickpea (ELMO and ORION) and soybean (YAKARTA and POCA) genotypes under controlled hydroponic conditions.
- Simulating salinity and water deficit stresses via applying specific concentrations of NaCl (25, 50, 75, and 100 mM) and PEG (-0.2, -0.4 and -0.6 MPa) to replicate salt and water deficit stress.
- Utilizing multi-trait analysis – employing a comprehensive screening method that includes morphological, physiological, and biochemical traits to capture various plant responses.

- Addressing regional research gaps, contributing to the limited research on early-stage salinity and water deficit screening of legumes in Hungary and similar agro-ecological. This research establishes a basis for effective early-stage screening of stress tolerance and improves the comprehension of genetic responses to controlled abiotic stress, thereby aiding breeding programs for stress-resistant legumes.

3. MATERIAL AND METHODS

3.1 Study location and growth conditions

This study was conducted between 2022 and 2024 at the Institute of Crop Sciences, Department of Applied Plant Biology, University of Debrecen, Hungary. All experimental procedures were performed in a controlled-climate growth chamber utilizing a hydroponic system. The chamber environment was maintained at 24/18°C (day/night), with 45% relative humidity, a 10/14-hour light/dark cycle, and a photosynthetic photon flux density (PPFD) of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Plants were cultivated in a static hydroponic setup, with four seedlings supported per 1.7 L plastic pot (Figure 4).



Figure 4. Soybean and chickpea genotypes were grown under hydroponic conditions in a controlled climatic chamber at the University of Debrecen.

3.1.1 Nutrient solution preparation

A modified Hoagland solution, as detailed by Marschner et al. (1990), was used to supply nutrients. The composition was as follows: 2.0 mM $\text{Ca}(\text{NO}_3)_2$, 0.7 mM K_2SO_4 , 0.5 mM

MgSO₄, 0.1 mM KH₂PO₄, 0.1 mM KCl, 10 µM H₃BO₃, 0.5 µM MnSO₄, 0.5 µM ZnSO₄, 0.2 µM CuSO₄, and 10⁻⁴ M Fe-EDTA. The nutrient solution's pH was maintained between 5.8 and 6.2, with adjustments and a complete solution replacement performed every three days to ensure consistent nutrient availability and freshness.

3.2 Evaluating Morpho-Physiological and Biochemical Responses of Selected Chickpea and Soybean Genotypes to NaCl-Induced Salinity Stress in a Hydroponic System

Objective

This study aims to evaluate the response of selected chickpea (*Cicer arietinum* L.) and soybean (*Glycine max* L. Merrill) genotypes to NaCl-induced salinity in a controlled hydroponic environment. Through the analysis of key morphological, physiological, and biochemical traits, the research seeks to identify salt-tolerant genotypes and establish the most effective diagnostic indicators for use in future screening and breeding programs.

Plant Material

Four genotypes were used in this study, two from each species:

- Chickpea: ELMO and ORION
- Soybean: YAKARTA and POCA

Chickpea (*Cicer arietinum* L.) seeds were provided by the Department of Applied Plant Biology at the University of Debrecen, Hungary, while soybean (*Glycine max* L. Merr.) seeds were obtained from the National Soybean Germplasm Bank of the Hungarian Research Institute of Plant Breeding and Crop Production (HIPP). The genotypes were selected based on preliminary observations of contrasting physiological vigor under abiotic stress, with YAKARTA (soybean) and ORION (chickpea) typically displaying stronger vegetative growth, and POCA (soybean) and ELMO (chickpea) showing relatively lower stress resilience. Although detailed published characterizations of these specific lines are limited, the use of physiologically divergent genotypes is a standard and widely validated methodology for investigating salinity tolerance mechanisms (Essa, 2002; Ashraf & Harris, 2004; Phang et al., 2008; Khan et al., 2014). Their intentional selection therefore enables a comparative analysis of distinct stress-response phenotypes. Seedlings were established hydroponically in a controlled environment. NaCl-induced

salinity stress was initiated two weeks post-germination, coinciding with the early vegetative stage (VE–VC in chickpea; V1 in soybean), and maintained until approximately V4–V6 in chickpea and V4–V5 in soybean.

Seed sterilization and germination

To ensure a sterile start, seeds of both crops were surface-sterilized using 6% (v/v) hydrogen peroxide (H₂O₂) for 20 minutes and then thoroughly rinsed with deionized water. Germination proceeded geotropically between moistened filter papers in Petri dishes under controlled conditions (24 ± 2°C). After five days, uniform seedlings at the VE stage were selected for subsequent transplantation into the hydroponic system.

Transplantation and growth

One week after sowing, uniformly germinated seedlings at the early vegetative stage (VE for chickpea and V1 for soybean) were selected based on uniformity and vigor. These seedlings were transplanted into 1.7 L plastic pots under a static hydroponic system, with each pot containing four plants supported by foam plugs to maintain upright positioning. Initially, all genotypes were exposed to five salinity levels (0, 25, 50, 75, and 100 mM NaCl). However, preliminary observations revealed that all chickpea plants could not survive under 100 mM NaCl stress. Therefore, this treatment level was excluded for chickpea due to complete mortality during early exposure. As a result, each chickpea genotype was assigned to four NaCl concentrations (0, 25, 50, and 75 mM), with three replicates per treatment, totaling 24 pots. Soybean genotypes were maintained under all five salinity levels with the same replication scheme, totaling 30 pots. Overall, the experiment consisted of 54 pots and 216 plants. The plants were grown under controlled environmental conditions in a growth chamber. The nutrient solution was replaced every three days, and its pH was regularly monitored and adjusted to remain between 5.8 and 6.2 throughout the experiment. The NaCl concentrations selected for our experiment (25, 50, 75, and 100 mM) were chosen to provide a gradient of increasing salt stress, ranging from mild/moderate osmotic stress to severe ion toxicity. This approach is widely used in hydroponic screening of soybean and chickpea for salt tolerance. For example, Khan et al. (2013) treated 10-day-old soybean seedlings with 0, 25, 50, 75, 100, 125, and 150 mM NaCl and successfully captured a progressive physiological response. Similarly, Ghirardelli et al. (2021) tested preliminary NaCl concentrations from 25 to 150 mM and selected 100 mM as the treatment level where clear stress effects were observed in

soybean. Nguyen et al. (2021) and El Sabagh et al. (2015) also used similar salinity levels (100 mM) in hydroponic and soil-based experiments, demonstrating that these concentrations are effective for evaluating early and severe salt-stress responses. Thus, our chosen gradient is consistent with established protocols and allows assessment of both early physiological changes and threshold responses relevant for salt-tolerance evaluation.

Salinity treatment application

Salinity stress treatments were initiated two weeks after sowing, once the seedlings had reached early vegetative growth stages (VE–VC for chickpea and V1 for soybean). Sodium chloride (NaCl) was used to simulate salinity stress under hydroponic conditions. NaCl was gradually added to the nutrient solution over a 6-hour period to avoid osmotic shock. The following treatment levels were applied:

Chickpea Treatments:

Control (0 mM NaCl)

25 mM NaCl

50 mM NaCl

75 mM NaCl

Soybean Treatments:

Control (0 mM NaCl)

25 mM NaCl

50 mM NaCl

75 mM NaCl

100 mM NaCl

Each genotype (two chickpea and two soybean genotypes) was subjected to all respective salinity treatments in a completely randomized design (CRD) with three biological replicates per treatment. Each replicate consisted of one pot containing four plants, resulting in a total of 54 pots and 216 plants across the entire experiment.

Stress was maintained until approximately V4–V6 in chickpea and V4–V5 in soybean. Electrical conductivity (EC) of the nutrient solution was regularly monitored to ensure stable salt concentrations. The nutrient solution was replaced and pH adjusted (5.8–6.2) every three days to maintain consistent growth conditions.

Experimental Design

A factorial experiment was arranged in a completely randomized design (CRD). The two factors studied were genotype and NaCl treatment level. The design included three replications per treatment combination, where each replication was one hydroponic pot containing four plants.

Chickpea:

2 genotypes $\times$ 4 NaCl levels $\times$ 3 replicates = **24 pots**

Soybean:

2 genotypes $\times$ 5 NaCl levels $\times$ 3 replicates = **30 pots**

In total, the experiment comprised **54 pots** and **216 plants**.

Measurements

On the 30th day after sowing, key physiological parameters including relative chlorophyll content (SPAD values) and stomatal conductance were recorded to assess plant responses to salinity stress. Subsequently, plants were harvested on the 35th day to analyze various morphological, physiological and biochemical traits.

Statistical analysis

All recorded data were statistically analyzed using MSTAT-C software. A factorial analysis of variance (ANOVA) was applied to evaluate the individual and interactive effects of the main factors: genotype and salinity level. Mean separation for significant effects was performed using the Least Significant Difference (LSD) test at the 1% probability level. The coefficient of variation (CV%) was calculated to assess the precision and variability within the experimental data. Furthermore, Pearson's correlation analysis was conducted to determine the relationships among the measured morpho-physiological and biochemical traits.

3.3 Evaluating the response of chickpea and soybean genotypes to PEG-induced water deficit under hydroponic conditions

Objective

1. To identify water deficit-tolerant genotypes by evaluating the morphological, physiological, and biochemical responses of two chickpea (*Cicer arietinum* L.) and two soybean (*Glycine max* L. Merrill) genotypes to PEG-induced osmotic stress under controlled hydroponic conditions.
2. To determine the key morphological, physiological, and biochemical traits that are most strongly associated with water deficit tolerance in the studied legume genotypes.

Plant Material

1. The same genotypes as described in Experiment I were used:
 - Chickpea: ELMO and ORION
 - Soybean: YAKARTA and POCA
2. Details on seed source and selection criteria are provided in Section 3.2.

Seed Sterilization and Germination

Seeds were sterilized, soaked in 0.01 M CaSO₄, and germinated following the same procedure as in Experiment 1.

Transplantation and Growth

One week after sowing, uniformly germinated seedlings at the early vegetative stage (VE for chickpea and V1 for soybean) were selected based on uniformity and vigor. These seedlings were transplanted into 1.7 L plastic pots under a static hydroponic system, with four plants per pot supported by foam plugs to maintain upright positioning. Initially, all genotypes were subjected to four levels of PEG-induced osmotic stress: control (0 MPa), -0.2 MPa, -0.4 MPa, and -0.6 MPa. However, chickpea plants exhibited complete mortality at -0.6 MPa during early exposure. As a result, this treatment was excluded from the chickpea experiment to avoid data inconsistency due to total plant loss.

For chickpea, three levels of PEG-induced water deficit were maintained: 0 MPa (control), -0.2 MPa, and -0.4 MPa, with three replicates per treatment, totaling 18 pots.

Soybean genotypes continued under all four treatments, including -0.6 MPa, with three replicates per treatment, totaling 24 pots. Overall, the experiment consisted of 42 pots and 168 plants. Plants were maintained under controlled environmental conditions in a growth chamber. The nutrient solution was replaced every three days, and its pH was regularly monitored and adjusted to remain between 5.8 and 6.2 throughout the experiment.

PEG-Water Deficit Treatment Implementation

Water deficit treatments were initiated for two weeks post-sowing, coinciding with the early vegetative stages of the seedlings (VE for chickpea and V1 for soybean). To simulate osmotic stress under controlled hydroponic conditions, polyethylene glycol 6000 (PEG 6000; VWR International bvba, Leuven, Belgium) was used. To prevent osmotic shock, PEG was gradually introduced into the nutrient solution over a 6-hour period. The following target osmotic potentials were applied:

Chickpea Treatments: Control (0 MPa), -0.2 MPa PEG, -0.4 MPa PEG.

Soybean Treatments: Control (0 MPa), -0.2 MPa PEG, -0.4 MPa PEG, -0.6 MPa PEG.

Each genotype (two chickpea and two soybean genotypes) was exposed to all respective PEG treatments in a completely randomized design (CRD) with three biological replicates per treatment. Each replicate consisted of one pot containing four plants, resulting in a total of 42 pots and 168 plants in the experiment. Stress was maintained until approximately V4 – V6 in chickpea and V4 – V5 in soybean. The nutrient solution was replaced and its pH adjusted (5.8–6.2) every three days to ensure stable and consistent growth conditions.

Experimental Design

The experiment was arranged in a completely randomized design (CRD) with a factorial treatment structure, incorporating two main factors: genotype and PEG-induced water deficit level. Each combination of genotype and stress level was replicated three times. An individual experimental unit was defined as a single hydroponic pot containing four plants.

- **Chickpea:**

2 genotypes $\times$ 3 PEG levels (-0.2, -0.4, and control) $\times$ 3 replicates = **18 pots**

- **Soybean:**

2 genotypes $\times$ 4 PEG levels (-0.2, -0.4, -0.6, and control) $\times$ 3 replicates = **24 pots**

In total, the experiment comprised **42 pots** and **168 plants**.

Measurements

On day 30 after sowing, key physiological indicators—including relative chlorophyll content (SPAD value) and stomatal conductance—were measured to assess the plants' response to PEG-induced water deficit. Final harvest was conducted on day 35 to collect comprehensive data on morphological, physiological, and biochemical characteristics.

Statistical Analysis

All collected data were analyzed with MSTAT-C software. A two-way factorial analysis of variance (ANOVA) was conducted to determine the individual and interactive effects of the main factors: genotype and water deficit level (PEG). For significant treatment effects, mean comparisons were made using the Least Significant Difference (LSD) test at the 5% probability level. The coefficient of variation (CV%) was computed to assess experimental precision and data variability across replicates. Furthermore, Pearson's correlation analysis was employed to examine the relationships among the measured morpho-physiological and biochemical traits.

3.4 Methodology

3.4.1 Root and shoot length

Root and shoot lengths were measured manually using a standard ruler. Shoot length was determined from the stem base to the apical meristem, while root length was measured from the root-shoot junction to the primary root tip.

3.4.2 Fresh and dry weight

Shoot and root dry weights (g) were determined 35 days after treatment. Three seedlings per treatment were harvested, and the shoot and root parts were separated. Samples were oven-dried at 70 °C (Memmert GmbH, Germany) for four days until a constant weight was achieved. Dry weights were measured using an analytical balance with 0.001 g precision.

3.4.3 Root-to-shoot dry weight ratio

To assess biomass partitioning under stress, the root-to-shoot ratio was determined from dry weights as follows:

$$\mathbf{R:S = Root\ dry\ weight\ (g) / Shoot\ dry\ weight\ (g)}$$

3.4.4 Absolute growth rate (AGR)

was calculated to quantify the increase in plant height during the stress period imposed by PEG-induced water deficit. Measurements were taken on Day 0 (start of stress treatment) and Day 35 (end of treatment) using the formula described by Blackman (1919):

$$\text{AGR (cm day}^{-1}\text{)} = \frac{h_2 - h_1}{t_2 - t_1}$$

Where:

h_1 = plant height at Day 0 of water deficit treatment

h_2 = plant height at Day 35

$(t_2 - t_1)$ = 35 days

3.4.5 Leaf area duration (LAD)

was calculated to assess the persistence of leaf area over the stress period. LAD was determined using the formula proposed by Power et al. (1967), with leaf area index (LAI) measurements taken at Day 0 and Day 35:

$$\text{LAD (days)} = \frac{L_1 + L_2}{2} \times (t_2 - t_1)$$

Where:

L_1 = leaf area index at Day 0

L_2 = leaf area index at Day 35

$(t_2 - t_1)$ = 35 days

3.4.6 Relative growth rate (RGR)

was used to evaluate the rate of biomass accumulation under PEG-induced water deficit. RGR was calculated following Williams (1946) using dry biomass measurements at the start and end of the stress period:

$$\text{RGR (g g}^{-1}\text{ day}^{-1}\text{)} = \frac{(\text{Ln}W_2 - \text{Ln}W_1)}{t_2 - t_1}$$

Where:

W_1 = total plant dry weight at Day 0

W_2 = total plant dry weight at Day 35

$(T_2 - T_1) = 35$ days

3.4.7 Leaf area ratio (LAR)

was calculated to estimate the leaf area per unit of total plant dry weight, indicating the plant's investment in photosynthetic surface relative to biomass. LAR was determined using the formula by Radford (1967):

$$\text{LAR (cm}^2 \cdot \text{g}^{-1}) = A/W$$

Where:

A = total leaf area (cm²) per plant

W = total plant dry weight (g)

3.4.8 Relative chlorophyll content

Relative chlorophyll content (SPAD value) was quantified with a SPAD-502Plus chlorophyll meter (Konica Minolta, Japan). From the most recently fully expanded leaf of each plant, three readings were recorded and averaged to produce a single data point per replicate.

3.4.9 Stomatal Conductance (g_s , mmol H₂O m⁻² s⁻¹)

Stomatal conductance (g_s) was measured 30 days following the initiation of salinity stress. Measurements were taken on the uppermost, fully expanded, healthy leaves using an AP4 porometer (Delta-T Devices, UK), with three plants sampled per treatment. To minimize diurnal variation, all readings were consistently performed between 9:00 and 11:00 AM.

3.4.10 Quantitative measurement of photosynthetic pigments

Chlorophyll-a, chlorophyll-b, and total carotenoid concentrations were determined following the method of Moran and Porath (1980). Leaf discs were incubated in N, N-dimethylformamide for 48 hours in darkness at room temperature to facilitate pigment extraction. The absorbance of the resulting supernatant was then measured using a UV-VIS spectrophotometer (Metertech SP-830 PLUS, Taiwan). Final pigment concentrations, expressed as mg·g⁻¹ fresh weight (FW), were calculated using the equations established by Wellburn (1994).

3.4.11 Measurement of leaf relative water content (RWC%)

At harvest, trifoliate leaf samples were collected to determine relative water content (RWC) according to the method described by Barrs and Weatherley (1962). The procedure consisted of the following steps: 1) measuring fresh weight (FW), 2) submerging the samples in distilled water for 4 hours to achieve full turgor and recording the turgid weight (TW), and 3) oven-drying at 70°C for 24 hours to obtain a constant dry weight (DW). RWC was calculated using the formula:

$$\text{RWC (\%)} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100$$

3.4.12 Measurement of proline content ($\mu\text{mol g}^{-1}$ FW)

Leaf proline content was quantified following the method of Bates et al. (1973). In brief, 0.2 g of fresh leaf tissue was homogenized in 4 mL of 3% sulfosalicylic acid and centrifuged. A 2 mL aliquot of the supernatant was reacted with 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid. The mixture was incubated at 100°C for 1 hour. The reaction was then terminated and the chromophore extracted using 4 mL of toluene. The absorbance of the toluene phase was measured at 520 nm. Proline concentration was determined by comparison to an L-proline standard curve and expressed as $\mu\text{mol g}^{-1}$ FW).

3.4.13 Measurement of membrane integrity (solute leakage, %)

Membrane stability was assessed through the measurement of solute leakage, following the method of Leopold et al. (1981). Fresh leaf samples (0.5 g) were rinsed with deionized water and placed in test tubes containing 10 mL of deionized water. After a 3-hour incubation at 25°C, the initial electrical conductivity (EC_1) of the bathing solution was measured. The samples were then autoclaved at 100°C for 20 minutes to induce complete cell lysis, and the final electrical conductivity (EC_2) was recorded. Solute leakage was calculated as a percentage using the formula:

$$\text{SL (\%)} = (\text{A}_1/\text{A}_2) \times 100$$

A_1 = Absorbance after 3 hours shaking at 25 °C

A_2 = Absorbance after boiling for 20 minutes at 100 °C

The results were expressed as a percentage of total solute content leaked, indicating the degree of membrane injury.

3.4.14 Lipid peroxidation assay (MDA content, $\mu\text{mol g}^{-1}$ FW)

The degree of membrane lipid peroxidation was quantified by determining the malondialdehyde (MDA) content according to the method of Heath and Packer (1968). Fresh leaf tissue (0.2 g) was homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged. An aliquot of the supernatant was reacted with a solution containing 20% TCA and 0.5% thiobarbituric acid (TBA). The mixture was heated at 90°C for 30 minutes and then cooled. MDA concentration was calculated using the formula:

$$\text{MDA (M)} = (A_{532} - A_{600}) / (\epsilon \times b)$$

where A_{532} and A_{600} are the absorbance values at 532 nm and 600 nm, respectively, ϵ is the molar extinction coefficient for the MDA-TBA complex ($1.55 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), and b is the path length of the cuvette (1 cm).

3.4.15 Measurement of Na^+ and K^+ ratio

Sodium (Na^+) and potassium (K^+) concentrations were determined from 1.0 g of oven-dried leaf tissue following acid digestion. The tissue was digested in a mixture of perchloric (HClO_4) and nitric (HNO_3) acids until a clear solution was obtained. The digest was diluted to a final volume of 100 mL with distilled water, and the ion concentrations were quantified using a flame photometer according to the method of Wolf (1982). The potassium to sodium ratio (K^+/Na^+) was subsequently calculated from the measured concentrations.

3.4.16 Measurement of ascorbic acid content (AsA, $\mu\text{g g}^{-1}$)

Ascorbic acid (vitamin C) concentration was determined for all genotypes, treatments, and replicates following the protocol of De Pinto et al. (1999). A 0.5 g sample of frozen, ground tissue was homogenized in 1 mL of 6% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 11,000 rpm for 15 minutes at 4°C. A 100 μL aliquot of the supernatant was mixed with 400 μL of 0.2 mM phosphate buffer (pH 7.4) and incubated at 42°C for 15 minutes. Subsequently, 1.5 mL of freshly prepared color reagent was added to each sample. The reagent was composed of a mixture containing 5 mL of 10% TCA, 4 mL of 42% orthophosphoric acid (H_3PO_4), 4 mL of 4% 2,2'-dipyridyl solution, and 2 mL of ferric chloride (FeCl_3) solution. After vortexing, the mixture was incubated again at 42°C for 40 minutes. Absorbance was measured at 525 nm using a UV-Vis spectrophotometer. Sample ascorbic acid concentrations were quantified by

comparison to a standard curve generated from known concentrations of commercial ascorbic acid.

3.4.17 Measurement of hydrogen peroxide (H_2O_2 , $\mu\text{mol g}^{-1}$ FW)

Hydrogen peroxide (H_2O_2) content was determined using the method of Velikova et al. (2000). Approximately 0.25 g of frozen leaf powder was homogenized in 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at $12,000 \times g$ for 15 minutes at 4°C . A 500 μL aliquot of the supernatant was mixed with 250 μL of 10 mM potassium phosphate buffer (pH 7.0) and 500 μL of 1 M potassium iodide (KI) solution. Following a 30-minute incubation in darkness, the absorbance was measured at 390 nm. H_2O_2 concentration was determined by comparison to a standard curve and expressed as μmol per gram fresh weight ($\mu\text{mol g}^{-1}$ FW).

4. RESULTS AND DISCUSSION

4.1 Morphological responses to NaCl-induced salinity stress

4.1.1 Shoot and root length (cm)

The effects of NaCl-induced salinity stress on shoot and root length were evaluated in two chickpea genotypes (ELMO and ORION) and two soybean genotypes (YAKARTA and POCA) under hydroponic conditions. In both crops, salinity significantly reduced shoot and root lengths ($p \leq 0.01$), with the magnitude of reduction increasing at higher salt concentrations. However, genotype differences and their interaction with salinity varied between species.

In chickpea, no significant difference was observed between genotypes in shoot length ($p > 0.01$), although salinity and genotype $\times$ salinity interactions were significant. Shoot length declined steadily from an average of 32.0 cm under control conditions to 14.5 cm at 75 mM NaCl. Both genotypes responded similarly, with ELMO showing a slightly higher but non-significant shoot length across salinity levels (Table 3). Soybean exhibited significant differences in shoot length between genotypes ($p \leq 0.01$). YAKARTA consistently outperformed POCA, maintaining longer shoots across all salinity treatments. Shoot length decreased from 37.5 cm (control) to 16.6 cm at 100 mM NaCl. Notably, YAKARTA demonstrated better tolerance, especially under severe salinity (100 mM NaCl), with shoots averaging 19.9 cm compared to POCA's 13.3 cm (Table 4).

Root length in chickpea was similarly unaffected by genotype ($p > 0.01$) but significantly influenced by salinity and genotype $\times$ salinity interaction. Root length averaged 46.8 cm under control and 39.2 cm at 25 mM NaCl, declining sharply to 20.7 cm at 75 mM NaCl. Both genotypes exhibited comparable sensitivity (Table 3). In soybean, root length showed no significant genotypic difference ($p > 0.01$), but salinity and interaction effects were significant. Root length decreased from an average of 54.7 cm (control) to 27.1 cm at 100 mM NaCl. While both genotypes had similar root lengths under control and mild stress, YAKARTA's root length declined more at 100 mM NaCl (21.9 cm) compared to POCA (32.3 cm), indicating differential root growth responses at high salinity (Table 4). Overall, both chickpea and soybean showed marked reductions in root and shoot growth with increasing salinity, indicating sensitivity to salt stress. However, soybean genotypes—particularly YAKARTA—generally maintained longer shoot lengths than chickpea genotypes under comparable or higher salinity levels, suggesting better shoot growth tolerance to salinity. At the highest stress levels, average shoot length reductions

were 54.9% in chickpea (75 mM NaCl) and 56% in soybean (100 mM NaCl), with YAKARTA showing a relatively higher absolute shoot length (19.9 cm) than chickpea genotypes (e.g., ELMO at 16.7 cm), reinforcing its superior shoot performance under salt stress. Conversely, chickpea maintained relatively stable root lengths under mild salinity (up to 25 mM NaCl), but suffered more drastic root length reductions at higher salinity, particularly in the ELMO genotype, which showed a 64.3% reduction compared to 47.8% in ORION. In contrast, soybean exhibited a more gradual root length decline, with average reductions of 50.4%, although variability between genotypes was evident—YAKARTA experienced a 58.7% reduction, while POCA was less affected (42.7%). These findings suggest that soybean, especially YAKARTA, exhibits comparatively greater shoot length tolerance under salinity stress, a trait closely linked to above-ground biomass accumulation and yield potential. On the other hand, chickpea's root system appears more sensitive, particularly under high salinity conditions, which may impair water uptake and overall plant resilience. Therefore, under hydroponic conditions, soybean may be considered more tolerant overall, primarily due to its ability to sustain shoot growth across a wider range of salt concentrations.

Table 3. Effect of NaCl-induced salinity stress on shoot and root length (cm) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions. Different lowercase letters (a–f) within a column indicate significant differences ($p \leq 0.01$) between salt levels for the same genotype. Capital letters (A–C) indicate differences among mean values for salinity levels. No significant differences were observed between genotypes.

Salinity level (mM NaCl)	ELMO Shoot	ORION Shoot	Avg. Shoot	ELMO Root	ORION Root	Avg. Root
Control	33.5 ^a	30.5 ^{ab}	32.0 ^A	45.7 ^a	47.9 ^a	46.8 ^A
25	23.7 ^{cd}	25.4 ^{bc}	24.6 ^B	36.7 ^{ab}	41.6 ^{ab}	39.2 ^A
50	19.8 ^{de}	22.7 ^{cd}	21.3 ^B	25.0 ^{cd}	31.3 ^{bc}	28.2 ^B
75	16.7 ^{ef}	12.3 ^f	14.5 ^C	16.3 ^d	25.0 ^{cd}	20.7 ^B
Average	23.4 ^A	22.7 ^A	—	30.92 ^A	36.46 ^A	—
Variable	Genotypes		Salinity Levels		Interaction	
LSD (0.01) – Shoot	4.20		3.65		5.16	
LSD (0.01) – Root	9.86		8.21		11.60	
CV (%) – Shoot	8.96					
CV (%) – Root	13.80					

Table 4. Effect of NaCl-induced salinity stress on shoot length (cm) and root length (cm) of two soybean genotypes (YAKARTA and POCA) under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments.

Salinity level (mM NaCl)	YAKARTA Shoot	POCA Shoot	Avg. Shoot	YAKARTA Root	POCA Root	Avg. Root
Control	41.8 ^a	33.1 ^b	37.5 ^A	53.0 ^a	56.4 ^a	54.7 ^A
25	38.2 ^a	30.3 ^{bc}	34.3 ^B	49.0 ^{ab}	50.7 ^{ab}	49.8 ^A
50	28.4 ^{cd}	27.3 ^{cd}	27.9 ^C	38.0 ^{cd}	33.6 ^d	35.8 ^B
75	25.5 ^d	25.4 ^d	25.5 ^C	35.2 ^{cd}	43.0 ^{bc}	39.1 ^B
100	19.9 ^e	13.3 ^f	16.6 ^D	21.9 ^e	32.3 ^d	27.1 ^C
Average	30.8 ^A	25.9 ^B	—	39.4 ^A	43.2 ^A	—
Variable	Genotypes		Salinity Levels		Interaction	
LSD (0.01) – Shoot	4.20		3.65		5.16	
LSD (0.01) – Root	9.86		8.21		11.60	
CV (%) – Shoot	8.96					
CV (%) – Root	13.80					

4.1.2 Biomass accumulation (fresh and dry weight, g)

Salinity stress significantly reduced shoot fresh weight in chickpea (ELMO and ORION), soybean genotypes (YAKARTA and POCA), with notable effects of salinity and genotype $\times$ salinity interactions ($p \leq 0.01$), though no significant main genotypic differences were found. In chickpea, the highest average shoot fresh weight (6.02 g) was recorded under control conditions, decreasing sharply with increasing salinity. At 25 mM NaCl, shoot fresh weight declined by 53.2% to 2.82 g, and further dropped to 1.01 g (83.2% reduction) at 50 mM and 0.55 g (90.9% reduction) at 75 mM, with no significant difference between these two levels. ELMO had significantly higher fresh weight under control (7.48 g) than ORION (4.56 g), but both genotypes exhibited similar reductions under salt stress. In soybean, the maximum shoot fresh weight (10.5 g average) was recorded under control conditions, which progressively declined with increasing NaCl levels: 7.47 g (28.9% reduction) at 25 mM, 4.93 g (53.0%) at 50 mM, 3.87 g (63.1%) at 75 mM, and 2.15 g (79.5%) at 100 mM. YAKARTA maintained higher shoot fresh weight at 25 mM (7.0 g), while POCA performed better at 50 and 75 mM. At 100 mM, POCA exhibited the lowest shoot fresh weight (0.89 g) versus 3.41 g for YAKARTA, highlighting differential salt tolerance (Table 5).

Table 5. Effect of NaCl-induced salinity stress on shoot fresh weight (g) of two chickpea (ELMO and ORION) and soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	ELMO	ORION	Avg. Chickpea	YAKARTA	POCA	Avg. Soybean
Control	7.48 ^a	4.56 ^b	6.02 ^A	10.8 ^a	10.3 ^{ab}	10.5 ^A
25	2.66 ^{bcd}	2.98 ^{bc}	2.82 ^B	7.0 ^{bcd}	7.94 ^{abc}	7.47 ^B
50	0.89 ^{cde}	1.13 ^{cde}	1.01 ^C	3.47 ^{def}	6.38 ^{cde}	4.93 ^{BC}
75	0.46 ^e	0.64 ^{de}	0.55 ^C	3.13 ^{ef}	4.61 ^{cde}	3.87 ^{CD}
100	-	-	-	3.41 ^{def}	0.89 ^f	2.15 ^D
Average	2.88 ^A	2.33 ^A	-	5.65 ^A	6.02 ^A	-
Variable	Genotypes LSD (0.01)		Salinity LSD (0.01)	Interaction LSD (0.01)		CV (%)
Chickpea	2.07		1.55	2.19		23.8
Soybean	1.80		2.55	3.60		26.1

Salinity stress significantly reduced shoot dry weights in chickpea (ELMO and ORION) and soybean (YAKARTA and POCA) genotypes, with marked effects of salinity levels and their interaction with genotype ($p \leq 0.01$), while genotypic differences alone were not significant ($p > 0.01$). In chickpea, shoot dry weight followed a similar declining trend, with the highest value (0.92 g) under control conditions, significantly dropping to 0.54 g at 25 mM and 0.34 g at 50 mM, and reaching the lowest value of 0.24 g at 75 mM NaCl. ELMO again showed a higher dry weight (1.14 g) than ORION (0.69 g) under control conditions, but both genotypes had comparably low weights under high salinity. Soybean shoot dry weight was highest under control (1.50 g), declining significantly to 1.08 g at 25 mM, and further to 0.69 g and 0.50 g at 50 and 75 mM, respectively, with the lowest value at 0.18 g under 100 mM NaCl. Both soybean genotypes showed a significant decline in shoot dry weight under increasing salinity. At 75 mM NaCl, shoot dry weight decreased to 0.52 g in POCA and 0.48 g in YAKARTA. The reduction was most severe at 100 mM NaCl, with values falling to 0.17 g for POCA and 0.18 g for YAKARTA (Table 6).

Table 6. Effect of NaCl-induced salinity stress on shoot dry weight (g) of two chickpea (ELMO and ORION) and soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	ELMO	ORION	Avg. Chickpea	YAKARTA	POCA	Avg. Soybean
Control	1.14 ^a	0.69 ^b	0.92 ^A	1.64 ^a	1.36 ^{ab}	1.50 ^A
25	0.48 ^{bcd}	0.60 ^{bc}	0.54 ^B	1.11 ^{bc}	1.04 ^c	1.08 ^B
50	0.36 ^{bcd}	0.33 ^{bcd}	0.34 ^{BC}	0.56 ^{de}	0.82 ^{cd}	0.69 ^C
75	0.27 ^{cd}	0.21 ^d	0.24 ^C	0.48 ^{ef}	0.52 ^{de}	0.50 ^C
100	—	—	—	0.17 ^f	0.18 ^f	0.18 ^D
Average	0.5633 ^A	0.4567 ^A	—	0.79 ^A	0.78 ^A	—
Variable	Genotypes LSD (0.01)		Salinity LSD (0.01)	Interaction LSD (0.01)		CV (%)
Chickpea	0.3664		0.2556	0.3614		18.37
Soybean	0.2711		0.2262	0.3200		16.83

Salinity stress markedly reduced shoot fresh and dry weights in both chickpea and soybean, though the severity differed between species. Chickpea exhibited stronger biomass losses, particularly beyond moderate salinity, indicating high sensitivity. Soybean maintained comparatively better shoot growth, with YAKARTA consistently outperforming POCA and both chickpea genotypes under stress. While ELMO produced relatively high biomass under control conditions, it could not sustain growth under salinity. Overall, soybean demonstrated greater resilience in shoot biomass retention, with genotype-specific differences highlighting potential for breeding salt-tolerant cultivars. In contrast, chickpea's pronounced sensitivity underscores the need for targeted management and genetic improvement for saline environments.

4.1.3 Root-to-shoot dry weight ratio (%)

In both chickpea and soybean genotypes, statistical analysis showed no significant differences in the average root-to-shoot dry weight ratio between the genotypes within each species. However, salinity level had a highly significant effect ($p \leq 0.01$) in both crops, as did the interaction between genotype and salinity level.

In chickpea, the highest average root-to-shoot ratios were observed under control (42.11%) and 25 mM NaCl (40.20%) treatments, with no significant difference between them. The lowest ratios were recorded under 50 and 75 mM NaCl (21.99% and 22.51%, respectively), also statistically similar. Regarding genotype $\times$ salinity interaction, ORION showed significantly higher values under control and 25 mM NaCl (47.98% and 43.30%),

while ELMO also exhibited elevated ratios under these conditions (36.25% and 37.10%). All other salinity levels resulted in significantly lower values, with no differences among them (Fig. 5). In contrast, soybean genotypes exhibited a different pattern. Root-to-shoot ratios were greatest under elevated salinity treatments (50, 75, and 100 mM NaCl), with no statistically significant variation among these stress levels. Lower mean ratios, which also did not differ significantly, were recorded for the control and 25 mM NaCl conditions (Fig. 6). Genotypic differences were evident under severe stress. YAKARTA exhibited significantly higher ratios at 100 mM (38.42%) and 75 mM (32.77%) NaCl. In contrast, the lowest ratios were observed in YAKARTA at 25 mM NaCl (26.84%) and in POCA at 100 mM (27.82%) and 50 mM (30.69%) NaCl.

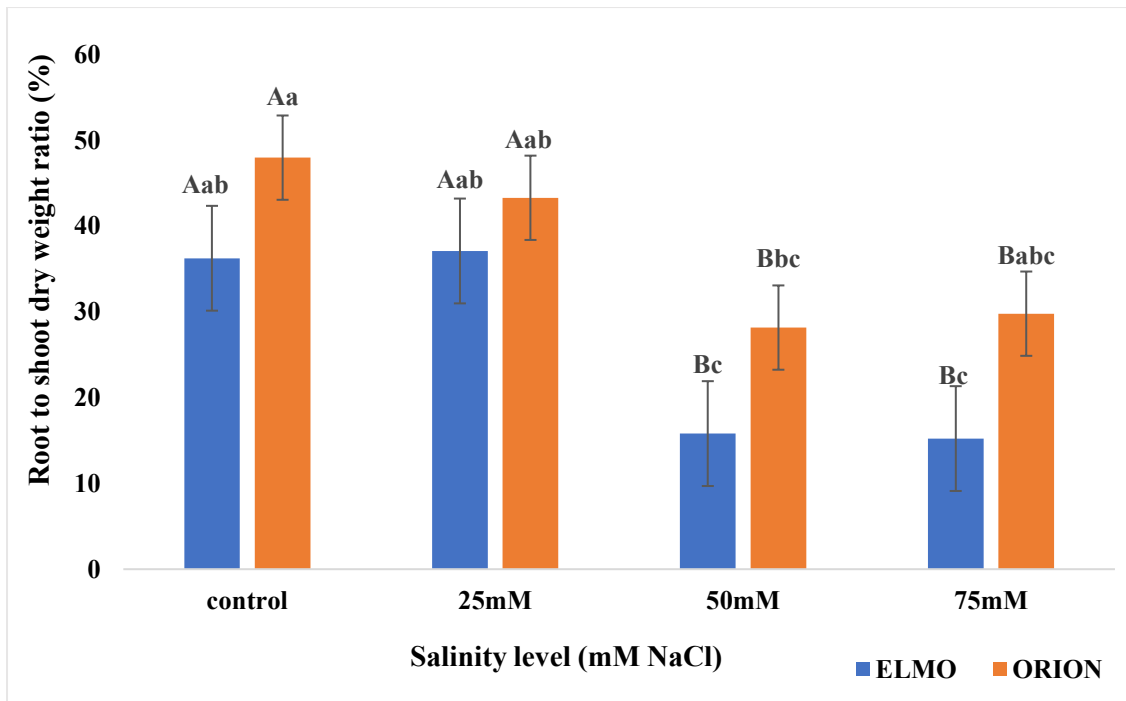


Fig. 5. Effect of NaCl-induced salinity stress on root-to-shoot dry weight ratio (%) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.01$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.01$).

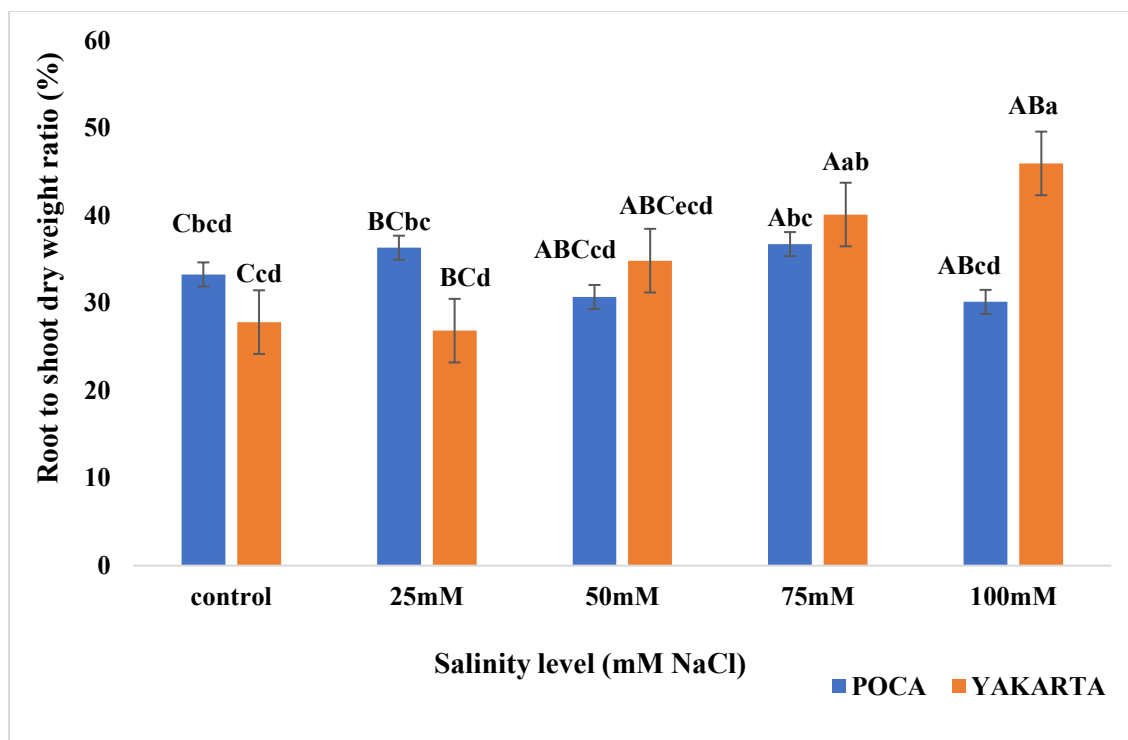


Fig. 6. Effect of NaCl-induced salinity stress on root-to-shoot dry weight ratio (%) of two soybean genotypes (POCA and YAKARTA) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.01$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.01$).

In chickpea, the highest root-to-shoot dry weight ratios were observed under non-saline and mildly saline conditions (control and 25 mM NaCl), suggesting that these genotypes favor root development when stress is minimal. This indicates a tendency to maintain balanced shoot and root biomass allocation under favorable conditions. In contrast, soybean showed the highest root-to-shoot ratios under moderate to high salinity levels (50–100 mM NaCl). This pattern may reflect an adaptive response in which the plant allocates more biomass to root development under stress, potentially to enhance water and nutrient uptake during saline conditions. Although both species exhibited significant genotype $\times$ salinity level interactions, their responses followed different trends. Chickpea genotypes performed better in terms of root-to-shoot allocation at lower salinity levels, whereas soybean genotypes responded more favorably under higher salinity. These findings suggest a species-specific adaptive strategy to salinity stress: chickpea emphasizes root development under non-stressful environments, while soybean increases root allocation in response to salinity. Such contrasting responses highlight the importance of understanding crop-specific mechanisms for developing salt-tolerant cultivars.

Salinity stress significantly disrupts plant water relations by decreasing external water potential, leading to cellular dehydration and shrinkage. As water content declines, turgor pressure (ψ_p) in plant cells diminishes, reducing cell wall extensibility and impairing cell division and expansion processes, particularly critical in meristematic regions such as root and shoot apices (Farooq et al., 2009; Farooq et al., 2012). When salinity stress is imposed during early developmental stages, it limits leaf expansion and reduces total photosynthetic area, ultimately resulting in stunted shoot and root growth.

In the present study, NaCl-induced salinity caused a marked reduction in shoot and root growth parameters, such as length, fresh and dry biomass—in both chickpea and soybean genotypes. These adverse effects intensified with increasing salinity levels, indicating that both osmotic and ionic components of salt stress contributed to growth inhibition. The initial osmotic phase reduced water availability by lowering the overall water potential (ψ), thereby restricting root water uptake and triggering downstream physiological stress responses (Munns and Tester, 2008; Farooq et al., 2009).

Our results align with previous studies on salinity stress in legumes, where diminished shoot and root elongation and reduced biomass accumulation are closely linked to decreased water uptake and suppressed cellular expansion (Munns and Tester, 2008). As salinity limits the ability of roots to meet transpirational demand, a drop-in leaf turgor pressure (ψ_p) ensues, inhibiting cell enlargement and thus constraining overall plant growth (Cosgrove, 1989).

This physiological impairment was evident in our findings, where both fresh and dry weights of shoot and root tissues declined significantly across increasing NaCl concentrations. These effects are further exacerbated by reduced stomatal conductance (g_s) under salinity, leading to increased stomatal resistance (r_s) and restricted CO_2 diffusion into chloroplasts. Consequently, the intercellular CO_2 concentration (C_i) declines, lowering photosynthetic carbon assimilation and ultimately limiting the production and allocation of photo-assimilates to growing tissues (Xiancan et al., 2018; Carminati and Javaux, 2020). Evidence from soybean and other glycophytes, including the studies by Essa (2002), Phang et al. (2008), Hafeez et al. (2023), and Amirjani (2010), is cited to substantiate the physiological basis of biomass decline, ensuring the statement is fully supported by literature.

The reduction in leaf area observed in both species under salinity can also be attributed to the inhibition of cell expansion due to osmotic stress and ion toxicity, particularly from

Na^+ and Cl^- accumulation in metabolically active tissues. This decrease in photosynthetically active surface area compromises overall carbon assimilation and limits assimilate translocation to root systems. Our observations are consistent with previous reports indicating that salinity suppresses both cell division and elongation, leading to reduced plant size and biomass (Cisse et al., 2019). Notably, differences in root-to-shoot ratio (R:S) responses between species suggest divergent adaptive strategies. Soybean exhibited an increased R:S ratio at 50 and 100 mM NaCl, implying greater tolerance of root systems relative to shoots, likely supporting continued water uptake under stress. In contrast, chickpea showed a decreased R:S ratio at 50 and 75 mM NaCl, indicating that root growth was equally or more suppressed than shoot growth, limiting its ability to compensate for shoot water loss. These contrasting patterns further emphasize the superior physiological resilience of soybean compared to chickpea. These findings corroborate with previous studies suggesting that root plasticity, efficient water transport, and osmotic adjustment contribute to salinity tolerance in soybean (Otie et al., 2021). Conversely, chickpea has been characterized as salt-sensitive, displaying early reductions in root growth and impaired water uptake under even moderate salt concentrations (Demir et al., 2003). The mortality observed in chickpea at 100 mM NaCl further reflects its limited capacity for ionic homeostasis and water balance, likely due to inefficient exclusion or compartmentalization of toxic ions such as Na^+ and Cl^- (Toker et al., 2021).

The genotypic variation observed among chickpea and soybean accessions in this study may be attributed to differences in maintaining higher turgor pressure, cell wall extensibility (Φ), and stomatal regulation. Genotypes such as YAKARTA (soybean) and ORION (chickpea) exhibited improved water status and biomass retention under saline conditions, suggesting their superior adaptive capacity. These physiological traits, particularly turgor maintenance and stomatal conductance regulation, are well-documented as central mechanisms underlying salt stress tolerance (Xiancan et al., 2018; Carminati and Javaux, 2020). Overall, salinity stress led to significant morphological and physiological impairments in both chickpea and soybean. However, soybean genotypes demonstrated greater resilience, as evidenced by higher R:S ratios and better root biomass retention under moderate and high salinity levels. In contrast, chickpea exhibited greater sensitivity, especially at 75 and 100 mM NaCl, where growth was severely restricted, and mortality occurred. These findings underscore the importance of genotype selection for

saline environments and suggest that soybean possesses a more robust physiological capacity to withstand salinity compared to chickpea.

4.2 Physiological responses to NaCl-induced salinity stress

4.2.1 Relative chlorophyll content (SPAD value)

The relative chlorophyll content (SPAD value) showed no statistically significant differences between chickpea or soybean genotypes when averaged across salinity levels. However, salinity treatments ($p \leq 0.01$) and genotype $\times$ salinity interactions were significant in both species (Tables 7 and 8), indicating that while baseline chlorophyll levels were similar on average, genotypes responded differently to increasing salt concentrations. In chickpea, average SPAD values declined progressively from 39.72 under control conditions to 29.64, 21.57, and 12.67 at 25, 50, and 75 mM NaCl, respectively. Although Elmo exhibited the highest SPAD value under control (46.03) compared to Orion (33.42), both genotypes experienced sharp reductions at higher salt levels, with SPAD values falling below 16. The average chlorophyll contents of Elmo (25.42) and Orion (26.38) were statistically comparable, indicating similar overall sensitivity despite variation in individual responses (Table 7).

Table 7. Effect of NaCl-induced salinity stress on Relative chlorophyll content (SPAD) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	ELMO	ORION	Average
control	46.03 ^a	33.42 ^b	39.72 ^A
25	29.42 ^b	29.86 ^b	29.64 ^B
50	15.77 ^c	27.37 ^b	21.57 ^C
75	10.47 ^c	14.87 ^c	12.67 ^D
Average	25.42 ^A	26.38 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	7.928	6.607	9.343
CV (%)	14.47		

In soybean, a similar downward trend in SPAD values was observed with increasing salinity. The highest average SPAD (32.53) was recorded under control conditions, gradually declining to 30.37, 29.35, and 28.12 at 25, 50, and 75 mM NaCl, respectively, and reaching 16.48 at 100 mM. YAKARTA consistently maintained higher SPAD values than POCA, particularly under control (38.80 vs. 26.57). However, under severe salinity

(100 mM), both genotypes showed comparable SPAD values (14.67 and 18.30), reflecting similar levels of chlorophyll degradation under high salt stress (Table 8).

Table 8. Effect of NaCl-induced salinity stress on Relative chlorophyll content (SPAD) of two soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	YAKARTA	POCA	Average
control	38.80 ^a	26.57 ^d	32.53 ^A
25	32.87 ^b	27.87 ^{cd}	30.37 ^{AB}
50	32.13 ^{bc}	26.27 ^d	29.35 ^B
75	32.70 ^b	23.53 ^d	28.12 ^B
100	14.67 ^e	18.30 ^e	16.48 ^C
Average	30.23 ^A	24.51 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	6.357	3.087	4.366
CV (%)	6.69		

Comparatively, soybean genotypes exhibited greater average SPAD stability under moderate salinity (25–75 mM) than chickpea, suggesting better pigment retention and potential tolerance. Chickpea genotypes showed a steeper and more immediate decline, with significant chlorophyll loss already evident at 50 mM NaCl. This points to greater sensitivity of chickpea photosynthetic machinery to osmotic and ionic stress, possibly linked to less efficient antioxidant and nitrogen metabolism pathways. These results align with earlier findings in both crops, indicating that salinity impairs chlorophyll biosynthesis and promotes degradation through oxidative stress and metabolic disruption (Nakayama et al., 2022; Khan et al., 2022). The marked decline in SPAD values under high salinity reflects a reduction in photosynthetic potential, contributing to overall growth inhibition under salt stress conditions.

4.2.2 Stomatal conductance under NaCl-induced salinity stress

The analysis of stomatal conductance (g_s) in chickpea and soybean genotypes revealed significant effects of genotype, salinity level, and their interaction ($p \leq 0.01$) in both species (Fig. 7 and 8).

In chickpea, the ORION genotype exhibited a higher average g_s (335.40 mmol H₂O m⁻² s⁻¹) compared to ELMO (299.10 mmol H₂O m⁻² s⁻¹). Stomatal conductance decreased progressively with increasing NaCl concentration, from a high of 516.30 mmol H₂O m⁻² s⁻¹ under control to a low of 87.50 mmol H₂O m⁻² s⁻¹ at 75 mM NaCl. Both genotypes

showed similar g_s under control and severe salt stress, with marked stomatal closure at 75 mM. The higher g_s in ORION was associated with significantly greater relative water content (RWC) and proline accumulation—a key osmolyte whose detailed analysis will be presented in the subsequent proline results section—indicating enhanced osmotic adjustment and water retention under salt stress, which helped maintain stomatal function (Fig. 7).

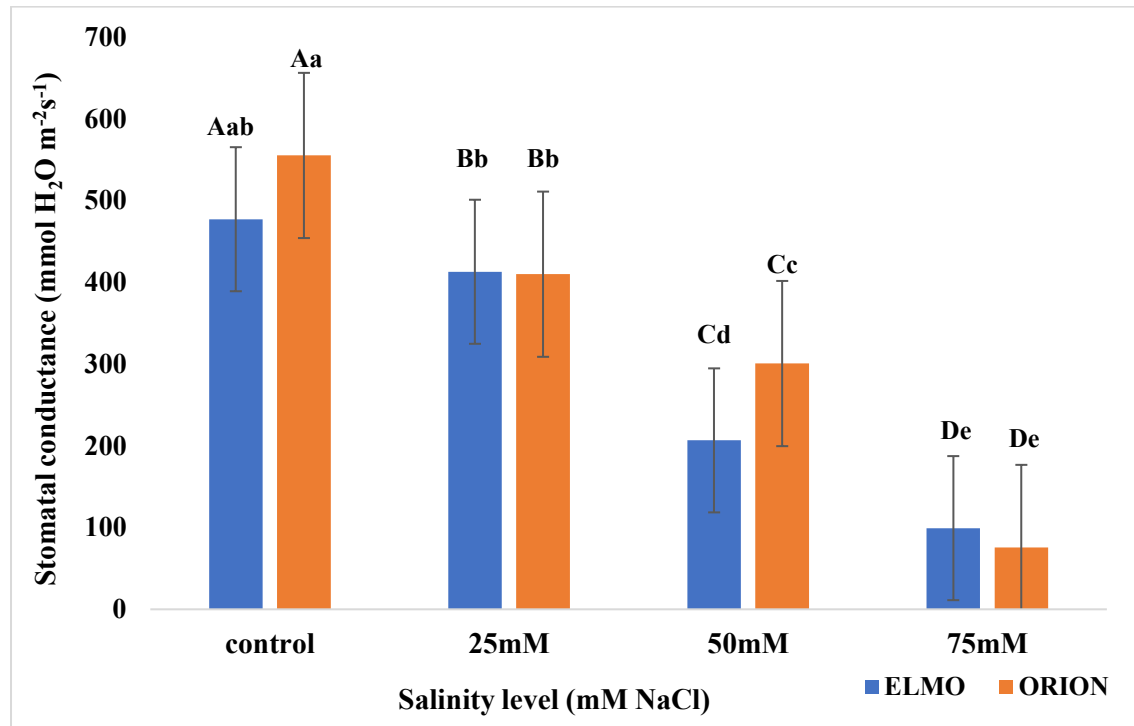


Fig. 7. Effect of NaCl-induced salinity stress on stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.01$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.01$).

In soybean, the genotype YAKARTA maintained a significantly higher average stomatal conductance (g_s) ($341.80 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) compared to POCA ($285.90 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$). Under control (non-saline) conditions, both genotypes exhibited similar g_s values (approximately $540 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$). However, under moderate to severe salinity stress (50–100 mM NaCl), YAKARTA consistently outperformed POCA, with g_s declining from 547.00 to $118.00 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$, compared to a drop from 536.70 to $78.67 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ in POCA. This pattern suggests YAKARTA possesses a superior capacity for stomatal regulation, which likely contributes to better maintenance of water-use efficiency and gas exchange under saline conditions (Fig. 8).

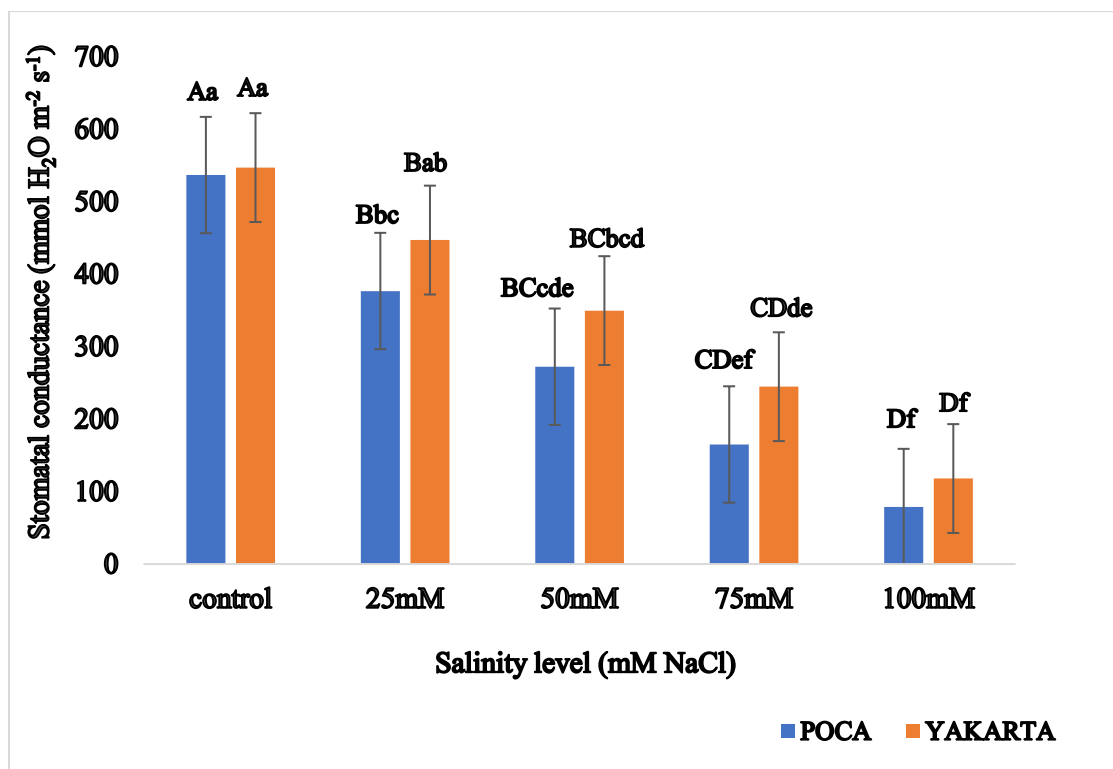


Fig. 8. Effect of NaCl-induced salinity stress on stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two soybean genotypes (YAKARTA and POCA) under hydroponic conditions ($n = 3, \pm \text{s.e.}$). Different capital letters among salinity levels indicate significant differences ($p \leq 0.01$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.01$).

Both chickpea and soybean plants showed a decrease in stomatal conductance (g_s) as salt levels increased. However, chickpea's g_s dropped sharply at a lower salt concentration (75 mM NaCl), indicating greater salt sensitivity earlier in the stress progression. In contrast, soybean maintained higher g_s values even at higher salt levels (up to 100 mM NaCl), demonstrating better tolerance to severe salt stress. In chickpea, the ORION genotype's superior osmotic adjustment, evidenced by higher RWC and proline accumulation (to be detailed later), helped sustain stomatal opening under salt stress. In soybean, the ability to retain stomatal conductance under salinity was genotype-dependent, with YAKARTA showing a genetic advantage over POCA. These findings align with previous studies emphasizing stomatal conductance as a critical physiological trait for salinity tolerance (Brugnoli and Lauteri, 1991; Khan et al., 2022; Liao et al., 2023), underscoring the importance of osmotic adjustment and water relations in mitigating salt stress impacts in legumes.

4.2.3 Leaf relative water content dynamics under NaCl-induced salinity stress

Analysis of leaf relative water content (RWC) revealed significant effects of genotype, salinity level, and their interaction ($p \leq 0.01$) in both chickpea and soybean (Tables 9 and 10). The chickpea genotype ORION maintained a significantly higher average RWC (88.01%) compared to ELMO (84.09%), while in soybean, YAKARTA exhibited superior RWC relative to POCA across all salt treatments.

In chickpea, RWC remained relatively stable under control and mild salinity (0 and 25 mM NaCl), with values of 93.17% and 89.89%, respectively, before declining sharply at 75 mM NaCl to 77.45%. ORION sustained higher hydration under severe stress (81.74% vs. 73.16% in ELMO) (Table 9), suggesting more effective osmotic adjustment mechanisms supporting physiological resilience, including stomatal conductance. These findings are consistent with recent chickpea studies highlighting the importance of osmotic regulation and leaf water status in salinity tolerance (Navyashree et al., 2024; Kaur et al., 2021).

Table 9. Effect of NaCl-induced salinity stress on leaf relative water content (%) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	ELMO	ORION	Mean
control	91.25 ^{ab}	95.10 ^a	93.17 ^A
25	88.45 ^{abc}	91.34 ^{ab}	89.89 ^A
50	83.49 ^{bc}	83.87 ^{bc}	83.68 ^B
75	73.16 ^d	81.74 ^c	77.45 ^C
Mean	84.09 ^B	88.01 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	3.572	5.643	7.980
CV (%)	3.72		

Both soybean genotypes exhibited a decline in relative water content (RWC) with increasing salinity; however, YAKARTA maintained significantly higher RWC than POCA at moderate to high salt concentrations (50–100 mM NaCl). While YAKARTA's RWC decreased from 94.25% (control) to 57.83% (100 mM NaCl), POCA's RWC showed a more severe reduction, falling from 89.83% to 44.35% under the same conditions (Table 10). This indicates that YAKARTA possesses a greater capacity to

maintain cellular hydration under salinity stress, likely due to more effective osmotic adjustment and membrane protection. These findings are consistent with recent studies highlighting significant genotypic variation in water retention and membrane stability among soybean cultivars under saline conditions (Mannan et al., 2013; Ismail & Horie, 2017; Parveen et al., 2016; Khan et al., 2022).

Table 10. Effect of NaCl-induced salinity stress on leaf relative water content (%) of two soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	YAKARTA	POCA	Mean
Control	94.25 ^a	89.83 ^{ab}	92.04 ^A
25	87.90 ^{ab}	80.62 ^{bc}	84.26 ^B
50	75.18 ^c	66.43 ^d	70.80 ^C
75	62.39 ^d	53.25 ^e	57.82 ^D
100	57.83 ^e	44.35 ^f	51.09 ^E
Mean	75.51 ^A	66.97 ^B	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	9.75	4.78	6.77
CV (%)	3.85		

Comparatively, chickpea genotypes exhibited higher baseline RWC and better water retention at lower to moderate salinity levels, while soybean genotypes, especially YAKARTA, showed stronger maintenance of RWC under more severe salt stress. These differences highlight distinct physiological strategies between species and support the role of osmotic adjustment and water relations in legume salt tolerance (Shawquat et al., 2015). Maintaining leaf water content is essential for sustaining photosynthetic activity and overall plant productivity under salinity stress. This genotype-specific RWC variation complements findings on stomatal conductance and will be further linked with proline accumulation in subsequent sections, providing an integrated view of salt tolerance mechanisms.

4.3 Changes of photosynthetic pigments under salinity stress

The influence of salinity stress on chlorophylls and carotenoids in chickpea leaves was evaluated by assessing chlorophyll-a, chlorophyll-b, the chlorophyll a/b ratio, and carotenoid content across two genotypes (ELMO and ORION) under hydroponic conditions. The results revealed distinct responses in chlorophyll pigment concentrations as a function of salinity level and genotype, providing insight into the physiological tolerance mechanisms of the studied cultivars. Salinity stress significantly affected chlorophyll-a, chlorophyll-b, carotenoids, and their interactions with genotype ($p \leq 0.01$). Chlorophyll- a content peaked under control and 25 mM NaCl treatments (15.18 –16.46 mg·g⁻¹ FW), declining sharply at higher salinity, especially in the ELMO genotype. ORION maintained higher chlorophyll-a level across all salt treatments, indicating greater tolerance. Chlorophyll-b content showed a similar trend, highest at control and 25 mM NaCl (6.24–6.74 mg·g⁻¹ FW) and reduced significantly at 50 and 75 mM NaCl. Although genotypic differences were not significant overall, ORION retained more chlorophyll-b under moderate stress, whereas ELMO suffered greater losses at elevated salinity. The chlorophyll a/b ratio remained stable across genotypes and salinity levels (2.15 – 2.56), suggesting proportional regulation of pigment biosynthesis or degradation under stress (Table 11).

Table 11. Effect of NaCl-induced salinity stress on chlorophyll pigments of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

and genotypes.

Salinity Level (mM NaCl)	Chl.a (ELMO)	Chl.a (ORION)	Chl.b (ELMO)	Chl.b (ORION)	Chl a/b (ELMO)	Chl a/b (ORION)
Control	14.44 ^a	15.93 ^a	5.76 ^a	6.71 ^a	2.62 ^a	2.40 ^a
25	15.81 ^a	17.11 ^a	5.44 ^a	8.05 ^a	2.10 ^a	2.13 ^a
50	1.960 ^b	13.07 ^a	0.96 ^b	4.44 ^b	2.05 ^a	2.96 ^a
75	1.329 ^b	5.490 ^b	0.74 ^b	2.13 ^b	1.79 ^a	2.52 ^a
Average	8.38 ^A	12.90 ^A	3.22 ^A	5.33 ^A	2.36 ^A	2.50 ^A
Variable	Genotypes		Salinity Levels		Interaction	
Chlorophyll-a	4.744		2.713		3.863	
Chlorophyll-b	2.332		1.662		2.350	
Chl a/b Ratio	0.7908		0.7142		1.010	
CV (%) Chl.a	14.45					
CV (%) Chl.b	22.03					

Carotenoid content was significantly higher in ORION (4.32 mg·g⁻¹ FW) than in ELMO (2.45 mg·g⁻¹ FW), with maximal levels at control and 25 mM NaCl (~5.09 mg·g⁻¹ FW) and marked declines at 50 and 75 mM NaCl. The genotype × salinity interaction revealed ORION's superior carotenoid retention under salt stress (Table 12). These results confirm that salinity induces pigment degradation in chickpea leaves, reducing chlorophyll and carotenoid contents, consistent with reports of salt-induced oxidative damage to chloroplasts (Wang et al., 2024; Hameed et al., 2021). The superior retention of chlorophyll-a and carotenoids by ORION suggests enhanced photoprotection and antioxidative capacity, aligning with findings in salt-tolerant legumes (Hasanuzzaman et al., 2020). The stable chlorophyll a/b ratio despite overall pigment decline supports a coordinated downregulation of photosynthetic complexes to maintain functional balance under stress (Demmig-Adams and Adams, 2018). Carotenoids' role as antioxidants likely contributes to ORION's resilience by mitigating reactive oxygen species, as also observed in other stress-tolerant cultivars (Gill and Tuteja, 2010). Overall, ORION's pigment profile under salinity stress reflects greater physiological adaptation, providing a useful trait for breeding salt-tolerant chickpea varieties.

Table 12. Effect of NaCl-induced salinity stress on carotenoids (mg·g⁻¹ FW) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	ELMO	ORION	Average
control	4.75 ^{ab}	5.44 ^{ab}	5.09 ^A
25	4.13 ^b	6.06 ^a	5.09 ^A
50	0.49 ^c	4.27 ^{ab}	2.38 ^B
75	0.42 ^c	1.52 ^c	0.97 ^C
Average	2.45 ^B	4.32 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	1.606	1.317	1.863
CV (%)	22.07		

Salinity significantly reduced chlorophyll *a* content ($p \leq 0.01$) in both soybean genotypes. YAKARTA showed a higher average chlorophyll-*a* (15.39 mg·g⁻¹ FW) than POCA (11.04 mg·g⁻¹ FW). The highest values occurred in control and 25 mM NaCl treatments, while the lowest were at 100 mM NaCl. YAKARTA maintained chlorophyll-*a* better

under low to moderate salinity, whereas POCA showed a sharp decline at higher salt levels. Chlorophyll-*b* also decreased significantly with increasing salinity ($p \leq 0.01$). YAKARTA had a higher average chlorophyll-*b* ($6.83 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$) compared to POCA ($4.18 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$). The lowest chlorophyll-*b* contents were at 75 and 100 mM NaCl, especially in POCA. The chlorophyll *a/b* ratio did not differ significantly between genotypes but increased significantly at 50, 75, and 100 mM NaCl compared to control and 25 mM NaCl ($p \leq 0.01$) (Table 13).

Table 13. Effect of NaCl-induced salinity stress on photosynthetic pigments of two soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity Level (mM NaCl)	Chl.a (YAKARTA)	Chl.a (POCA)	Chl b (YAKARTA)	Chl b (POCA)	Chl a/b (YAKARTA)	Chl a/b (POCA)
Control	18.25 ^a	15.68 ^b _c	10.50 ^a	6.47 ^c	1.75 ^c	2.45 ^{ab}
25	17.36 ^{ab}	14.91 ^c	8.52 ^b	5.82 ^{cd}	2.01 ^{bc}	2.58 ^{ab}
50	15.49 ^{bc}	12.45 ^d	5.99 ^{cd}	4.30 ^d	2.60 ^{ab}	2.89 ^a
75	13.77 ^{cd}	7.05 ^e	4.66 ^d	2.22 ^e	2.95 ^a	3.05 ^a
100	12.10 ^d	5.12 ^e	4.46 ^d	1.97 ^e	2.75 ^a	2.61 ^{ab}
Average	15.39 ^A	11.04 ^B	6.83 ^A	4.18 ^B	2.41 ^A	2.72 ^A
Variable	LSD (Genotypes)		LSD (Salinity)		LSD (Interaction)	
Chlorophyll-a	2.010		1.656		3.341	
Chlorophyll-b	0.666		1.248		1.765	
Chl a/b Ratio	0.4843		0.4299		0.6080	
CV (%) Chl.a	7.43					
CV (%) Chl.a	13.46					
CV (%) Chl a/b	9.96					

Salinity stress induced a significant reduction in chlorophyll-a and chlorophyll-b content in the leaves of both soybean and chickpea, aligning with established findings on salt-induced oxidative damage to photosynthetic pigments (Parida and Das, 2005; Ashraf and Harris, 2013). In soybean, the genotype YAKARTA demonstrated greater chlorophyll retention, maintaining concentrations of $15.39 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ for chlorophyll-a and $6.83 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ for chlorophyll-b, compared to POCA, especially under conditions of low to moderate salinity. Similarly, in chickpea, the ORION genotype maintained higher pigment levels than ELMO, indicating genotype-dependent tolerance mechanisms. The chlorophyll *a/b* ratio increased with salinity in soybean, reflecting adaptive modifications in photosystem composition (Zhao et al., 2024). In contrast, chickpea maintained a relatively stable chlorophyll *a/b* ratio across salinity levels (2.15–2.56), suggesting a more coordinated regulation of pigment biosynthesis and degradation (Demmig-Adams et al.,

2014). No significant differences were found between genotypes in carotenoid content, but salinity affected levels significantly ($p \leq 0.01$). The highest carotenoid content was at 50 mM NaCl and control treatments, while lower contents occurred at other salt levels. YAKARTA showed higher carotenoids at moderate salinity, and POCA had higher levels at low salinity (Table 14). Carotenoid content exhibited contrasting trends: soybean showed a peak at moderate salinity (50 mM NaCl), especially in YAKARTA, followed by declines at higher salinity, whereas chickpea (ORION) maintained higher carotenoid levels at control and mild stress conditions, with sharper reductions at elevated salinity. These differences imply that chickpea may possess a more robust antioxidant defense mediated by carotenoids, contributing to enhanced salt tolerance (Gill and Tuteja, 2010). Overall, both crops display pigment degradation under salinity, but chickpea's more stable chlorophyll a/b ratio and superior carotenoid retention highlight a stronger physiological resilience. These findings align with previous research indicating legume species vary in their photosynthetic and antioxidative responses to salt stress (Hasanuzzaman et al., 2020). The enhanced pigment stability in chickpea, particularly in ORION, suggests valuable traits for breeding programs aiming to improve salt tolerance.

Table 14. Effect of NaCl-induced salinity stress on carotenoids ($\text{mg}\cdot\text{g}^{-1}$ FW) of two soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	YAKARTA	POCA	Average
control	2.35 ^c	5.24 ^{ab}	3.79 ^{AB}
25	2.15 ^c	5.07 ^{ab}	3.61 ^B
50	5.42 ^a	4.18 ^{ab}	4.80 ^A
75	4.17 ^{ab}	2.30 ^c	3.24 ^B
100	3.83 ^b	1.91 ^c	2.87 ^B
Average	3.58 ^A	3.74 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	0.8896	1.041	1.472
CV (%)	16.86		

4.4 Osmolyte accumulation and membrane stability

4.4.1 Proline accumulation ($\mu\text{mol}\cdot\text{g}^{-1}$ FW)

Proline accumulation in chickpea leaves was significantly affected by genotype, salinity level, and their interaction ($p \leq 0.01$). The genotype ORION demonstrated a notably higher mean proline concentration ($7.96 \mu\text{mol}\cdot\text{g}^{-1}$ FW) than ELMO ($5.76 \mu\text{mol}\cdot\text{g}^{-1}$ FW), reflecting a stronger osmotic adjustment response under salinity stress. Proline content increased progressively with salinity, reaching a maximum at 75 mM NaCl ($15.94 \mu\text{mol}\cdot\text{g}^{-1}$ FW), followed by a moderate accumulation at 50 mM NaCl ($8.06 \mu\text{mol}\cdot\text{g}^{-1}$ FW). In contrast, the lowest levels were recorded under control and 25 mM NaCl treatments (1.31 and $2.14 \mu\text{mol}\cdot\text{g}^{-1}$ FW, respectively), with no significant difference between them.

A significant genotype $\times$ salinity interaction was observed. At 75 mM NaCl, both genotypes exhibited the highest proline levels, $16.25 \mu\text{mol}\cdot\text{g}^{-1}$ FW in ELMO and $15.63 \mu\text{mol}\cdot\text{g}^{-1}$ FW in ORION—without a statistically significant difference. However, at 50 mM NaCl, ORION maintained a markedly higher proline content ($11.61 \mu\text{mol}\cdot\text{g}^{-1}$ FW) compared to ELMO ($4.51 \mu\text{mol}\cdot\text{g}^{-1}$ FW) (Table 15). Under lower salinity levels (control and 25 mM), both genotypes showed similarly low proline accumulation with no significant variation, suggesting a threshold response in proline synthesis under moderate to severe stress.

Table 15. Effect of NaCl-induced salinity stress on proline content ($\mu\text{mol g}^{-1}$ FW) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	ELMO	ORION	Average
Control	0.80 ^d	1.81 ^d	1.31 ^C
25	1.49 ^d	2.79 ^{cd}	2.14 ^C
50	4.51 ^c	11.61 ^b	8.06 ^B
75	16.25 ^a	15.63 ^a	15.94 ^A
Average	5.76 ^B	7.96 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	1.659	1.587	2.346
CV (%)	13.50		

Proline content in soybean leaves was significantly influenced by genotype, salinity level, and their interaction ($p \leq 0.01$). A marked increase in proline accumulation was observed with increasing NaCl concentration in both genotypes. YAKARTA exhibited a

significantly higher average proline content ($6.15 \mu\text{mol}\cdot\text{g}^{-1}$ FW) compared to POCA ($4.34 \mu\text{mol}\cdot\text{g}^{-1}$ FW), suggesting superior osmotic adjustment. At low salinity levels (0–50 mM NaCl), proline accumulation remained low and statistically similar in both genotypes. However, under high salinity stress (75 and 100 mM NaCl), YAKARTA accumulated significantly more proline (14.04 and $15.25 \mu\text{mol}\cdot\text{g}^{-1}$ FW, respectively) than POCA (7.76 and $12.76 \mu\text{mol}\cdot\text{g}^{-1}$ FW, respectively) (Table 16). This represents a 1880.52% increase in YAKARTA from control to 100 mM NaCl, compared to a 5230% increase in POCA, reflecting a stronger stress response in YAKARTA. These findings highlight YAKARTA's enhanced ability to accumulate proline under salt stress, supporting its potential as a more salt-tolerant genotype through osmotic regulation mechanisms.

Table 16. Effect of NaCl-induced salinity stress on proline content ($\mu\text{mol g}^{-1}$ FW) of two soybean (POCA and YAKARAT) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	YAKARTA	POCA	Average
Control	0.77 ^d	0.24 ^d	0.51 ^C
25	0.45 ^d	0.35 ^d	0.40 ^C
50	0.25 ^d	0.67 ^d	0.46 ^C
75	14.04 ^{ab}	7.76 ^c	10.90 ^B
100	15.25 ^a	12.76 ^b	14.01 ^A
Average	6.15 ^A	4.34 ^B	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	1.563	1.425	2.015
CV (%)	16.08		

The accumulation of proline serves as a key physiological adaptation to salinity, functioning as both an osmoprotectant and an antioxidant (Amini and Ehsanpour, 2005; Gill and Tuteja, 2010). Under salt stress, both chickpea and soybean exhibited a significant rise in proline levels, highlighting its role in osmotic adjustment and oxidative defense.

In chickpea, the ORION genotype accumulated higher proline concentrations than ELMO, particularly at 50 and 75 mM NaCl. This aligns with prior research associating greater proline synthesis with improved salt tolerance, as it helps protect cellular structures and preserve membrane integrity (Najaphy et al., 2010; Turan et al., 2009; Per et al., 2017).

A similar pattern was observed in soybean, where YAKARTA showed more pronounced proline accumulation than POCA under severe salinity, indicating a stronger adaptive response consistent with findings by Mannan et al. (2013). Proline's multifunctional role, maintaining osmotic balance and scavenging reactive oxygen species, which was evident in both crops (Bidabadi et al., 2012). These results emphasize the central importance of proline in the salinity response of legumes and support its use as a reliable biochemical marker in breeding programs aimed at developing salt-tolerant cultivars (Gill and Tuteja, 2010; Per et al., 2017).

4.4.2 MDA ($\mu\text{mol}\cdot\text{g}^{-1}$ FW) (Lipid peroxidation)

The analysis of variance revealed highly significant effects ($p \leq 0.01$) of genotype, salinity level, and their interaction on malondialdehyde (MDA) accumulation in chickpea leaves. MDA content, an indicator of lipid peroxidation and oxidative stress, increased significantly with salinity. Across genotypes, ELMO exhibited higher average MDA content ($132.20 \mu\text{mol}\cdot\text{g}^{-1}$ FW) than ORION ($115.50 \mu\text{mol}\cdot\text{g}^{-1}$ FW), suggesting a greater degree of membrane damage under stress. Regarding salinity levels, the highest MDA content was observed at 75 mM NaCl ($163.60 \mu\text{mol}\cdot\text{g}^{-1}$ FW), while the lowest values were recorded under control and 25 mM NaCl treatments (92.78 and $102.20 \mu\text{mol}\cdot\text{g}^{-1}$ FW, respectively), with no significant difference between them. A significant interaction was also detected. The ELMO genotype at 75 mM NaCl exhibited the highest MDA accumulation ($177.00 \mu\text{mol}\cdot\text{g}^{-1}$ FW), followed by ELMO at 50 mM ($154.00 \mu\text{mol}\cdot\text{g}^{-1}$ FW) and ORION at 75 mM ($150.10 \mu\text{mol}\cdot\text{g}^{-1}$ FW). In contrast, the lowest MDA levels were recorded under control and 25 mM treatments in both genotypes, without significant differences (Fig. 9). These results indicate that ELMO is more susceptible to oxidative damage under salinity stress than ORION, reflected by its higher MDA accumulation, particularly at elevated salt concentrations.

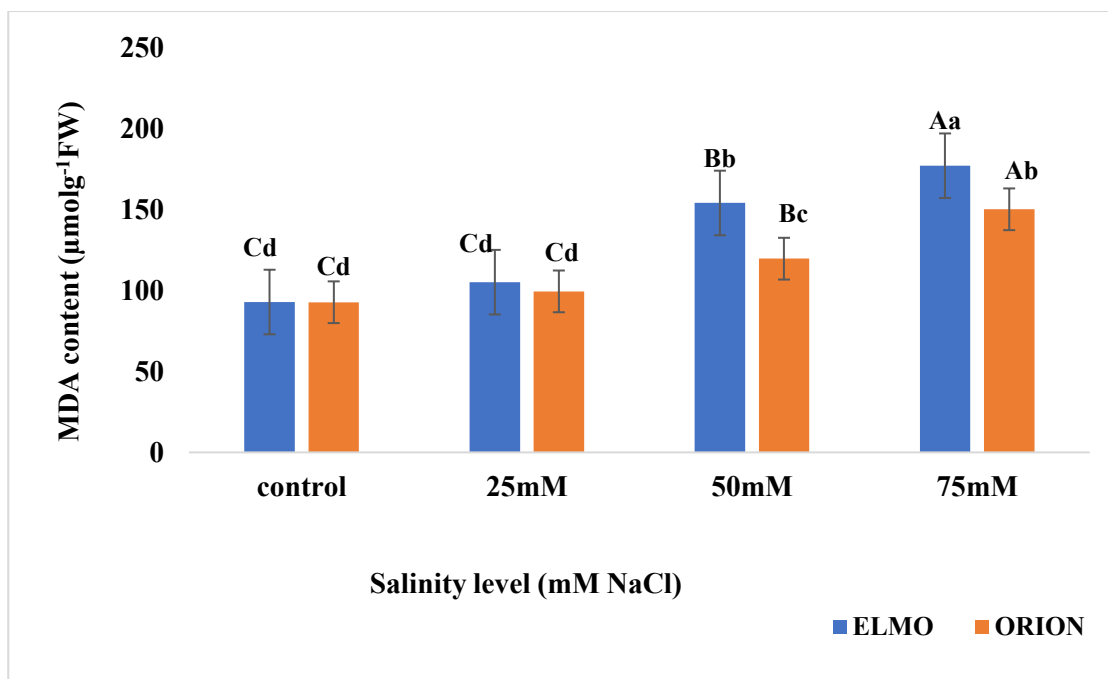


Fig. 9. Effect of NaCl-induced salinity on MDA ($\mu\text{mol g}^{-1}$ fw.) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions ($n=3$, $\pm$ s.e.). Different small letters indicate significant differences ($p \leq 0.01$) for the interactions between salt levels and genotypes, while the capital letters indicate significant differences ($p \leq 0.01$) for the general means of salt treatments and genotype.

Statistical analysis indicated highly significant effects ($p \leq 0.01$) for genotype, salinity level, and their interaction on malondialdehyde (MDA) content. As a primary indicator of lipid peroxidation and membrane integrity, MDA concentrations increased progressively with higher salinity in both genotypes. Across all treatments, the POCA genotype accumulated significantly more MDA ($122.0 \mu\text{mol} \cdot \text{g}^{-1}$ FW) than YAKARTA ($111.8 \mu\text{mol} \cdot \text{g}^{-1}$ FW), reflecting a higher degree of oxidative injury and membrane damage in POCA under saline stress. Under control conditions, both genotypes recorded the lowest MDA levels (YAKARTA: $24.40 \mu\text{mol} \cdot \text{g}^{-1}$ FW; POCA: $21.05 \mu\text{mol} \cdot \text{g}^{-1}$ FW), with no significant differences between them. However, salinity stress induced a sharp increase, with maximum MDA concentrations observed at 100 mM NaCl (POCA: $270.90 \mu\text{mol} \cdot \text{g}^{-1}$ FW; YAKARTA: $255.00 \mu\text{mol} \cdot \text{g}^{-1}$ FW) (Fig. 10). These results suggest that NaCl stress triggers substantial membrane lipid peroxidation in both genotypes, with POCA experiencing more severe oxidative damage. This pattern aligns with the observed variations in relative water content and proline levels, both established indicators of a plant's ability to manage osmotic pressure and mitigate oxidative damage under stress.

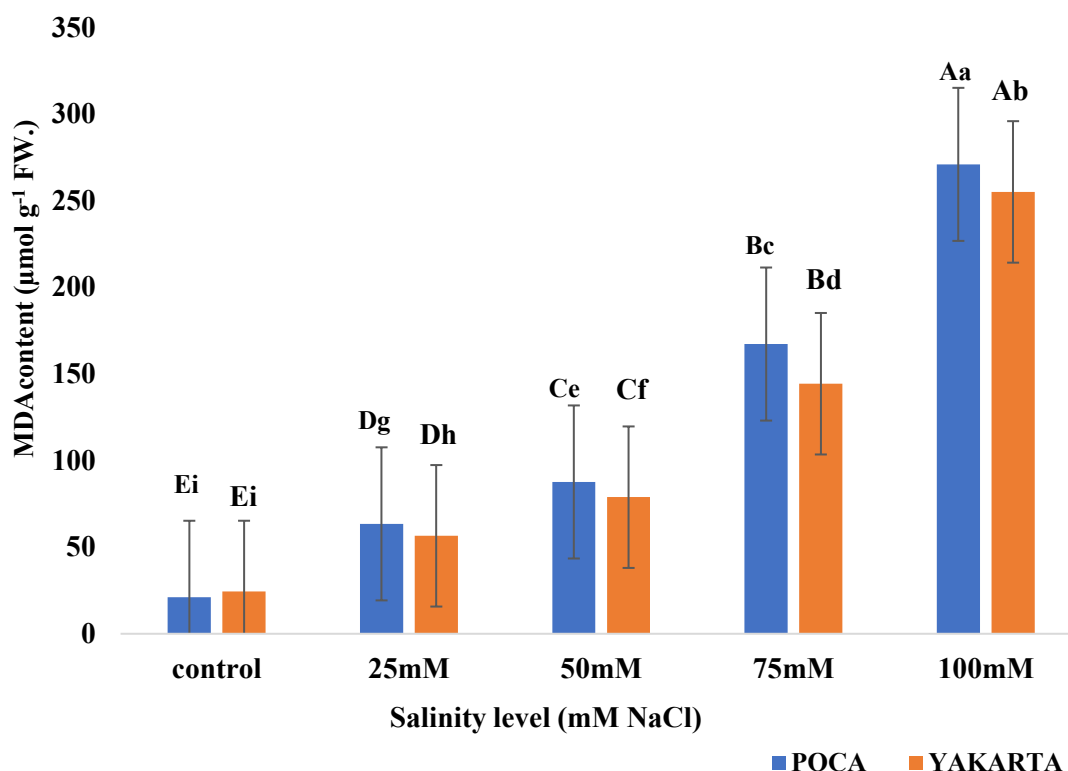


Fig. 10. Effect of NaCl-induced salinity on MDA ($\mu\text{mol g}^{-1}$ FW) of two soybean genotypes (YAKARTA and POCA) under hydroponic conditions ($n=3$, $\pm$ s.e.). Different small letters indicate significant differences ($p \leq 0.01$) for the interactions between salt levels and genotypes, while the capital letters indicate significant differences ($p \leq 0.01$) for the general means of salt treatments and genotype.

Malondialdehyde (MDA) serves as a key indicator of lipid peroxidation and oxidative damage in plant tissues subjected to salinity. In both chickpea and soybean, MDA content increased significantly with rising salt concentrations, confirming the adverse effects of NaCl stress on membrane integrity. This trend was especially pronounced in salt-sensitive genotypes such as ‘ELMO’ (chickpea) and ‘POCA’ (soybean), which exhibited consistently higher MDA levels than the more tolerant ‘ORION’ and ‘YAKARTA’, respectively. These differences reflect the genotypic variation in oxidative stress tolerance and membrane stability (Bandeoglu et al., 2004; Ahmad et al., 2018; Sadak et al., 2020). In chickpea, MDA accumulation was most notable at 75 mM NaCl, particularly in ‘ELMO’, suggesting that prolonged exposure to high salinity exacerbates lipid peroxidation. Similarly, in soybean, MDA levels peaked at 100 mM NaCl, indicating that oxidative damage intensifies with salt severity. This aligns with findings by Negrão et al. (2016), who attributed increased MDA under salinity to the breakdown of membrane-associated phosphorylated fatty acids caused by ROS. Additionally, higher MDA content was accompanied by increased electrolyte leakage in both crops,

reinforcing the role of membrane lipid peroxidation as a primary mechanism of salt-induced injury. These physiological responses were more evident in genotypes with lower relative water content and weaker osmotic adjustment capacity, underscoring the importance of maintaining cellular hydration for membrane protection (Arefian et al., 2014; Ahmad et al., 2010). The results also highlight the role of compatible solutes such as proline in membrane stability. Genotypes like ‘Orion’ and ‘YAKARTA’, which accumulated higher proline under stress, showed lower MDA levels, suggesting more effective protection against oxidative membrane degradation (Gill and Tuteja, 2010; Per et al., 2017). These findings are consistent with previous reports in chickpea (Bandeoglu et al., 2004) and soybean (Razmi et al., 2017), where salinity stress was linked to increased MDA levels and membrane instability. These results underscore that MDA accumulation serves as a reliable biochemical marker of oxidative damage under salt stress, and its differential expression among genotypes provides valuable insights into their salinity tolerance. Genotypes exhibiting lower MDA levels under high salinity conditions are better equipped to preserve membrane integrity and function, contributing to enhanced stress resilience.

4.4.3 Solute leakage (Membrane integrity)

Salinity stress significantly affected membrane stability in chickpea, as reflected by solute leakage (%), with notable differences between genotypes, salt levels, and their interaction ($p \leq 0.01$). The salt-sensitive genotype ‘ELMO’ exhibited higher membrane damage (72.2%) compared to the more tolerant ‘ORION’ (60.3%). Solute leakage increased progressively with salt concentration, reaching 89.0% at 75 mM NaCl, compared to 44.6% in the control. At 75 mM, both genotypes recorded high leakage values, 92.9% for ‘Elmo’ and 85.1% for ‘Orion’, with the lowest leakage observed in ‘Orion’ under control conditions (35.9%) (Table 17).

Table 17. Effect of NaCl-induced salinity stress on Membrane Integrity (Solute Leakage) (%) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	Elmo	Orion	Average
control	53.3 ^b	35.9 ^c	44.6 ^D
25	59.9 ^b	54.3 ^b	57.1 ^C
50	82.9 ^a	66.0 ^b	74.5 ^B
75	92.9 ^a	85.1 ^a	89.0 ^A
Average	72.2 ^A	60.3 ^B	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	6.50	10.2	14.4
CV (%)	8.74		

Significant effects ($p \leq 0.01$) of genotype, salinity level, and their interaction were observed on solute leakage in soybean. The POCA genotype exhibited significantly higher average solute leakage (87.14%) compared to YAKARTA (79.17%), indicating greater membrane damage and lower salt tolerance. The average Solute leakage increased progressively with salinity, reaching the highest levels at 100 and 75 mM NaCl (92.71% and 88.37%, respectively), with no significant difference between them. In contrast, the lowest leakage values were recorded under control (72.52%) and 25 mM NaCl (78.61%) treatments. Both genotypes showed significantly increased leakage at higher salt levels (100 and 75 mM), while POCA also recorded high leakage at 50 mM (85.19%). The lowest leakage was observed in YAKARTA under control conditions (63.42%) (Table 18), suggesting superior membrane stability and salt stress resilience.

Table 18. Effect of NaCl-induced salinity stress on Membrane Integrity (Solute Leakage) (%) of two soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	YAKARTA	POCA	Average
control	63.42 ^d	81.62 ^{bc}	72.52 ^D
25	73.42 ^c	83.81 ^b	78.61 ^{CD}
50	81.99 ^{bc}	85.19 ^{ab}	83.59 ^{BC}
75	85.88 ^{ab}	90.86 ^{ab}	88.37 ^{AB}
100	91.22 ^{ab}	94.20 ^a	92.71 ^A
Average	79.17 ^B	87.14 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	5.844	6.797	9.613
CV (%)	4.85		

Electrolyte leakage, a standard physiological marker for membrane integrity and oxidative stress under salinity, was evaluated alongside malondialdehyde (MDA) as an indicator of lipid peroxidation (Bandeoglu et al., 2004). Significant genotypic differences in membrane stability were observed in both crops, corresponding to increasing salt concentrations.

In chickpea, genotype ELMO exhibited significantly greater solute leakage than ORION, reflecting higher membrane susceptibility. This impairment correlated with ELMO's lower relative water content and reduced proline accumulation, traits essential for preserving membrane structure under osmotic and ionic stress.

A parallel response was recorded in soybean, where genotype POCA showed higher electrolyte leakage than YAKARTA, particularly under severe salinity. This indicates a comparatively weaker ability in POCA to maintain cellular membrane integrity under stress conditions.

Across both species, solute leakage increased significantly with rising NaCl concentrations, especially beyond 50 mM. This aligns with the observed rise in MDA content, particularly in the more sensitive genotypes (ELMO and POCA), suggesting increased lipid peroxidation and oxidative membrane damage. The degradation of cellular membranes under salt stress is primarily driven by oxidative processes, including the peroxidation of membrane lipids and the accumulation of reactive oxygen species (ROS) (Negrao et al., 2016; Arefian et al., 2014). The strong correlation observed between elevated malondialdehyde (MDA) levels and increased electrolyte leakage underscores the central role of oxidative damage in destabilizing membrane integrity. These findings align with earlier research on chickpea, soybean, and faba bean (Ahmad et al., 2010, 2018; Razmi et al., 2017), confirming the effectiveness of MDA content and solute leakage as robust biomarkers for evaluating salinity tolerance in breeding programs. Overall, the ORION and YAKARTA genotypes demonstrated greater tolerance to salinity by maintaining lower solute leakage and MDA levels, highlighting the importance of osmotic adjustment (e.g., proline accumulation) and antioxidant defense mechanisms in protecting membrane integrity under salt stress.

4.4.4 H₂O₂ levels

The results revealed significant effects ($p \leq 0.01$) of genotype, salinity level, and their interaction on hydrogen peroxide (H₂O₂) content. Across treatments, the ORION genotype accumulated significantly higher H₂O₂ levels (654.00 $\mu\text{mol g}^{-1}$ FW) compared

to ELMO (638.70 $\mu\text{mol g}^{-1}$ FW). H_2O_2 content decreased progressively with increasing salinity, recording the highest value under control conditions (850.70 $\mu\text{mol g}^{-1}$) and the lowest at 75 mM NaCl (483.10 $\mu\text{mol g}^{-1}$). At the control level, both genotypes showed high H_2O_2 concentrations (872.80 $\mu\text{mol g}^{-1}$ in ELMO and 828.60 $\mu\text{mol g}^{-1}$ in ORION) without significant differences. However, at 75 mM NaCl, both genotypes recorded significantly reduced H_2O_2 content (469.50 $\mu\text{mol g}^{-1}$ in ELMO and 496.60 $\mu\text{mol g}^{-1}$ in ORION), indicating that salinity stress led to a notable reduction in H_2O_2 accumulation (Table 19). These findings suggest a possible impairment or downregulation of ROS-generating mechanisms under high salt conditions or an enhanced detoxification response, potentially through antioxidant systems.

Table 19. Effect of NaCl-induced salinity stress on H_2O_2 Content ($\mu\text{mol g}^{-1}$) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	ELMO	ORION	Average
control	872.80 ^a	828.60 ^{ab}	850.70 ^A
25	635.60 ^c	758.90 ^b	697.20 ^B
50	576.90 ^{cd}	531.90 ^{de}	545.40 ^C
75	469.50 ^e	496.60 ^{de}	483.10 ^D
Average	638.70 ^B	654.00 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	10.71	68.52	96.90
CV (%)	6.01		

A factorial ANOVA revealed highly significant ($p \leq 0.01$) effects of genotype, salinity level, and their interaction on hydrogen peroxide (H_2O_2) content. On average, the YAKARTA genotype accumulated significantly more H_2O_2 (833.80 $\mu\text{mol g}^{-1}$ FW) than POCA (720.20 $\mu\text{mol g}^{-1}$ FW), suggesting a genotype-specific oxidative response. Across salinity levels, H_2O_2 concentration peaked at 25 mM NaCl (1228.00 $\mu\text{mol g}^{-1}$ FW) and then declined progressively with increasing salt concentration, reaching a minimum at 100 mM NaCl (406.50 $\mu\text{mol g}^{-1}$ FW). No statistically significant differences were observed among the control, 50 mM, and 75 mM NaCl treatments, which maintained moderate H_2O_2 levels (ranging from 767.90 to 805.50 $\mu\text{mol g}^{-1}$ FW). The most pronounced genotype $\times$ salinity interaction was detected at 25 mM NaCl, where YAKARTA exhibited the highest H_2O_2 accumulation (1289.00 $\mu\text{mol g}^{-1}$ FW), followed by POCA (1167.00 $\mu\text{mol g}^{-1}$ FW). In contrast, both genotypes exhibited minimal H_2O_2 accumulation at 100 mM NaCl, with POCA at 323.00 and YAKARTA at 490.00 $\mu\text{mol g}^{-1}$

without significant difference (Table 20). Specifically, it is proposed that the sharp decline of H_2O_2 at 100 mM NaCl likely reflects metabolic collapse due to severe cellular damage, including chloroplast degradation that limits ROS production. Furthermore, the loss of cellular compartmentalization and the subsequent release of phenolic compounds could neutralize residual H_2O_2 or interfere with its chemical detection. It is therefore emphasized that the low H_2O_2 concentrations measured at severe stress likely reflect the functional collapse of reactive oxygen species (ROS)-generating metabolic pathways, rather than an efficient detoxification process. At moderate salinity, YAKARTA demonstrates an adaptive oxidative burst, which initiates critical stress signaling and upregulates antioxidant defenses, a key factor in its superior tolerance. In contrast, POCA displays dysregulated ROS metabolism, aligning with its observed higher oxidative injury. This interpretation is supported by established literature on ROS signaling, oxidative stress, and antioxidant function in plants (Das and Roychoudhury, 2014; Choudhury et al., 2017; Mittler, 2002; Apel and Hirt, 2004; Gill and Tuteja, 2010; Blokhina et al., 2003; Mittler, 2017; Baxter et al., 2014). These results demonstrate that H_2O_2 accumulation is highly context-dependent: in the tolerant genotype YAKARTA, moderate H_2O_2 under 25–50 mM NaCl represents a controlled oxidative signal that activates antioxidant defenses, maintains membrane integrity, and supports overall physiological performance. In contrast, the elevated H_2O_2 levels in POCA indicate dysregulated ROS metabolism, overwhelming detoxification systems, and contributing directly to oxidative damage. This mechanistic interpretation explains why a higher H_2O_2 level in YAKARTA does not correspond to greater damage; rather, it reflects an adaptive oxidative burst that primes stress responses. Conversely, POCA's H_2O_2 accumulation reflects loss of regulatory control, consistent with its higher MDA levels and oxidative damage. By explicitly discussing the biphasic nature of ROS dynamics, where moderate ROS acts as a signaling molecule and excessive ROS becomes cytotoxic.

Table 20. Effect of NaCl-induced salinity stress on H₂O₂ Content (μmol g⁻¹) of two soybean (POCA and YAKARTA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	YAKARTA	POCA	Average
control	777.70 ^d	833.90 ^{cd}	805.80 ^B
25	1289.00 ^a	1167.00 ^{ab}	1228.00 ^A
50	883.00 ^{cd}	720.80 ^{de}	801.90 ^B
75	979.20 ^{bc}	556.50 ^{ef}	767.90 ^B
100	490.00 ^{fg}	323.00 ^g	406.50 ^C
Average	883.80 ^A	720.20 ^B	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	155.90	138.30	195.60
CV (%)	10.23		

Hydrogen peroxide (H₂O₂), a reactive oxygen species (ROS), plays a dual role in plants—acting as a signaling molecule at low concentrations and a cytotoxic agent at high concentrations (Mittler, 2017). In both chickpea and soybean, H₂O₂ content significantly declined with increasing salinity, likely due to enhanced antioxidant activity, particularly the accumulation of ascorbic acid (AA), which scavenges excess H₂O₂ via the ASC-GSH cycle (Hossain et al., 2015). In chickpea, H₂O₂ levels were higher in the salt-tolerant genotype ORION compared to ELMO, especially under control and mild salt stress (25 mM NaCl), indicating more active signaling. However, levels dropped significantly under severe stress (75 mM NaCl), suggesting either reduced ROS generation or more efficient detoxification. Ascorbic acid content was highest at 75 mM NaCl, particularly in ORION (529.30 μg g⁻¹), supporting the role of AA in moderating oxidative stress. Similarly, in soybean, the more tolerant genotype YAKARTA showed significantly higher H₂O₂ content than POCA, especially at moderate salinity (25 mM NaCl), pointing to an effective ROS signaling role. Despite higher H₂O₂ levels, YAKARTA had lower MDA and solute leakage, suggesting controlled ROS accumulation for adaptive responses. In contrast, POCA exhibited lower H₂O₂ but higher oxidative damage markers, indicating a weaker antioxidant system. The positive correlation between H₂O₂ and stomatal conductance in soybean supports its signaling role in maintaining stomatal function under stress. In both crops, genotypic variation in H₂O₂ content correlated with ascorbic acid levels, reinforcing the idea that tolerant genotypes can better modulate ROS to balance stress signaling and damage prevention (Ye et al., 2011). These findings highlight that controlled H₂O₂ accumulation, rather than total suppression, may be key to salinity tolerance.

4.5 Antioxidant and ionic homeostasis

4.5.1 Ascorbic acid

The ascorbic acid content differed significantly ($p \leq 0.01$) between the two chickpea genotypes. The ORION genotype had a higher average ascorbic acid content ($440.50 \mu\text{g g}^{-1}$) than the ELMO genotype ($395.60 \mu\text{g g}^{-1}$). Across salinity treatments, the highest ascorbic acid content was recorded at 75 mM NaCl ($464.70 \mu\text{g g}^{-1}$), while the control and 50 mM NaCl treatments had significantly lower values (375.30 and $391.80 \mu\text{g g}^{-1}$, respectively) with no significant difference between them. Regarding the interaction between genotype and salinity levels, the highest ascorbic acid content ($529.30 \mu\text{g g}^{-1}$) was observed in the ORION genotype at 75 mM NaCl. Other genotype and salinity level combinations showed significantly lower ascorbic acid contents with no significant differences among them (Fig. 11).

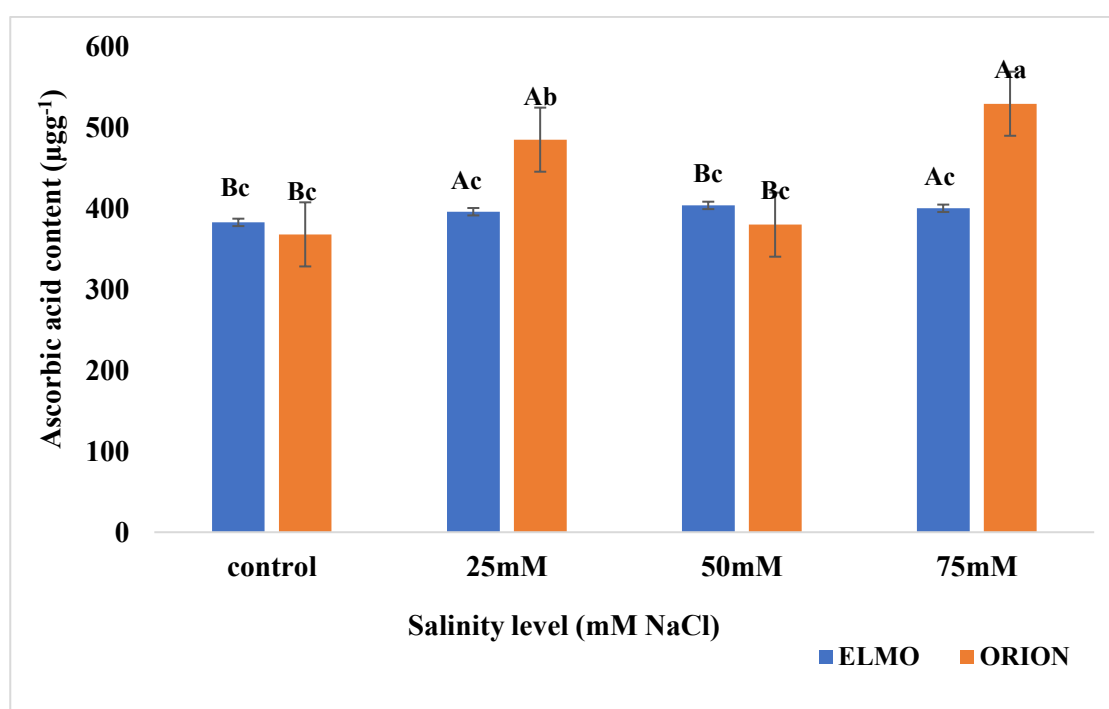


Fig. 11. Effect of NaCl-induced salinity stress on ascorbic acid ($\mu\text{g g}^{-1}$) of two chickpea genotypes (ELMO AND ORION) under hydroponic conditions ($n = 3, \pm \text{s.e.}$). Different capital letters among salinity levels indicate significant differences ($p \leq 0.01$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.01$).

The statistical analysis indicated highly significant ($p \leq 0.01$) effects of genotype, salinity level, and their interaction on ascorbic acid (AsA) content. Across all treatments, POCA accumulated significantly more AsA ($334.30 \mu\text{g g}^{-1}$) than YAKARTA ($292.80 \mu\text{g g}^{-1}$), suggesting a more pronounced antioxidant mobilization in response to salinity.

AsA concentration rose significantly with increasing salt stress, peaking at 100 mM (376.90 $\mu\text{g g}^{-1}$) and 75 mM (364.30 $\mu\text{g g}^{-1}$) NaCl, with no significant difference between these two highest levels. The lowest AsA content was recorded under control (non-saline) conditions (224.20 $\mu\text{g g}^{-1}$).

In the genotype $\times$ salinity interaction, both genotypes showed their highest AsA levels at 75 and 100 mM NaCl. Notably, POCA also maintained significantly elevated AsA at moderate salinity levels (25 and 50 mM NaCl), with values of 333.10 and 336.90 $\mu\text{g g}^{-1}$, respectively. In contrast, YAKARTA exhibited its lowest AsA content under control conditions (197.30 $\mu\text{g g}^{-1}$) (Fig. 12).

These results imply that the higher AsA accumulation in POCA likely constitutes a stress-induced antioxidant response, potentially activated by its greater physiological sensitivity to salinity. This elevated antioxidant activity may serve as a compensatory mechanism to counteract the heightened oxidative damage experienced by this more susceptible genotype.

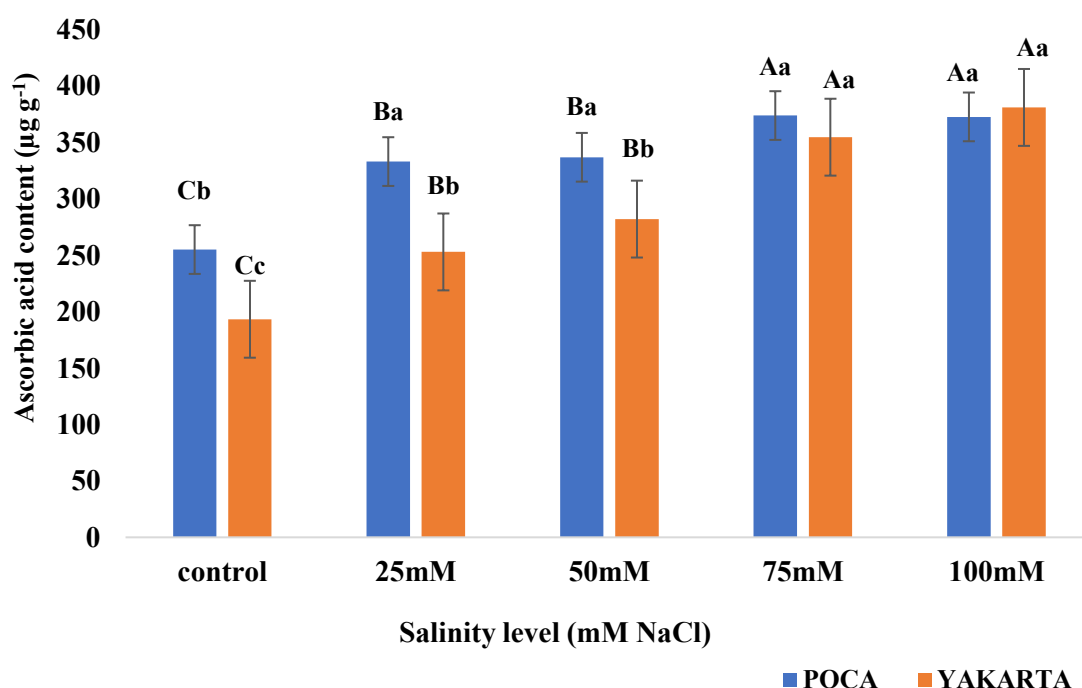


Fig. 12. Effect of NaCl-induced salinity stress on ascorbic acid ($\mu\text{g g}^{-1}$) content of two soybean genotypes (YAKARTA and POCA) ($n=3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.01$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.01$).

Salinity stress significantly influenced hydrogen peroxide (H_2O_2) and ascorbic acid (AsA) contents in both chickpea and soybean, reflecting their roles in oxidative stress response and tolerance. In chickpea, H_2O_2 content declined with increasing salinity, while

AsA content increased, especially in the ORION genotype at 75 mM NaCl. This suggests that ORION may possess a stronger antioxidant response, likely helping it mitigate oxidative damage under high salinity. The inverse relationship between H₂O₂ and AsA indicates the possible role of AsA in scavenging reactive oxygen species and enhancing salt tolerance, as supported by earlier studies (Manurung and Rahmawati, 2018; Kim et al., 2017). In soybean, moderate salinity (25 mM NaCl) triggered the highest H₂O₂ levels, particularly in the YAKARTA genotype, suggesting a signaling role for H₂O₂ under stress. Meanwhile, AsA content increased significantly under higher salinity (75 and 100 mM NaCl), with the POCA genotype showing higher AsA levels than YAKARTA. The elevated AsA may contribute to H₂O₂ detoxification, helping limit oxidative damage at high salinity. This dual pattern, moderate H₂O₂ accumulation and increased AsA under stress—supports the idea that controlled ROS buildup aids in signaling, while antioxidants maintain redox balance (Hossain et al., 2015; Mittler, 2017; Ye et al., 2011). The results demonstrate that salinity stress induces significant changes in hydrogen peroxide and ascorbic acid levels in both chickpea and soybean. Increased AsA content, particularly under higher salt concentrations, suggests its vital role in mitigating oxidative stress. Genotypic differences in H₂O₂ and AsA accumulation highlight the potential of using these traits as indicators of salt tolerance, with ORION (chickpea) and YAKARTA (soybean) showing stronger antioxidant responses.

4.5.2 K^+/Na^+ ratio

The statistical analysis showed significant effects ($p \leq 0.01$) of genotype, salinity level, and their interaction on K^+/Na^+ selectivity. The ORION genotype exhibited significantly higher selectivity (7.497) compared to ELMO (5.939). Selectivity was highest under control conditions (18.46), followed by a sharp decrease at 50 mM NaCl (1.202), and the lowest at 75 mM (0.4892). The highest K^+/Na^+ ratio was recorded in ORION under control (20.13), while the lowest was in ELMO at 75 mM (0.4274) (Table 21).

Table 21. Effect of NaCl-induced salinity stress on K⁺/Na of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	ELMO	ORION	Mean
control	16.80 ^b	20.13 ^a	18.46 ^A
25	16.66 ^b	20.11 ^a	18.25 ^A
50	0.5926 ^d	1.811 ^c	1.202 ^B
75	0.4274 ^d	0.5510 ^d	0.4892 ^C
Mean	5.939 ^B	7.497 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	0.1105	0.3172	0.1915
CV (%)	6.5		

The statistical analysis showed highly significant ($p \leq 0.01$) effects of genotype, salinity level, and their interaction on the potassium to sodium (K⁺/Na⁺) ratio in soybean. The K⁺/Na⁺ ratio declined substantially with rising salinity in both genotypes. The highest ratios were recorded under control and 25 mM NaCl conditions (154 and 151 in YAKARTA; 79.5 and 78.3 in POCA), with no significant difference between these two levels. The lowest ratios were found at 100 mM NaCl, dropping to 0.27 in YAKARTA and 0.22 in POCA, reflecting severe Na⁺ accumulation and ion homeostasis disruption. Across all treatments, YAKARTA maintained a significantly higher average K⁺/Na⁺ ratio (61.8) than POCA (32.2) (Table 22). This consistent advantage indicates a superior capacity for selective K⁺ uptake and/or Na⁺ exclusion, a key physiological mechanism linked to salinity tolerance. The results suggest that YAKARTA possesses a more effective ion regulatory system under salt stress.

Table 22. Effect of NaCl-induced salinity stress on K⁺/Na of two soybean (POCA and YAKARTA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.01$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity level (mM NaCl)	YAKARTA	POCA	Mean
Control	154.00 ^a	79.50 ^b	117 ^A
25	151.00 ^a	78.30 ^b	115 ^A
50	2.41 ^c	2.12 ^c	2.27 ^B
75	1.32 ^c	0.67 ^c	1.00 ^B
100	0.27 ^c	0.22 ^c	0.24 ^B
Mean	61.8 ^A	32.20 ^B	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.01)	3.52	3.54	7.03
CV (%)	8.53		

Ion homeostasis is a critical determinant of plant salt tolerance. High salinity disrupts nutrient balance primarily by promoting Na^+ accumulation, which interferes with K^+ uptake—an essential ion for enzyme function, osmotic regulation, and cellular integrity (Arif et al., 2020). A high K^+/Na^+ ratio is thus a reliable physiological marker of salinity tolerance (Essa, 2002; Amirjani, 2010; Wu et al., 2014). In this study, both soybean and chickpea genotypes exhibited a significant reduction in K^+/Na^+ ratios with increasing salinity, highlighting the adverse effects of NaCl on Ion selectivity. However, strong genotypic differences were observed. In soybean, YAKARTA maintained a significantly higher K^+/Na^+ ratio than POCA across all salt levels, suggesting better Na^+ exclusion or K^+ retention. Even under severe salinity (100 mM NaCl), YAKARTA sustained a higher average ratio (61.8) than POCA (32.2). Similarly, in chickpea, ORION showed superior ion regulation compared to ELMO, particularly under moderate and high salinity (50–75 mM NaCl), maintaining higher K^+/Na^+ ratios and indicating stronger ion selectivity and Na^+ exclusion capacity. These findings confirm that YAKARTA and ORION possess more efficient mechanisms for ion homeostasis under salt stress, likely involving selective root ion transport, Na^+ sequestration into vacuoles, or active Na^+ extrusion. Such traits are essential for maintaining cellular function and supporting growth under saline conditions. These findings align with previous studies report that salt-tolerant soybean cultivars maintain higher K^+/Na^+ ratios under saline conditions by regulating Na^+ uptake and enhancing K^+ retention mechanisms (Essa, 2002; Wu et al., 2014) (Fig. 13).



Soybean (POCA)



Soybean (YAKARTA)



Chickpea (ELMO)

Chickpea (ORION)

Figure 13. Effect of NaCl-induced salinity stress (0.0, 25, 50, 75 and 100 mM NaCl) on the performance of different soybean and chickpea genotypes under controlled conditions.

4.6 Correlation among physio-biochemical traits under NaCl-induced salinity stress

4.6.1 Chickpea correlations among the investigated traits

In chickpea genotypes under salinity stress, relative water content (RWC) showed strong positive associations with stomatal conductance, chlorophyll pigments (a and b), SPAD values, carotenoids, and the K^+/Na^+ ratio—while negatively correlating with proline levels, membrane damage, MDA, suggesting that plants maintaining better hydration sustain photosynthesis and ionic balance while minimizing oxidative injury, consistent with earlier findings that salt-tolerant plants preserve higher RWC and membrane integrity under stress (Farooq et al., 2015; Munns and Tester, 2008). Proline, conversely, was positively linked to oxidative stress markers but negatively to RWC and pigments, implying its accumulation signals stress severity rather than confers tolerance, which agrees with the argument that proline often reflects stress exposure rather than true adaptation (Ashraf and Foolad, 2007). Oxidative stress indicators such as MDA and H_2O_2 were strongly interrelated and negatively correlated with photosynthetic pigments, underscoring their detrimental impact on the photosynthetic machinery as previously reported under saline conditions (Taïbi et al., 2016). The strong positive linkage among chlorophylls, carotenoids, SPAD, and K^+/Na^+ ratio reinforces the significance of ion homeostasis and pigment integrity in salinity adaptation, with carotenoids likely offering photoprotection through antioxidant activity (Munns and Tester, 2008; Taïbi et al., 2016). Finally, ascorbic acid showed weak, nonsignificant correlations with most traits, suggesting a less consistent role compared to carotenoids and other antioxidative systems, in agreement with reports that its regulation may be highly genotype- and stress-specific (Hasanuzzaman et al., 2020). Overall, salinity tolerance in chickpea appears to be rooted

in the plant's ability to sustain water status, ionic equilibrium, and pigment stability while mitigating oxidative damage, with proline acting more as a stress indicator than a tolerance mechanism (Fig. 14).

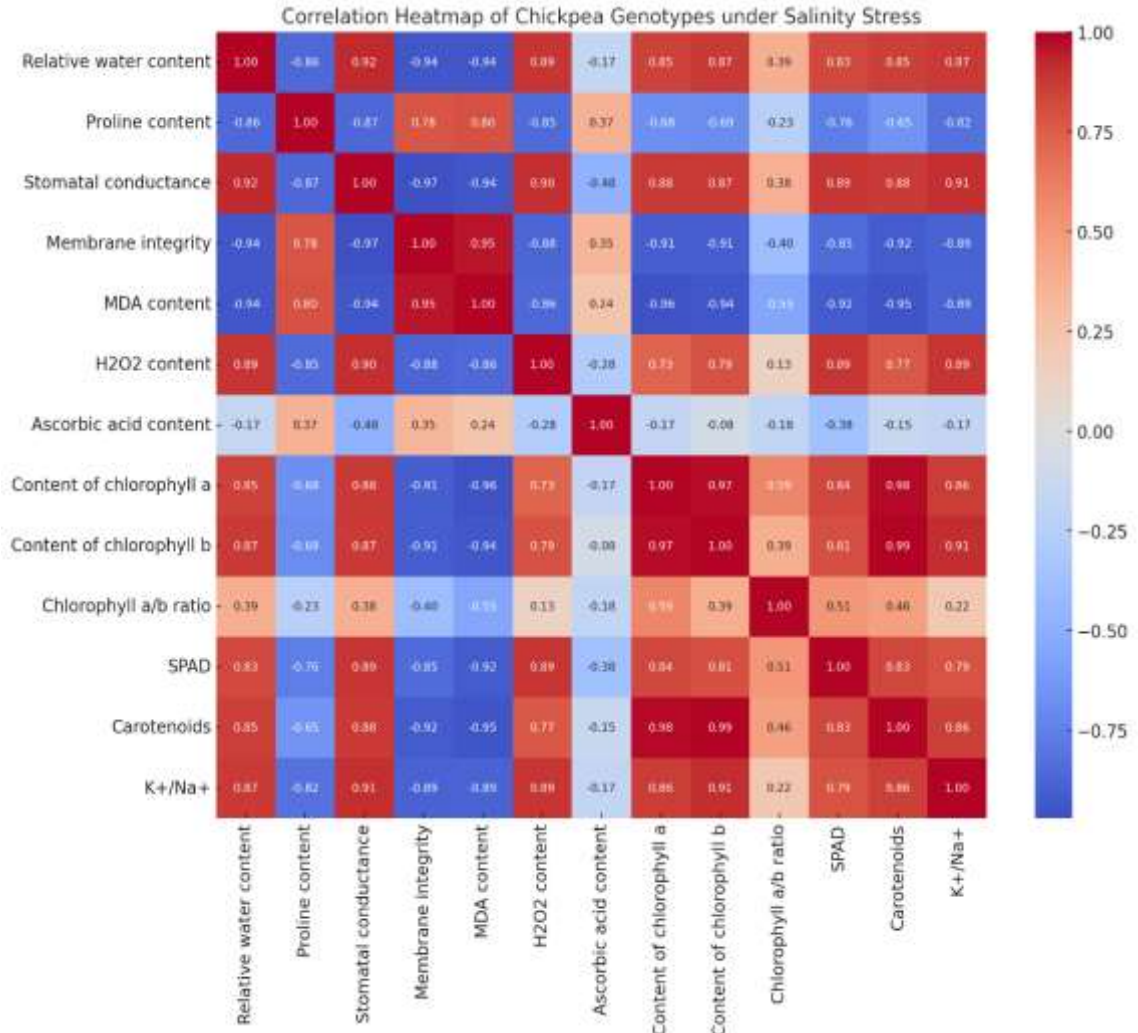


Fig. 14. Correlations among physio-biochemical traits under NaCl-induced salinity stress. Significant positive correlations are show in red, negative in blue; *, ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

4.6.2 Soybean correlations among the investigated traits

The correlation analysis revealed that relative water content (RWC) exhibited a very strong positive correlation with stomatal conductance (g_s ; $r = 0.985^{**}$) and chlorophyll-b content ($r = 0.875^{**}$), underscoring the critical role of adequate hydration in maintaining gas exchange and photosynthetic pigment stability. Chlorophyll-a content was strongly correlated with SPAD values ($r = 0.856^{**}$) and moderately with carotenoids

($r = 0.756^*$), while the chlorophyll a/b ratio also correlated positively with carotenoid content ($r = 0.712^*$), indicating coordinated regulation of light-harvesting and photoprotective pigments. Hydrogen peroxide (H_2O_2) content showed a very strong positive correlation with ascorbic acid ($r = 0.957^{**}$), reflecting the activation of antioxidant defenses in response to oxidative stress (Ahmad et al., 2010). In contrast, classical stress indicators such as proline and malondialdehyde (MDA) exhibited weak or non-significant correlations with other traits, suggesting that osmolyte accumulation and lipid peroxidation may respond independently to stress conditions (Anjum et al., 2011). Overall, these results highlight that water status and stomatal function are key determinants of photosynthetic performance, while oxidative stress and antioxidant responses operate in a tightly coupled manner, whereas proline accumulation and membrane damage appear more variable, reflecting the complex and multi-layered nature of plant stress physiology (Fig. 15).

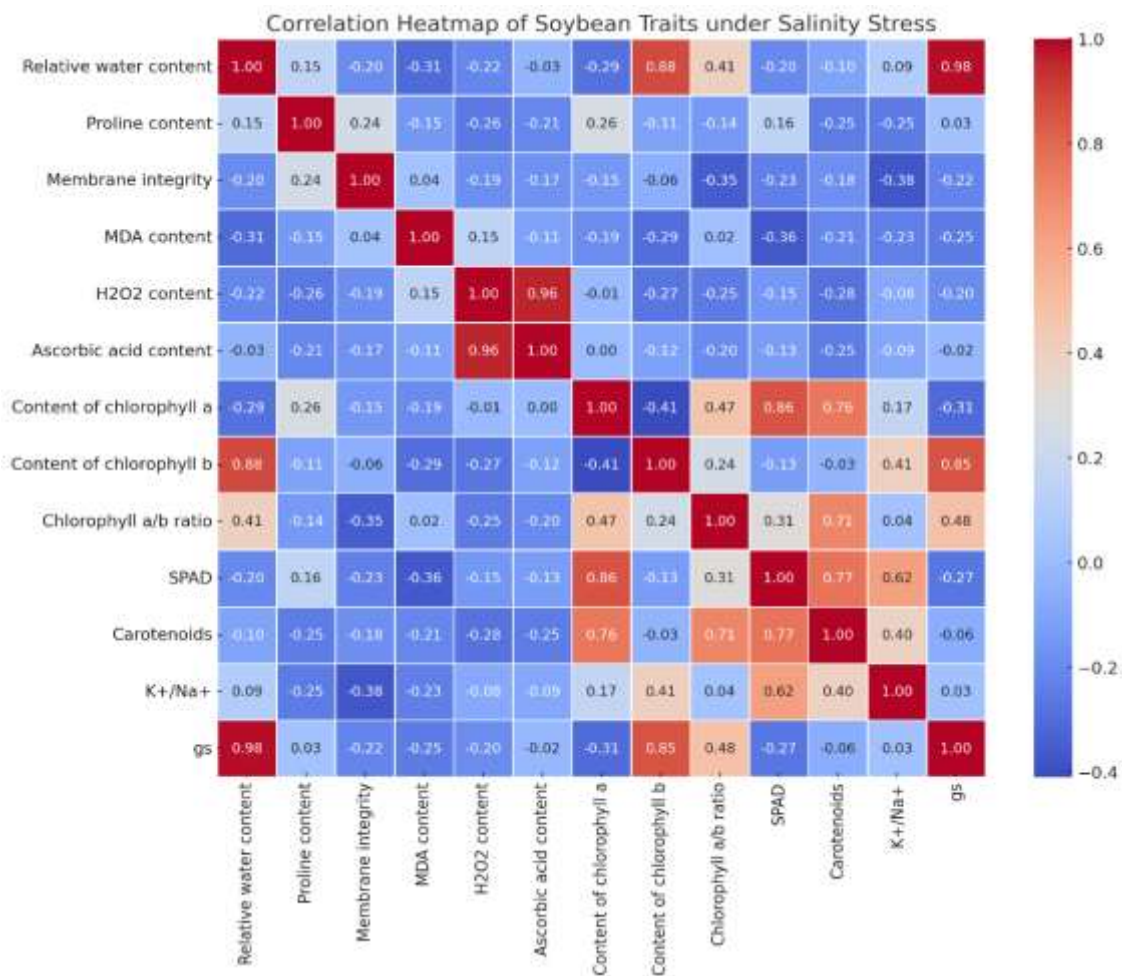


Fig. 15. Correlations among physio-biochemical traits under NaCl-induced salinity stress. Significant positive correlations are show in red, negative in blue; *, ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

4.7 Comparative tolerance between species and genotypes

To provide a clear overview of genotypic responses, a comparative schematic was developed to illustrate the contrasting performance of the tolerant genotypes (ORION in chickpea and YAKARTA in soybean) and the sensitive genotypes (ELMO in chickpea and POCA in soybean) across all measured parameters. This visual summary highlights key physiological and biochemical traits that underpin salinity tolerance, serving as a reference framework for interpreting the detailed data presented in the following tables (23 and 24) and figure (16):

Table 23. Comparative responses of chickpea and soybean genotypes to salinity stress across morpho-physiological and biochemical parameters.

Parameter	Chickpea (ORION)	Chickpea (ELMO)	Soybean (YAKARTA)	Soybean (POCA)	Comparative Insight
Growth	Moderate reduction; better growth retention under stress	Strong reduction, highly sensitive	Higher growth retention under stress	Strong reduction	Soybean > Chickpea; (ORION, YAKARTA) outperform sensitive ones
Photosynthetic pigments	Maintained higher pigments	Severe decline	Higher pigment retention	Significant decline	YAKARTA > ORION > POCA ≈ ELMO
Oxidative stress markers	Lower accumulation	Higher accumulation	Lower accumulation	Higher accumulation	Tolerant genotypes accumulate fewer oxidative stress markers
Ascorbic acid (AsA)	Higher maintenance under stress	Lower levels	lower in YAKARTA	Higher in POCA	Tolerance relies on multiple defenses, not just AsA
K⁺/Na⁺ ratio	7.50 (better Na ⁺ exclusion and K ⁺ selectivity)	5.94 (poor ion regulation)	61.8 (very efficient ion homeostasis)	32.2 (weaker ion regulation)	Soybean genotypes generally maintain higher K ⁺ /Na ⁺ than chickpea
Genotypic variation	ORION is clearly more tolerant than ELMO	—	YAKARTA is clearly more tolerant than POCA	—	Intraspecific variation strongly influences tolerance
Overall tolerance ranking	Moderate tolerance	Sensitive	High tolerance	Sensitive	YAKARTA > ORION > POCA ≈ ELMO

Table 24. Comparative Schematic of Tolerance Mechanisms in Chickpea and Soybean Genotypes.

Trait / Parameter	Tolerant Genotypes (ORION – Chickpea, YAKARTA – Soybean)	Sensitive Genotypes (ELMO – Chickpea, POCA – Soybean)
Growth performance	Better maintenance of shoot and root biomass under salinity stress	Severe growth inhibition at moderate to high salinity
Chlorophyll stability	Higher retention of chlorophyll a, b, and total chlorophyll, preserving photosynthesis	Rapid decline in chlorophyll pigments, reduced photosynthetic activity
Proline accumulation	Strong induction of proline, supporting osmotic adjustment and stress protection	Limited proline accumulation, weaker osmotic regulation
Ascorbic acid (AsA)	Higher or well-maintained AsA levels contribute to antioxidant defense (ORION); YAKARTA moderate but effective).	Lower (ELMO) or stress-induced higher AsA (POCA), but not sufficient for effective protection.
Ion balance (K^+/Na^+ ratio)	Maintains significantly higher ratios, showing better Na^+ exclusion and K^+ retention	Sharp decline in K^+/Na^+ ratio, indicating poor ion selectivity
Integrated tolerance response	Efficient osmotic adjustment + antioxidant defense + ionic homeostasis → salt-tolerant	Poor ion regulation + weak antioxidant system + chlorophyll loss → salt-sensitive

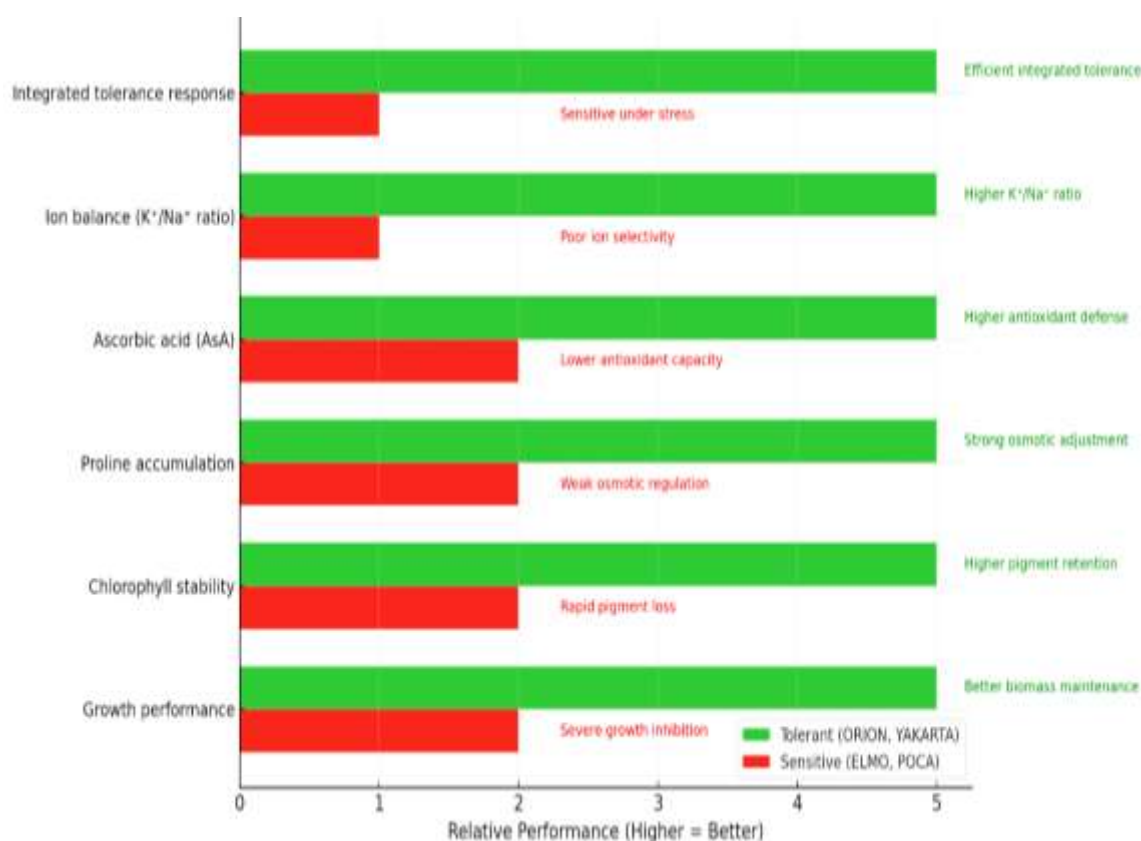


Fig. 16. Comparative schematic of tolerance mechanisms in chickpea and soybean.

4.8 Morphological response to PEG-induced water deficit

4.8.1 Shoot and root length

Analysis of variance revealed significant effects ($p \leq 0.05$) of genotype, PEG-induced water deficit levels, and their interaction on shoot and root growth in both chickpea and soybean. In chickpea, shoot length was highest under control conditions (43.41 cm) and decreased progressively with increasing PEG concentration, reaching a minimum of 26.91 cm at -0.4 MPa, while plants did not survive at -0.6 MPa. Among the genotypes, ORION maintained the longest shoots (39.33 cm) compared to ELMO (28.77 cm), with ORION consistently outperforming ELMO across all stress levels (Table 25). Similarly, in soybean, shoot length declined with increasing PEG-induced water deficit, from 69.83 cm in the control to 42.33 cm at -0.6 MPa. YAKARTA exhibited a significantly greater average shoot length (61.17 cm) than POCA (53.17 cm), particularly under control and moderate stress conditions (Table 26), though differences narrowed at severe stress. These results indicate that shoot growth in both crops is highly sensitive to water deficit, with ORION and YAKARTA demonstrating superior tolerance by maintaining comparatively longer shoots under osmotic stress. Root length showed similar trends: In chickpea, the longest roots were recorded under control conditions (57.33 cm), with progressive reductions observed as PEG concentration increased, reaching a minimum of 36.66 cm at -0.4 MPa. Among genotypes, ORION maintained significantly longer roots (51.00 cm) than ELMO (43.22 cm), particularly under severe stress (-0.4 MPa: 43.33 cm in ORION vs. 30.00 cm in ELMO) (Table 25), indicating superior tolerance and capacity to sustain root growth.

Table 25. Effect of PEG-induced water deficit on shoot and root length (cm) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions. Different lowercase letters (a–e) within a column indicate significant differences ($p \leq 0.05$) between PEG levels for the same genotype. Capital letters (A–C) indicate differences among mean values for PEG levels and genotypes.

PEG-induced stress (MPa)	ELMO Shoot	ORION Shoot	Avg. Shoot	ELMO Root	ORION Root	Avg. Root
Control	38.83 ^b	48.00 ^a	43.41 ^A	55.00 ^{ab}	59.67 ^a	57.33 ^A
-0.2	26.67 ^d	37.00 ^b	31.83 ^B	44.67 ^c	50.00 ^{bc}	47.33 ^B
-0.4	20.83 ^e	33.00 ^c	26.91 ^C	30.00 ^d	43.33 ^c	36.66 ^C
Average	28.77 ^B	39.33 ^A	—	43.22 ^B	51.00 ^A	—
Variable	Genotypes		PEG Levels		Interaction	
LSD (0.01) – Shoot	2.232		4.212		3.866	
LSD (0.01) – Root	4.477		4.350		7.754	
CV (%) – Shoot	6.57					
CV (%) – Root	9.91					

In soybean, root length was highest under control and mild stress (–0.2 MPa) conditions (87.50 cm and 81.50 cm, respectively) and decreased substantially at severe water deficit stress (–0.6 MPa: 52.50 cm). No significant differences were observed between the genotypes overall, both YAKARTA and POCA maintained comparable root lengths at control and mild stress levels (88.33, 86.67 cm; 83.00, 80.00 cm, respectively) and showed similar reductions under severe stress (53.33, 51.67 cm) (Table 26). These findings demonstrate that root growth in both crops is sensitive to water deficit, with chickpea ORION exhibiting a more pronounced ability to maintain root development under water deficit.

Table 26. Effect of PEG-induced water deficit on shoot and root length (cm) of two soybean genotypes (POCA and YAKARTA) under hydroponic conditions. Different lowercase letters (a–e) within a column indicate significant differences ($p \leq 0.05$) between PEG levels for the same genotype. Capital letters (A–C) indicate differences among values for PEG levels and genotypes.

PEG-induced stress (MPa)	POCA Shoot	YAKARTA Shoot	Avg. Shoot	POCA Root	YAKARTA Root	Avg. Root
Control	64.67 ^b	75.00 ^a	69.83 ^A	86.67 ^a	88.33 ^a	87.50 ^A
-0.2	58.00 ^{bc}	65.00 ^b	61.50 ^B	80.00 ^{ab}	83.00 ^a	81.50 ^A
-0.4	50.00 ^{cd}	60.00 ^b	55.00 ^C	60.00 ^{cd}	70.00 ^{bc}	65.00 ^B
-0.6	40.00 ^e	44.67 ^{de}	42.33 ^D	51.67 ^d	53.33 ^d	52.50 ^C
Average	53.17 ^B	61.17 ^A	—	69.58 ^A	73.67 ^A	—
Variable	Genotypes		PEG Levels		Interaction	
LSD (0.01) – Shoot	4.877		4.816		9.753	
LSD (0.01) – Root	5.565		10.56		11.33	
CV (%) – Shoot	9.06					
CV (%) – Root	8.40					

Both chickpea and soybean showed significant reductions in shoot and root growth under PEG-induced water deficit. In chickpea, shoot length decreased by 38.0% in ORION and 46.3% in ELMO at –0.4 MPa, while root length reductions were 27.4% in ORION and 45.5% in ELMO. In soybean, shoot length declined by 40.4% in YAKARTA and 38.2% in POCA at –0.6 MPa, whereas root length decreased by 39.6% in YAKARTA and 40.3% in POCA. These results indicate that soybean, particularly YAKARTA, maintains higher absolute shoot and root lengths under water deficit, whereas chickpea shows stronger genotype-dependent variability, with ORION moderately tolerant and ELMO highly sensitive.

4.8.2 Biomass accumulation (fresh and dry weight)

Shoot fresh weight was significantly affected ($p \leq 0.05$) by genotype, PEG-induced water deficit levels, and their interaction in both chickpea and soybean (Table 27). In chickpea, the average shoot fresh weight was highest under control conditions (11.31 g) and progressively declined with increasing PEG concentration, reaching the lowest value at -0.4 MPa (2.65 g). This corresponded to a reduction of approximately 76.56% compared to the control. Across genotypes, ORION maintained significantly higher shoot fresh weight (7.50 g) than ELMO (5.50 g). In soybean, a similar trend was observed. Shoot fresh weight was significantly greater in the control (23.33 g) and decreased progressively with increasing stress intensity, reaching 5.00 g at -0.6 MPa, which represented a reduction of 78.56% compared to the control. On average, POCA sustained significantly higher shoot fresh weight (15.92 g) compared to YAKARTA (11.75 g).

Table 27. Effect of PEG-induced water deficit stress on shoot fresh weight (g) of two chickpea (ELMO and ORION) and soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	Chickpea (Elmo)	Chickpea (Orion)	Average	Soybean (Poca)	Soybean (Yakarta)	Average
Control	9.80 ^b	12.83 ^a	11.31 ^A	26.67 ^a	20.00 ^{ab}	23.33 ^A
−0.2	4.74 ^{cd}	6.33 ^c	5.54 ^B	18.33 ^b	13.00 ^{bc}	15.67 ^B
−0.4	1.96 ^e	3.33 ^{de}	2.65 ^C	13.00 ^{bc}	9.67 ^{cd}	11.33 ^C
−0.6	—	—	—	5.67 ^{cd}	4.33 ^d	5.00 ^D
Average	5.50 ^B	7.50 ^A	—	15.92 ^A	11.75 ^B	—
Variable	Genotypes		PEG levels		Interaction	
LSD (0.05) Chickpea	1.085		0.7073		1.879	
LSD (0.05) Soybean	3.957		4.210		7.914	
CV (%) chickpea	16.48					
CV (%) soybean	15.38					

The shoot dry weight of both chickpea and soybean genotypes was significantly influenced by water deficit stress induced through varying concentrations of PEG-6000. In chickpea, the average shoot dry weight under control conditions was 1.77 g, which declined markedly to 0.40 g at the highest water deficit level (Table 28), representing a 77.40% reduction compared to the non-stress treatment. Similarly, soybean exhibited a higher baseline shoot dry weight under control conditions (5.75 g), which decreased significantly to 0.89 g under severe water stress, corresponding to an 84.52% reduction (Table 28). Statistical analysis revealed that, for both crops, water stress had a significant

negative effect on shoot dry weight; however, no significant differences were observed between the genotypes nor in the interaction between genotype and water stress treatment. Under non-stress conditions, soybean genotypes POCA and YAKARTA produced the highest shoot dry weights (6.17 g and 5.33 g, respectively), whereas under severe water deficit, shoot dry weight declined sharply in both genotypes (POCA: 0.96 g; YAKARTA: 0.81 g), with no significant difference between them (Table 28). Overall, these results indicate that increasing water deficit levels substantially reduced shoot biomass in both chickpea and soybean, while genotypic variation within each crop did not significantly influence shoot dry weight under either stress or control conditions.

Table 28. Effect of PEG-induced water deficit stress on shoot dry weight (g) of two chickpea (Elmo and Orion) and soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced Stress (MPa)	ELMO	ORION	Avg.	POCA	YAKARTA	Avg.
Control (0)	1.43	2.10	1.77 ^A	6.17 ^a	5.33 ^{ab}	5.75 ^A
-0.2	0.53	1.02	0.78 ^B	3.83 ^{bc}	2.53 ^{cd}	3.18 ^B
-0.4	0.14	0.66	0.40 ^C	2.48 ^{cd}	1.73 ^{cd}	2.11 ^C
-0.6	—	—	—	0.96 ^d	0.81 ^d	0.89 ^D
Average	0.70 ^A	1.26 ^A	—	3.36 ^A	2.60 ^A	—
Variable	Genotypes		PEG		Interaction	
LSD (0.05) Chickpea	5.900 ns		0.1459		10.22 ns	
LSD (0.05) Soybean	1.118		0.7437		2.236	
CV (%) Chickpea	17.86					
CV (%) Soybean	13.84					

4.8. 3 Root-to-shoot dry weight ratio

Chickpea exhibited a pronounced, genotype-dependent adjustment in root-to-shoot ratio under water deficit. Plants under stress (-0.4 and -0.2 MPa) had higher ratios (51.00% and 50.67%) than the control (35.22%), with ELMO showing the highest allocation to roots (61.41%) and markedly higher ratios under stress (88.00% and 69.33%), while ORION maintained lower ratios, particularly under control conditions (22.67%) (Fig. 17). In soybean, the responses were more genotype-specific: POCA showed a decrease in ratio from 41.33% under control to 24.33% at severe stress (-0.6 MPa), whereas YAKARTA increased its ratio from 32.33% to 40.33% under the same conditions (Fig. 18), reflecting adaptive root biomass allocation. Overall, chickpea displayed more consistent and substantial root investment under water-limited conditions, whereas

soybean exhibited selective, genotype-dependent adjustments, highlighting contrasting drought adaptation strategies between the two crops.

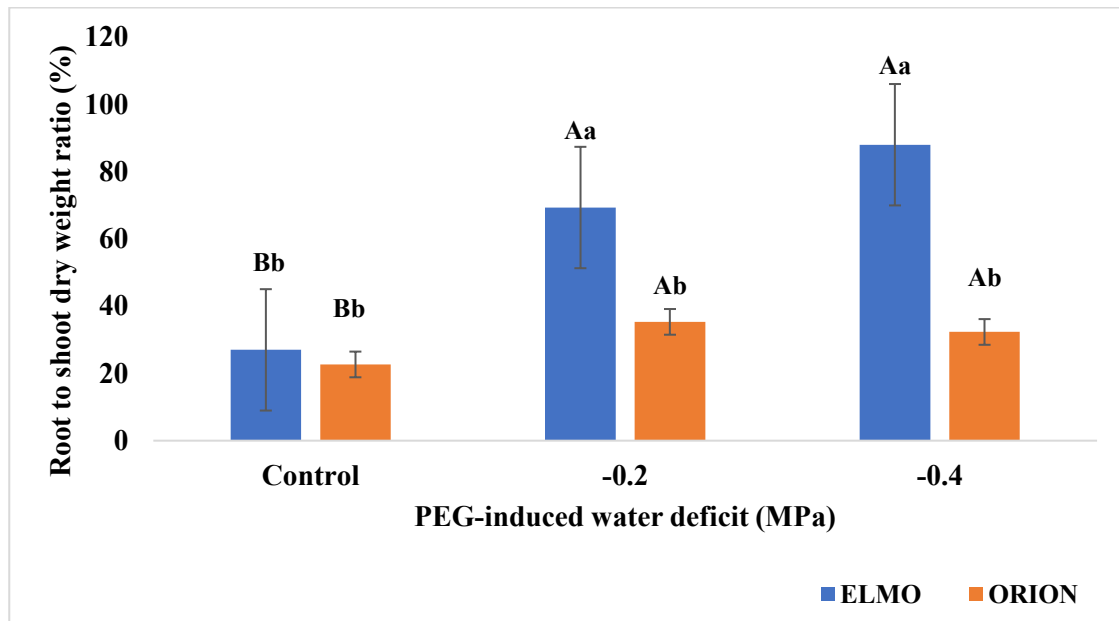


Fig.17. Effect of PEG-induced water deficit on root-to-shoot dry weight ratio (%) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

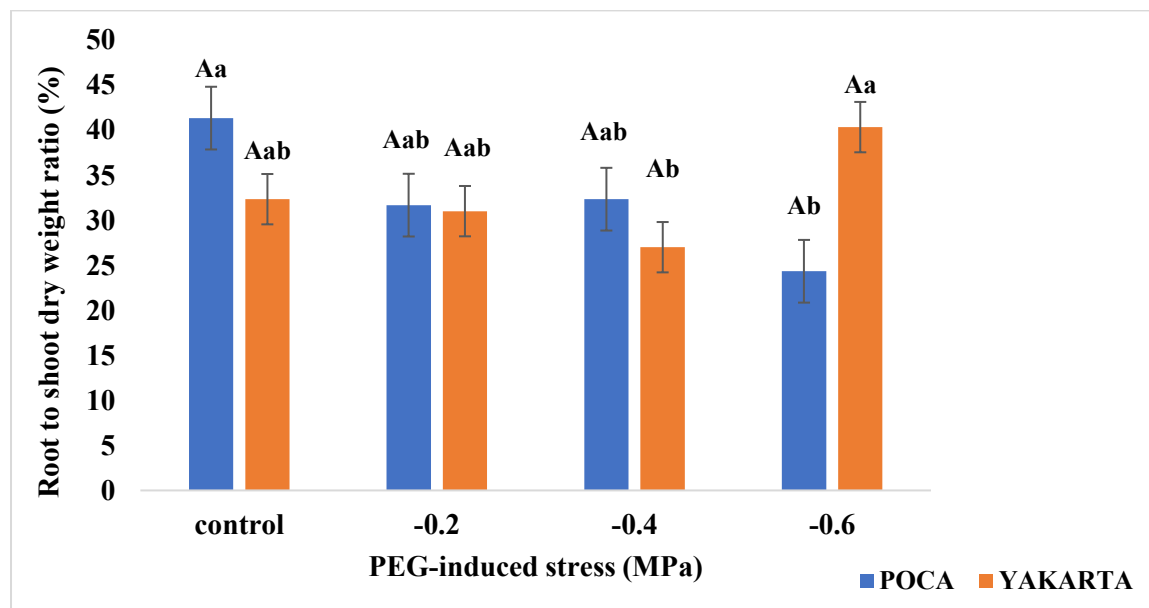


Fig.18. Effect of PEG-induced water deficit on root-to-shoot dry weight ratio (%) of two soybean genotypes (POCA and YAKARTA) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

4.8.4 Biomass accumulation: Absolute growth rate (AGR)

The absolute growth rate in both chickpea and soybean was significantly influenced ($p \leq 0.05$) by water deficit levels, genotypes, and their interactions (Table 29). However, the

response pattern differed between species, reflecting contrasting tolerance mechanisms. In chickpea, ORION consistently maintained a significantly higher AGR (0.38 cm day⁻¹) compared to Elmo (0.27 cm day⁻¹). AGR was highest under control conditions (0.49 cm day⁻¹) but declined sharply with increasing stress, reaching 0.20 cm day⁻¹ at -0.4 MPa. This corresponded to a reduction of 60% compared to the control. Genotypic differences were evident under stress: ORION sustained relatively higher AGR values at -0.2 and -0.4 MPa (0.30 and 0.28 cm day⁻¹, respectively) than ELMO (0.25 and 0.13 cm day⁻¹) (Table 29). In soybean, YAKARTA exhibited significantly higher AGR (0.91 cm day⁻¹) than POCA (0.66 cm day⁻¹). While the control treatment recorded the highest AGR (1.02 cm day⁻¹), severe stress (-0.6 MPa) reduced AGR by more than 52%, with values declining to 0.49 cm day⁻¹. YAKARTA outperformed POCA across all stress levels, maintaining AGR values of 1.02 and 0.82 cm day⁻¹ under moderate (-0.2 MPa) and severe (-0.4 MPa) stress, respectively, compared to 0.82 and 0.57 cm day⁻¹ in POCA. Comparing both species, soybean genotypes exhibited higher absolute AGR values overall compared to chickpea, but chickpea demonstrated relatively greater proportional reductions under stress, particularly in the sensitive genotype ELMO. In both crops, the tolerant genotypes (ORION and YAKARTA) consistently maintained higher AGR values under stress, reflecting stronger adaptive capacity for sustaining growth under water deficit conditions.

Table 29. Effect of PEG-induced water deficit stress on absolute growth rate (AGR) (cm day⁻¹) of two chickpea (ELMO and ORION) and soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	ELMO	ORION	Average	POCA	YAKARTA	Average
Control	0.42 ^{ab}	0.58 ^a	0.50 ^A	0.83 ^C	1.22 ^A	1.02 ^A
- 0.2	0.25 ^{bc}	0.30 ^{bc}	0.27 ^B	0.82 ^C	1.02 ^B	0.92 ^A
- 0.4	0.13 ^c	0.28 ^{bc}	0.20 ^B	0.57 ^D	0.82 ^C	0.70 ^B
-0.6	—	—	—	0.41 ^E	0.57 ^D	0.49 ^C
Average	0.27 ^B	0.38 ^A	—	0.66 ^B	0.91 ^A	—
Variable	Genotypes		PEG Levels		Interaction	
LSD (0.05) Chickpea	0.1094		0.1182		0.1895	
LSD (0.05) Soybean	0.0666		0.1396		0.1331	
CV (%) Chickpea	12.95					
CV (%) Soybean	9.39					

4.8.5 Leaf area persistence: Leaf area duration (LAD)

Both chickpea and soybean exhibited substantial reductions in leaf area duration (LAD) under water-limiting conditions, though genotypic responses and stress sensitivity differed. In chickpea, the ORION genotype maintained consistently higher LAD than ELMO across all treatments, with control and moderate water deficit values of 14,500 and 18,910, respectively, compared to severe stress reductions to 1,993 (ORION) and 1,205 (ELMO), representing an 82.73% decrease relative to control (Fig. 19). In soybean, YAKARTA displayed higher LAD than POCA under both control and PEG-induced stress, with a mean LAD of 13,590 versus 11,460. Progressive water deficit (-0.2 to -0.6 MPa) caused substantial LAD reductions in both genotypes, from 17,990 in the control to 7,020–5,845 under severe stress (Fig. 20). Comparatively, both crops demonstrated that genotypes with inherently higher LAD sustained growth more effectively under mild to moderate water deficit, while severe stress levels led to dramatic reductions in both species. Chickpea showed slightly higher relative LAD retention under moderate stress (ORION at 18,910) than soybean (YAKARTA at 16,590 under -0.2 MPa), suggesting species-specific resilience patterns. These results underscore the importance of selecting genotypes with prolonged leaf area duration to improve growth performance and water stress tolerance across leguminous crops.

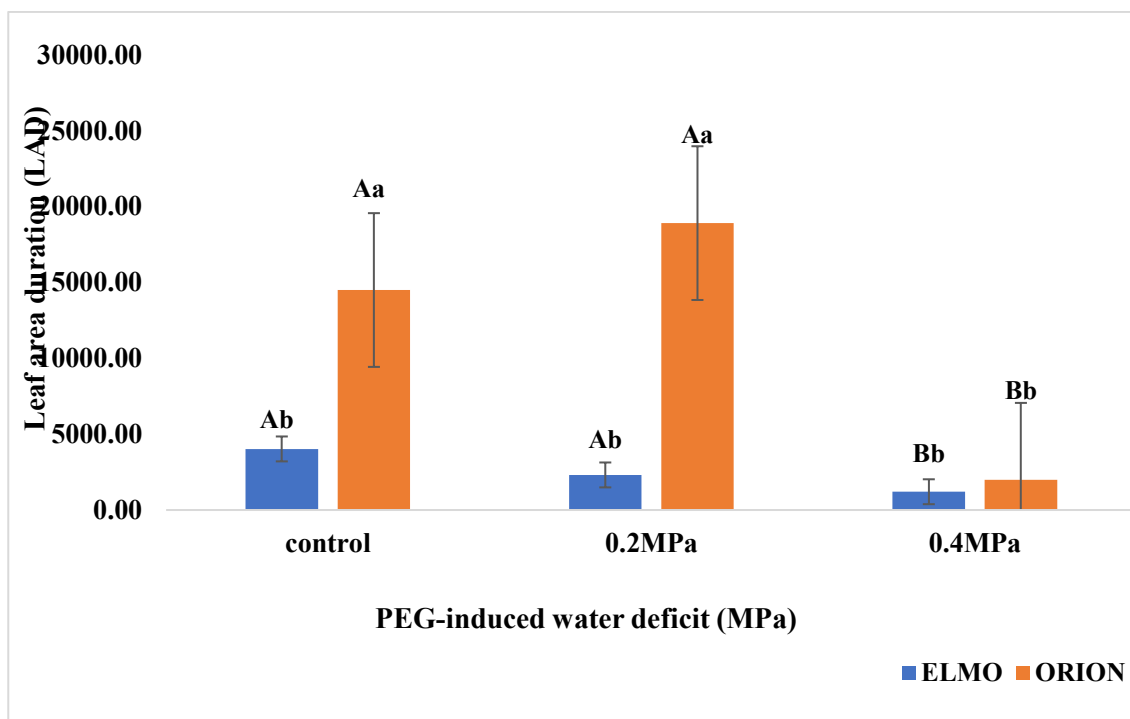


Fig. 19. Effect of PEG-induced water deficit on leaf area duration (LAD) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

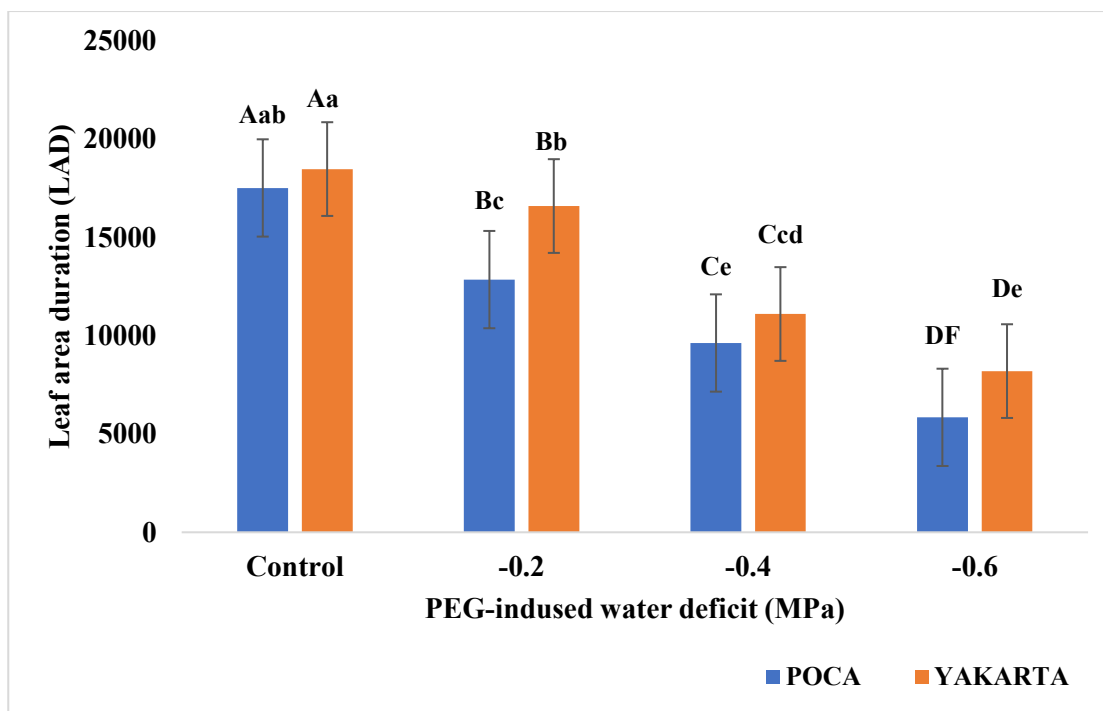


Fig. 20. Effect of PEG-induced water deficit on leaf area duration (LAD) of two soybean genotypes (POCA and YAKARTA) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

4.8.6 Leaf area allocation: Leaf area ratio (LAR)

Both chickpea and soybean exhibited significant modulation of leaf area ratio (LAR) in response to water deficit, although the patterns and magnitude of response differed between species and genotypes. In chickpea, the ORION genotype maintained a higher overall LAR ($190.8 \text{ cm}^2 \text{ g}^{-1}$) than ELMO ($107.1 \text{ cm}^2 \text{ g}^{-1}$). LAR decreased with increasing water deficit, from $208.5 \text{ cm}^2 \text{ g}^{-1}$ under control conditions to $95.78 \text{ cm}^2 \text{ g}^{-1}$ under severe stress, representing a 54.06% reduction. ORION had the highest LAR at control ($308.5 \text{ cm}^2 \text{ g}^{-1}$) but declined sharply to $92.45 \text{ cm}^2 \text{ g}^{-1}$ at severe stress, indicating higher sensitivity compared to ELMO (Fig. 21). In soybean, both genotypes showed a contrasting response, with LAR increasing under PEG-induced osmotic stress. The YAKARTA genotype consistently exhibited higher LAR than POCA, with mean values of 209.7 and $121.1 \text{ cm}^2 \text{ g}^{-1}$, respectively. LAR rose from $106.9 \text{ cm}^2 \text{ g}^{-1}$ under control to $227.0 \text{ cm}^2 \text{ g}^{-1}$ at -0.6 MPa , peaking at $286.2 \text{ cm}^2 \text{ g}^{-1}$ in YAKARTA, highlighting an adaptive leaf morphological response to stress (Fig. 22). Comparatively, chickpea and soybean show species-specific LAR responses to water deficit: chickpea genotypes exhibit a decline in LAR under stress, suggesting stress-induced limitation of leaf expansion, whereas soybean genotypes increase LAR, reflecting a compensatory strategy to maintain photosynthetic surface area. Across both species, genotypes with higher

baseline LAR (ORION in chickpea, YAKARTA in soybean) tend to sustain greater adaptive capacity under moderate stress, emphasizing the importance of genotype selection for water stress resilience.

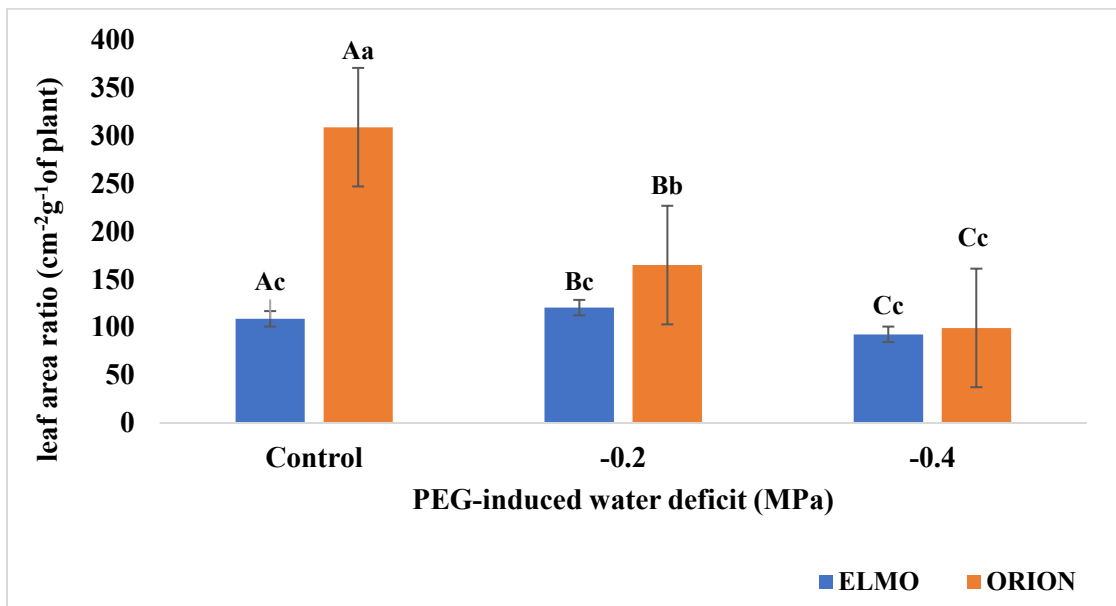


Fig. 21. Effect of PEG-induced water deficit on leaf area ratio (LAR) ($\text{cm}^2 \text{g}^{-1}$) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

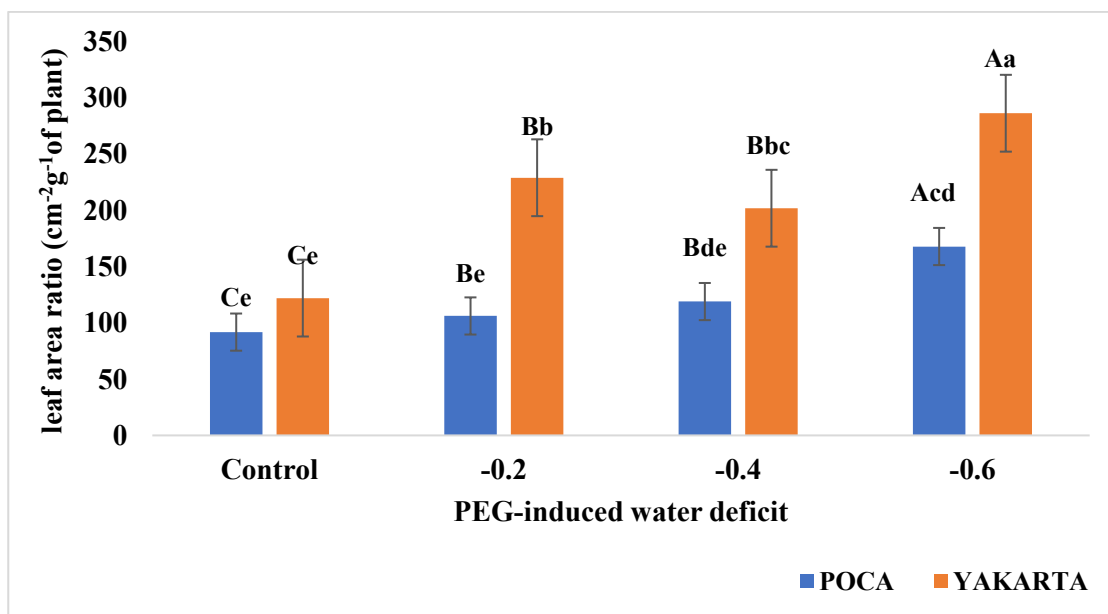


Fig. 22. Effect of PEG-induced water deficit on leaf area ratio (LAR) ($\text{cm}^2 \text{g}^{-1}$) of two soybean genotypes (POCA and YAKARTA) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

4.8.7 Biomass production efficiency: Relative growth rate (RGR)

PEG-induced water deficit significantly reduced the relative growth rate (RGR) in both chickpea and soybean, with genotypic differences in tolerance. In chickpea, ELMO had a slightly higher RGR under control conditions ($0.02293 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$) than ORION ($0.02060 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$), but under severe stress (-0.4 MPa), ELMO declined sharply ($0.00447 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$) while ORION maintained a higher RGR ($0.01230 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$), resulting in a higher average RGR for ORION ($0.01614 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$) (Table 30). Similarly, in soybean, POCA consistently outperformed YAKARTA, retaining higher RGR under stress (0.00927 vs. $0.00353 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ at -0.6 MPa) and a greater average RGR (0.01961 vs. $0.01488 \text{ g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$) (Table 30). Overall, both crops exhibited marked reductions in RGR under water deficit. Among the tested genotypes, ORION showed superior drought tolerance in chickpea, while POCA performed better than YAKARTA in soybean, although the overall stress impact on soybean was more pronounced.

Table 30. Effect of PEG-induced water deficit stress on relative growth rate (RGR) ($\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$) of two chickpea (ELMO and ORION) and soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	ELMO	ORION	Average	POCA	YAKARTA	Average
Control	0.02293 ^a	0.02060 ^{ab}	0.02177 ^A	0.02907 ^a	0.02417 ^a	0.02662 ^A
- 0.2	0.01217 ^c	0.01553 ^{bc}	0.01385 ^B	0.02340 ^a	0.01697 ^b	0.02018 ^B
- 0.4	0.00447 ^d	0.01230 ^c	0.00838 ^C	0.01670 ^b	0.01483 ^{bc}	0.01577 ^C
-0.6	—	—	—	0.00927 ^{cd}	0.00353 ^d	0.00640 ^D
Average	0.01319 ^A	0.01614 ^A	—	0.01961 ^A	0.01488 ^B	—
Variable	Genotypes		PEG Levels		Interaction	
LSD (0.05) Chickpea	0.003648		0.004467		0.006318	
LSD (0.05) Soybean	0.002971		0.004210		0.005954	
CV (%) Chickpea	11.72					
CV (%) Soybean	15.08					

PEG-induced water deficit had a pronounced impact on both morphological traits and growth parameters in chickpea and soybean, though the magnitude of response varied considerably among genotypes, reflecting their inherent genetic differences in water deficit tolerance. In both species, plant height, root length, shoot fresh and dry weights, and growth indices such as AGR, RGR, LAR, and LAD declined significantly as the PEG concentration increased, yet the degree of reduction differed between tolerant and

sensitive genotypes. For instance, in chickpea, ORION maintained higher shoot biomass and growth indices compared to the highly sensitive ELMO, highlighting superior drought tolerance. In soybean, the response was more complex. POCA accumulated a higher relative growth rate (RGR) under stress compared to YAKARTA, suggesting faster biomass gain in the short term. However, YAKARTA exhibited higher leaf area ratio (LAR) and leaf area duration (LAD), indicating greater persistence of photosynthetic leaf area despite slower relative growth. This contrast reflects a trade-off between rapid biomass accumulation and sustained leaf area maintenance under water deficit. While POCA's higher RGR may result from efficient allocation of resources to growth, YAKARTA's greater LAR and LAD could support prolonged photosynthesis and delayed senescence, offering potential advantages for long-term productivity under intermittent water stress. Thus, within soybean, genotypic differences reveal distinct adaptive strategies: POCA emphasizes short-term growth, whereas YAKARTA favors leaf area persistence and sustained physiological activity. These findings align with previous studies that have observed similar patterns in soybean genotypes under drought conditions. For instance, a study by Hossain et al. (2024) reported that water deficit led to substantial changes in soybean genotypes, including variations in leaf area and growth rates, supporting the notion of distinct adaptive strategies among genotypes under water deficit stress. Additionally, research by Aziez et al. (2022) demonstrated that water deficit significantly affected leaf area duration in soybean, with tolerant genotypes maintaining higher LAD, which is consistent with the observed persistence of leaf area in YAKARTA under stress.

Across species, shoot growth was generally more adversely affected than root growth, leading to an increased root-to-shoot ratio at higher water deficit levels, a well-documented adaptive strategy that promotes deeper and more extensive rooting, allowing plants to exploit residual soil moisture and sustain metabolic activity during water scarcity (Osmont et al., 2007; Ramamoorthy et al., 2017). Chickpea maintained root growth more effectively under stress, whereas soybean, particularly YAKARTA, tended to develop longer, and more branched fibrous roots under water deficit conditions, consistent with previous findings on water deficit-tolerant soybean lines (Zou et al., 2020; Fatema et al., 2023). These patterns indicate that chickpea adapts more through conservative resource allocation and restricted shoot growth, while soybean responds by investing heavily in root proliferation, explaining species-specific differences in water deficit tolerance strategies.

The overall reductions in shoot fresh weight and dry weight confirm that both crops experience severe biomass penalties under water deficit, largely due to impaired cell expansion, reduced leaf area, and inhibited photosynthesis driven by stomatal closure, lower CO₂ assimilation, and loss of turgor (Farooq et al., 2009; Munns and Tester, 2008). Yet, the tolerant genotypes maintained higher LAD and RGR under stress, suggesting better efficiency in assimilate partitioning and superior water-use efficiency, aligning with studies on chickpea and soybean genotypes that reported enhanced growth maintenance under water deficit (Seyed et al., 2015; Gullap et al., 2024; Petcu et al., 2024).

Collectively, these results underscore that chickpea and soybean, although both sensitive to PEG-induced water deficit, exhibit contrasting adaptive strategies. Chickpea genotypes like ORION sustain growth through conservative biomass allocation and improved shoot water status, whereas soybean genotypes display a spectrum of strategies: POCA emphasizes rapid biomass accumulation, while YAKARTA maintains leaf area and photosynthetic duration under stress. Exploiting such differential adaptive mechanisms could be a powerful breeding strategy, favoring root-based tolerance in soybean and shoot-water conservation in chickpea, to develop resilient cultivars capable of maintaining productivity under increasing drought scenarios associated with climate change.

4.9 Physiological response to PEG-induced water deficit

4.9.1 Relative chlorophyll content (SPAD)

The results demonstrated that water deficit stress significantly reduced the relative chlorophyll content (SPAD) in both chickpea and soybean, with clear genotypic differences in their ability to maintain leaf greenness under stress. In chickpea, ORION consistently recorded higher SPAD values (40.87) than ELMO (32.92). Compared to the control. Chlorophyll content decreased by 78% in ELMO and by 66% in ORION, with ORION maintaining significantly higher values across treatments (Table 31).

Table 31. Effect of PEG-induced water deficit on Relative chlorophyll content (SPAD) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments.

PEG-induced stress (MPa)	ELMO	ORION	Average
control	53.27 ^b	62.83 ^a	58.05 ^A
-0.2	33.73 ^c	38.07 ^c	35.9 ^B
-0.4	11.77 ^e	21.70 ^d	16.73 ^C
Average	32.92 ^B	40.87 ^A	-
Variable	Genotypes	PEG levels	Interaction
LSD (0.05)	2.856	2.596	4.946
CV (%)	7.69		

In soybean, YAKARTA maintained higher SPAD values (37.83) compared to POCA (34.83). Under severe stress (−0.6 MPa), the SPAD value decreased by 42% in YAKARTA and by 48% in POCA (Table 32).

Table 32. Effect of PEG-induced water deficit on Relative chlorophyll content (SPAD value) of two soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments.

PEG-induced stress (MPa)	POCA	YAKARTA	Average
control	42.33 ^b	47.33 ^a	44.83 ^A
-0.2	40.67 ^{bc}	39.67 ^{bc}	40.17 ^B
-0.4	34.33 ^d	37.00 ^{cd}	35.67 ^C
-0.6	22.00 ^f	27.33 ^e	24.67 ^D
Average	34.83 ^B	37.83 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.05)	2.415	3.805	4.831
CV (%)	7.06		

Our results show that ORION in chickpea and YAKARTA in soybean maintained higher chlorophyll content under PEG-induced water deficit compared to ELMO and POCA, indicating stronger protection of the photosynthetic apparatus and delayed chlorophyll degradation. This genotypic variation in SPAD under water deficit aligns with previous findings, where chickpea genotypes with higher chlorophyll retention exhibited greater drought tolerance (Ucak and Arslan, 2023), and soybean lines with stable chlorophyll content under PEG stress showed improved photosynthetic efficiency and resilience (Basal and Szabó, 2020). These results suggest that maintaining chlorophyll under water

deficit is a key adaptive mechanism contributing to better growth and stress tolerance in both species.

4.9.2 Stomatal conductance under PEG-induced water deficit

Stomatal conductance (g_s) is a highly sensitive physiological trait under water deficit, as it directly regulates transpiration and CO_2 assimilation. In the present study, g_s decreased significantly in both chickpea and soybean with increasing PEG-induced water deficit stress, confirming the responsiveness of stomatal behavior to water availability.

In chickpea, g_s was highest under control conditions ($501.7 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and declined sharply under severe stress ($70.8 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ at -0.4 MPa), corresponding to an 85.9% reduction. Among genotypes, ELMO maintained consistently higher g_s ($295.9 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than ORION ($224.8 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), particularly under non-stress conditions, although both genotypes exhibited pronounced stomatal closure under severe stress (Fig. 23).

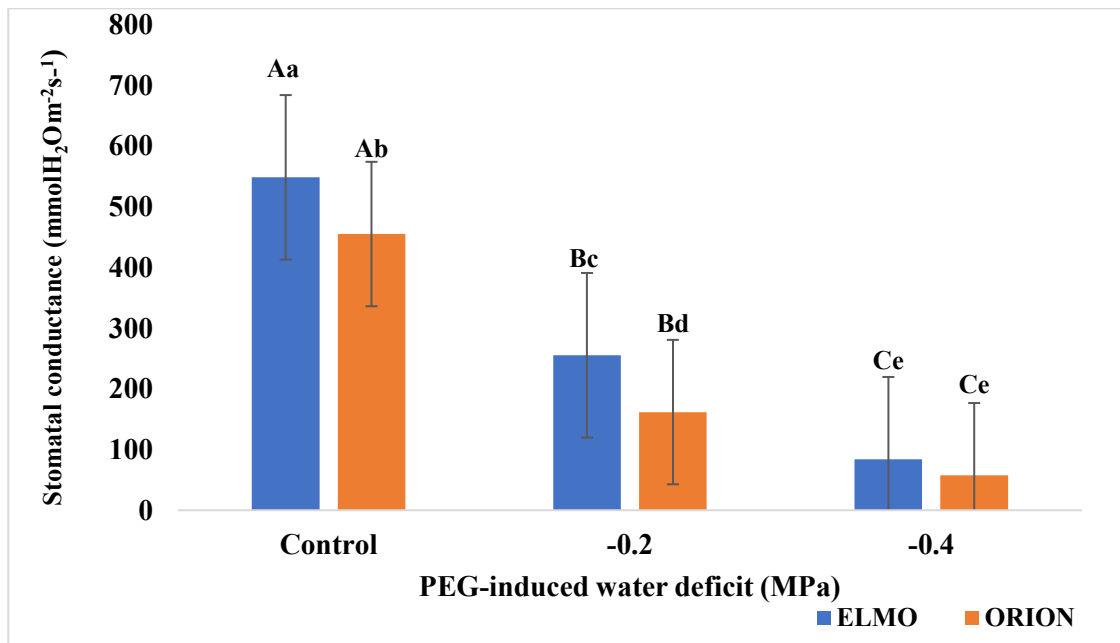


Fig. 23. Effect of PEG-induced water deficit on stomatal conductance (g_s) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

Similarly, in soybean, g_s decreased from $531.5 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ under control to $171.2 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ at -0.6 MPa , representing a 67.8% reduction. Genotype YAKARTA exhibited higher g_s ($399.7 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than POCA ($298.8 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), although both reached comparably low g_s values under severe water deficit (Fig. 24).

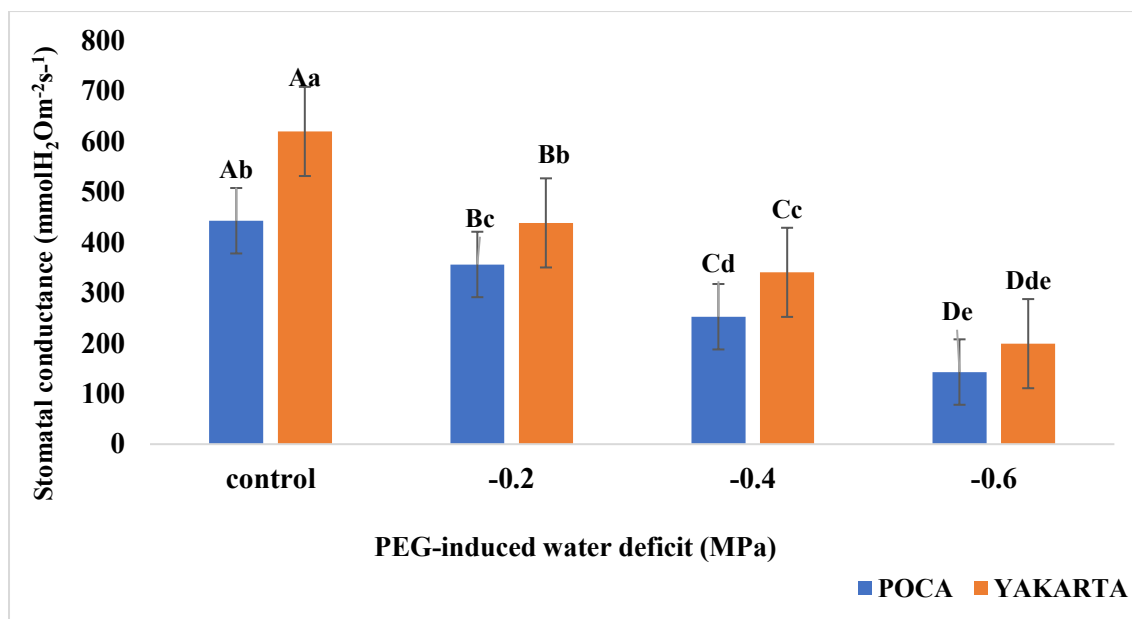


Fig. 24. Effect of PEG-induced water deficit on stomatal conductance (g_s) ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two chickpea genotypes (POCA and YAKARTA) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

These results indicate that chickpea genotypes experienced a larger relative reduction in g_s than soybean, yet genotype-specific differences were evident. ELMO (chickpea) and YAKARTA (soybean) maintained higher g_s under moderate stress, suggesting a capacity to support photosynthetic activity and biomass accumulation under mild water deficit. Conversely, genotypes with lower g_s under stress (ORION and POCA) likely adopt a more conservative water-use strategy, minimizing transpirational losses at the expense of carbon assimilation and growth. The observed reduction in g_s under PEG-induced stress is consistent with previous findings. Water deficit triggers a well-documented reduction in stomatal conductance in legumes like soybean and chickpea, a primary adaptation to conserve water and maintain leaf turgor (Ruppenthal et al., 2016; Farooq et al., 2009). This response is mediated by abscisic acid (ABA), which accumulates under soil moisture deficit and induces stomatal closure (Reddy et al., 2004). While this mechanism protects against dehydration, prolonged closure can severely limit carbon assimilation and reduce chlorophyll content, ultimately impairing productivity. Tolerant genotypes often exhibit superior regulatory control, as evidenced in soybean where higher stress-induced ABA levels correlate with more effective stomatal regulation and water conservation compared to sensitive lines (Mutava et al., 2015). Mechanistically, stomatal conductance is more directly coupled to transpiration than to photosynthetic rate, highlighting its primary role in managing water loss (Wang et al., 2006).

The genotypic differences observed in this study provide valuable insights for breeding programs. Selection for genotypes that balance sufficient g_s for photosynthesis while minimizing water loss may improve water deficit resilience in both chickpea and soybean. Such an approach could optimize water-use efficiency, maintain photosynthetic activity, and sustain productivity under water-limited conditions.

4.9.3 Leaf relative water content dynamics under PEG-induced water deficit

Leaf relative water content (RWC) differed significantly ($p \leq 0.05$) among genotypes, water deficit levels, and their interaction in both chickpea and soybean. In chickpea, RWC was highest under control conditions (83.17%) and declined progressively with increasing PEG-6000-induced water deficit stress, reaching 58.5% at the highest stress level (30% reduction). Among genotypes, ORION maintained a significantly higher RWC (76.22%) than ELMO (65.67%) under water deficit. Under control conditions, Orion also exhibited the highest RWC (87.67%) compared to Elmo (78.67%), indicating greater capacity to sustain leaf water status under stress (Table 33).

Table 33. Effect of PEG-induced water deficit on leaf relative water content (%) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	ELMO	ORION	Average
control	78.67 ^b	87.67 ^a	83.17 ^A
-0.2	65.00 ^c	77.33 ^b	71.17 ^B
-0.4	53.33 ^d	63.67 ^c	58.5 ^C
Average	65.67 ^B	76.22 ^A	-
Variable	Genotypes	PEG levels	Interaction
LSD (0.05)	1.817	2.970	3.147
CV (%)	2.57		

In soybean, RWC was highest under control conditions (88.17%) and decreased significantly with increasing water deficit stress, reaching 60.83% at the highest PEG-6000 concentration (31% reduction). Under water deficit, YAKARTA maintained a higher RWC (78.50%) than POCA (71.25%). under control conditions, RWC was higher in POCA (90.00%) than in YAKARTA (86.33%), whereas under severe stress, POCA exhibited the lowest RWC (53.67%). These findings suggest that YAKARTA sustains leaf water status more effectively under water deficit, reflecting higher water deficit tolerance (Table 34). Overall, ORION and YAKARTA demonstrate superior ability to

maintain leaf hydration under water deficit, highlighting their potential for cultivation in drought-prone environments.

Table 34. Effect of PEG-induced water deficit on leaf relative water content (%) of two soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	POCA	YAKARTA	Average
control	90.00 ^a	86.33 ^b	88.17 ^A
-0.2	72.00 ^c	82.00 ^c	77.00 ^B
-0.4	69.33 ^{ef}	77.67 ^d	73.50 ^C
-0.6	53.67 ^g	68.00 ^f	60.83 ^D
Average	71.25 ^B	78.50 ^A	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.05)	1.697	2.431	3.394
CV (%)	2.41		

Relative water content (RWC) is a key physiological parameter reflecting leaf water status, metabolic function, and overall plant performance under drought. In this investigation, water deficit significantly lowered RWC in all chickpea and soybean genotypes. However, the genotypes identified as tolerant—ORION in chickpea and YAKARTA in soybean—maintained significantly higher RWC values compared to their more susceptible counterparts, ELMO and POCA, respectively. This finding is consistent with prior research demonstrating that drought-resilient soybean varieties are characterized by a superior capacity to retain cellular water under stress conditions (Chowdhury et al., 2017; Souza et al., 2023; Dong et al., 2019). The observed genotypic differences in RWC are closely associated with osmotic adjustment mechanisms, particularly proline accumulation. In chickpea and soybean, the genotypes ORION and YAKARTA exhibited higher proline content (8.490 and 5.472 $\mu\text{mol g}^{-1}$ FW., respectively) than ELMO and POCA (4.22 and 4.171 $\mu\text{mol g}^{-1}$ FW., respectively) under water deficit, which likely contributed to maintaining higher leaf water potential and RWC. Proline functions as a compatible solute, reducing cell water potential and enhancing the water uptake gradient, thus supporting cellular hydration under drought (Ismail and Horie, 2017). These findings support the view that proline serves as an indicator of plant water status rather than a direct measure of tolerance, as its accumulation is influenced by genotype, developmental stage, and RWC. Maintaining high RWC is also critical for preserving cellular membrane integrity and ensuring

stomatal function under stress (Sinclair and Ludlow, 1985). Consistent with this, stomatal conductance (g_s) was higher in YAKARTA compared to POCA and declined with increasing water deficit, indicating that superior leaf hydration supports stomatal regulation and gas exchange under drought (Reynolds et al., 2000). Overall, the ability of ORION and YAKARTA to sustain higher RWC under water deficit highlights their drought-resilient physiological traits. These findings reinforce the importance of RWC, osmotic adjustment, and stomatal conductance as reliable indicators for screening water deficit tolerance in chickpea and soybean. Integrating these physiological traits in breeding programs can facilitate the selection of genotypes capable of maintaining productivity under water-limited conditions.

4.10 Chlorophyll pigments and carotenoids dynamics under water deficit

Statistical analysis revealed significant differences ($p \leq 0.05$) in chlorophyll a (Chl a) and chlorophyll b (Chl b) content between genotypes, PEG-induced water deficit levels, and their interaction, whereas the Chl a/b ratio showed variable responses. In chickpea, Chl a and Chl b decreased progressively with increasing water deficit. The highest Chl a (16.77 mg g⁻¹ FW) and Chl b (6.43 mg g⁻¹ FW) were recorded under control conditions, while the lowest values occurred at -0.4 MPa (Chl a: 6.85 mg g⁻¹ FW; Chl b: 3.37 mg g⁻¹ FW), representing reductions of 59% and 48%, respectively. Genotypic differences were evident: ORION maintained higher average Chl a (14.19 mg g⁻¹ FW) and Chl b (6.76 mg g⁻¹ FW) compared to ELMO (Chl a: 11.68 mg g⁻¹ FW; Chl b: 3.36 mg g⁻¹ FW), indicating superior retention of photosynthetic pigments under water deficit. Conversely, the Chl a/b ratio did not show significant variation between genotypes or stress levels, ranging from 1.97 to 2.20, suggesting proportional degradation of both pigments under stress conditions (Table 35).

Table 35. Effect of NaCl-induced salinity stress on photosynthetic pigments of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity Level (mM NaCl)	Chl.a (ELMO)	Chl.a (ORION)	Chl.b (ELMO)	Chl.b (ORION)	Chl a/b (ELMO)	Chl a/b (ORION)
Control	16.91 ^a	16.63 ^a	5.07 ^b	7.78 ^a	1.76	2.17
-0.2	13.77 ^b	16.59 ^a	3.01 ^c	7.78 ^a	2.18	2.13
-0.4	4.35 ^d	9.35 ^c	1.10 ^c	4.73 ^b	2.42	1.97
Average	11.68 ^B	14.19 ^A	3.361 ^B	6.76 ^A	2.12 ^A	2.09 ^A
Variable	Genotypes		Salinity Levels		Interaction	
Chlorophyll-a	0.558		0.726		0.966	
Chlorophyll-b	0.771		1.073		1.336	
Chl a/b Ratio	ns		ns		0.989	
CV (%) Chl.a	4.16					
CV (%) Chl.b	13.54					
CV (%) Chl a/b	15.49					

In soybean, Chl.-a and Chl.-b were also significantly affected by water deficit. The highest Chl.-a and Chl.-b were recorded under control conditions (Chl.-a: 18.81 mg g⁻¹ FW; Chl.-b: 11.13 mg g⁻¹ FW), while the lowest occurred at -0.6 MPa (Chl.-a: 6.94mg g⁻¹ FW; Chl.-b: 3.69 mg g⁻¹ FW), representing reductions of 63% and 67%, respectively. Genotypic differences showed that YAKARTA retained higher Chl.-b (10.18 mg g⁻¹ FW) and moderate Chl.-a (13.51 mg g⁻¹ FW average) compared to POCA (Chl.-b: 5.56 mg g⁻¹ FW; Chl.-a: 13.44 mg g⁻¹ FW average). The Chl a/b ratio was significantly higher in POCA (2.59) than in YAKARTA (1.43), particularly at moderate water deficit levels, indicating differences in pigment composition and potential stress adaptation mechanisms (Table 36). Overall, both species showed a progressive decrease in Chl.-a and Chl b under increasing PEG-induced water deficit, with genotypic differences highlighting superior pigment retention in ORION (chickpea) and YAKARTA (soybean), whereas the Chl a/b ratio remained relatively stable in chickpea but varied significantly in soybean under moderate stress. These results indicate that maintenance of chlorophyll content is an important physiological trait for water deficit tolerance in both species.

Table 36. Effect of NaCl-induced salinity stress on carotenoids (mg·g⁻¹ FW) of two soybean (POCA and YAKARTA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

Salinity Level (mM NaCl)	Chl.a (YAKARTA)	Chl.a (POCA)	Chl.b (YAKARTA)	Chl.b (POCA)	Chl a/b (YAKARTA)	Chl a/b (POCA)
Control	18.55 ^b	19.07 ^a	12.88 ^a	9.38 ^b	1.45 ^{bc}	2.04 ^{bc}
-0.2	13.39 ^d	17.22 ^c	11.66 ^{ab}	5.43 ^c	1.15 ^c	3.92 ^a
-0.4	12.59 ^e	13.12 ^d	11.31 ^{ab}	4.91 ^c	1.12 ^c	2.68 ^{ab}
-0.6	9.51 ^f	4.36 ^g	4.87 ^c	2.52 ^d	1.99 ^{bc}	1.73 ^{bc}
Average	13.51 ^A	13.44 ^A	10.18 ^A	5.56 ^B	1.43 ^B	2.59 ^A
Variable	Genotypes		Salinity Levels		Interaction	
Chlorophyll-a	ns		0.651		0.457	
Chlorophyll-b	10.18		8.541		2.451	
Chl a/b Ratio	0.658		ns		1.315	
CV (%) Chl.a	1.81					
CV (%) Chl.b	16.54					
CV (%) Chl.a/b	14.74					

These findings are in accordance with Wu et al. (2019), who reported that water deficit significantly decreases Chl.-a, Chl.-b, and total chlorophyll in soybean leaves. Similarly, Petcu et al., (2024) observed that fourteen days of water stress significantly reduced total chlorophyll content in soybean, although rehydration could rapidly reverse these effects. The Chl a/b ratio in chickpea remained relatively stable across genotypes and stress levels, indicating a proportional decline of both pigments under water deficit. In soybean, the Chl a/b ratio was significantly higher in POCA than in YAKARTA, particularly under moderate water deficit (−0.2 and −0.4 MPa), suggesting genotypic differences in the relative balance of chlorophyll pigments and potential adaptive mechanisms. The lack of consistent effect of water deficit on Chl a/b ratio indicates that Chl.-b is not necessarily more water deficit-sensitive than Chl.-a (Jampeetong and Brix, 2009). The reduction in chlorophyll content under water stress can be attributed to multiple physiological and biochemical mechanisms. Water deficit can impair chlorophyll synthesis or increase chlorophyll degradation via enhanced chlorophyllase activity (Santos, 2004). Moreover, water deficit-induced oxidative stress generates reactive oxygen species (ROS) in chloroplast thylakoid membranes, which accelerates pigment oxidation and degradation (Reddy et al., 2004). The observed higher Chl.-a compared to Chl.-b in both species at all stress levels aligns with previous reports that Chl.-a is generally more abundant and may be relatively more stable under water deficit (Jain et al., 2010; Mafakheri et al., 2010). Genotypic differences observed in this study, with ORION (chickpea) and

YAKARTA (soybean) maintaining higher chlorophyll contents under stress, suggest superior drought tolerance, likely associated with better maintenance of photosynthetic apparatus and lower oxidative damage. Overall, these results confirm that chlorophyll content is a rapid and reliable physiological indicator of plant response to water deficit, with implications for selecting water deficit-tolerant genotypes in chickpea and soybean breeding programs.

The statistical analysis showed significant differences ($p \leq 0.05$) in leaf carotenoid content between water deficit levels, genotypes, and their interaction in both chickpea and soybean. In chickpea, the average leaf carotenoid content decreased from 6.36 mg g⁻¹ FW in the control to 2.94 mg g⁻¹ FW at -0.4 MPa, representing a 54% reduction. Both ELMO and ORION maintained higher carotenoid levels (4.958 and 5.231 mg g⁻¹ FW, respectively), with ORION showing a smaller decline under severe stress, indicating higher adaptive capacity (Fig. 25).

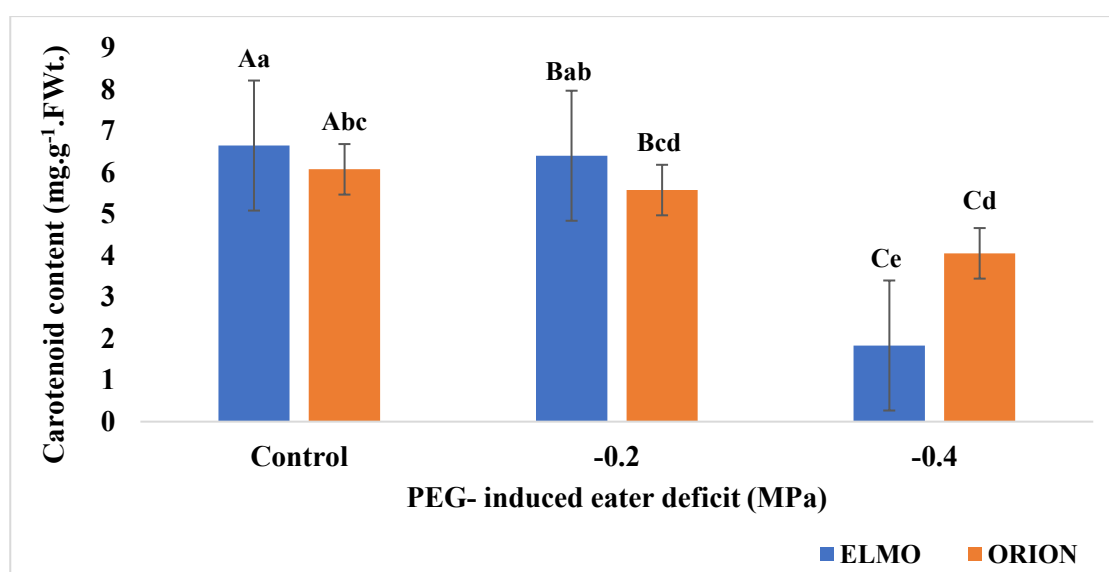


Fig. 25. Effect of PEG-induced water deficit on carotenoid content (mg g⁻¹ FW) of two chickpea genotypes (ELMO and ORION) under hydroponic conditions ($n = 3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

In soybean, carotenoids decreased more markedly from 5.562 mg g⁻¹ FW in the control to 1.657 mg g⁻¹ FW at -0.6 MPa, a reduction of 70%. The POCA genotype maintained higher carotenoid content (4.087 mg g⁻¹ FW) compared to YAKARTA (3.428 mg g⁻¹ FW), particularly under mild to moderate stress, while YAKARTA had a slower decline under severe stress (Fig. 26). These results indicate that water deficit negatively affects carotenoid biosynthesis in both species, but genotypic differences in maintenance of

carotenoid content suggest varying degrees of tolerance, likely associated with photoprotection and reduced photoinhibition under stress.

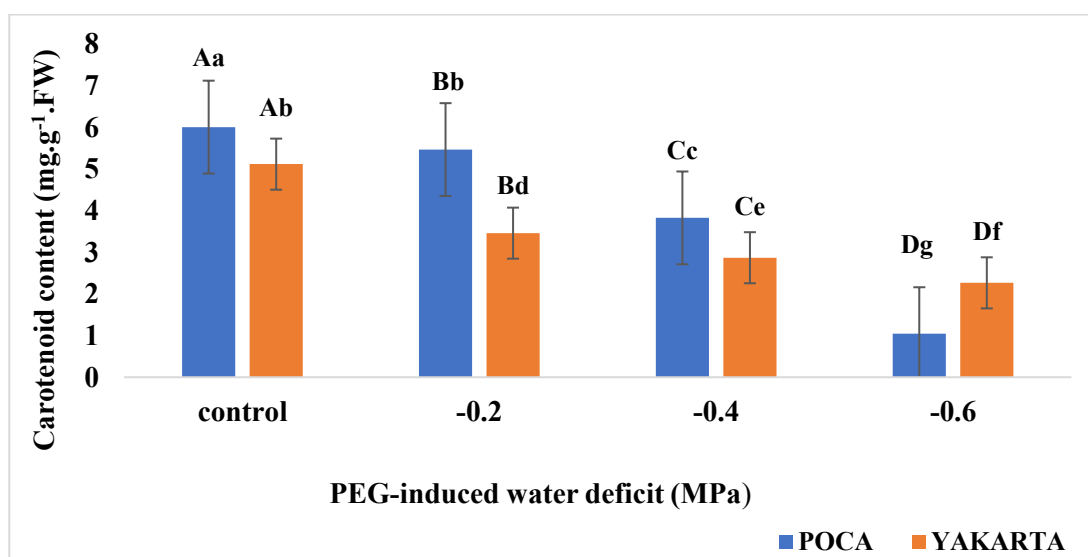


Fig. 26. Effect of PEG-induced water deficit on carotenoid content (mg g⁻¹ FW) of two soybean genotypes (YAKARTA and POCA) under hydroponic conditions (n = 3, ± s.e.). Different capital letters among salinity levels indicate significant differences (p ≤ 0.05). Different small letters at each salinity level between genotypes indicate significant differences (p ≤ 0.05).

The results revealed a significant decrease in leaf carotenoid content in both chickpea and soybean under increasing PEG-induced water deficit, with the lowest values recorded at the highest stress levels. Genotypic differences were evident: ORION (chickpea) and POCA (soybean) maintained higher carotenoid content under mild stress, while ELMO and YAKARTA showed lower levels. Although POCA exhibited relatively high carotenoid content under control and mild water deficit conditions (6.007 and 5.467 mg g⁻¹ FW, respectively). This likely reflects a baseline photoprotective mechanism, supporting normal photosynthetic activity and mitigating early oxidative stress. However, under severe water deficit (-0.6 MPa), POCA's carotenoid content declined sharply to 1.047 mg g⁻¹ FW., indicating that its adaptive capacity is limited and insufficient to sustain photoprotective carotenoids under prolonged stress. In contrast, more tolerant genotypes such as YAKARTA or ORION exhibited slower declines, maintaining higher carotenoid content under severe stress, highlighting their superior capacity for photoprotection. The decline in carotenoid content under higher water deficit levels is attributed to dehydration-induced beta-carotene catabolism and the conversion to zeaxanthins, which protect against photoinhibition (Sultana et al., 1999). Carotenoids are associated with chlorophyll molecules and photosystem proteins, acting as a safety valve that regulates energy transfer to chlorophyll, preserves photosynthetic efficiency,

and prevents photo-oxidative damage (Jaleel et al., 2009). Previous studies support these observations: carotenoid content can increase or decrease under drought depending on species, stress severity, and duration (Gajanayake and Reddy, 2016; Wijewardana et al., 2016). The reduction observed in this study aligns with the general effect of water deficit on photosynthetic pigments, contributing to decreased photosynthesis and potential growth limitation. Genotypic variation in carotenoid retention, especially under severe stress, emphasizes the importance of carotenoids as an adaptive trait for maintaining photosynthetic performance and mitigating photoinhibition.

4.11 Osmolyte accumulation and membrane stability

4.11.1 Proline accumulation

Leaf proline content differed significantly ($p \leq 0.05$) among genotypes, water deficit levels, and their interaction in both chickpea and soybean. In chickpea, proline accumulation increased progressively with increasing PEG-6000-induced water deficit, reaching the highest value in ORION at -0.4 MPa ($13.91 \mu\text{mol g}^{-1} \text{FW}$), while it was lowest under control. (Table 37).

Table 37. Effect of PEG-induced water deficit on proline content ($\mu\text{mol g}^{-1} \text{FW}$) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	ELMO	ORION	Average
control	2.29 ^f	3.59 ^e	2.94 ^C
-0.2	4.29 ^d	7.96 ^b	6.13 ^B
-0.4	6.11 ^c	13.91 ^a	10.01 ^A
Average	4.23 ^B	8.49 ^A	-
Variable	Genotypes	PEG levels	Interaction
LSD (0.05)	0.3248	0.5041	0.5626
CV (%)	5.16		

Similarly, in soybean, proline content increased with stress, reaching $9.66 \mu\text{mol g}^{-1} \text{FW}$ in YAKARTA at -0.6 MPa, while it was lowest under control conditions (Table 38). Overall, water deficit induced substantial increases in proline content, indicating enhanced osmotic adjustment in response to stress. Among genotypes, ORION (chickpea) and YAKARTA (soybean) accumulated significantly higher proline compared to ELMO and POCA, respectively, across all stress levels. This suggests that

these genotypes have a greater capacity for osmotic adjustment, which helps maintain cell turgor and relative water content under drought conditions.

Table 38. Effect of NaCl-induced salinity stress on proline content ($\mu\text{mol g}^{-1}$ FW) of two soybean (POCA and YAKARAT) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	POCA	YAKARTA	Average
control	1.37 ^f	2.11 ^e	1.74 ^D
-0.2	3.89 ^d	3.73 ^d	3.81 ^C
-0.4	5.24 ^c	6.40 ^b	5.82 ^B
-0.6	6.19 ^b	9.66 ^a	7.92 ^A
Average	4.17 ^B	5.47 ^A	-
Variable	Genotypes	PEG levels	Interaction
LSD (0.05)	0.3065	0.3904	0.6130
CV (%)	6.74		

Proline accumulation is a well-established osmotic adjustment mechanism that helps plants maintain turgor and leaf hydration under water deficit. In this study, both chickpea and soybean genotypes exhibited significant increases in leaf proline content under PEG-induced water deficit stress, with tolerant genotypes (ORION and YAKARTA) accumulating higher proline than susceptible ones (ELMO and POCA). These findings align with earlier studies reporting elevated proline levels in drought-tolerant chickpea and soybean genotypes under stress (Chowdhury et al., 2017; Souza et al., 2023; Kulundžić et al., 2022). Proline functions as a key osmolyte, reducing cellular water potential and strengthening the water potential gradient between the root zone and leaf tissues. This process enhances osmotic-driven water uptake during drought (Ismail & Horie, 2017). By supporting turgor maintenance, proline accumulation contributes to the preservation of relative water content (RWC) and stomatal conductance, which are critical for sustaining photosynthesis and metabolic function under water-limited conditions (Sinclair & Ludlow, 1985; Reynolds et al., 2000). The higher proline accumulation in ORION and YAKARTA aligns with their superior RWC observed under water deficit, highlighting the role of proline as a physiological indicator of plant water status. These findings suggest that proline content, together with RWC and stomatal conductance, can be effectively used to screen chickpea and soybean genotypes for water deficit tolerance and guide breeding programs aimed at developing stress-resilient cultivars.

4.11.2 MDA (lipid peroxidation)

Statistical analysis revealed significant differences ($p \leq 0.05$) in leaf malondialdehyde (MDA) content between water deficit levels, genotypes, and their interaction in both chickpea and soybean. In both crops, MDA content increased progressively with increasing PEG-6000 concentration, reaching the highest values at the most severe stress level, while the lowest values were recorded under control conditions. This pattern indicates enhanced lipid peroxidation and membrane damage under water deficit.

In chickpea, the highest MDA content was observed in ELMO (14.50 $\mu\text{mol g}^{-1}$ FW) at -0.4 MPa, while the lowest was recorded in ORION (3.46 $\mu\text{mol g}^{-1}$ FW) under control conditions. Across stress levels, ELMO maintained a significantly higher average MDA concentration (11.01 $\mu\text{mol g}^{-1}$ FW) compared to ORION (7.16 $\mu\text{mol g}^{-1}$ FW), suggesting greater oxidative membrane damage in Elmo (Fig. 27).

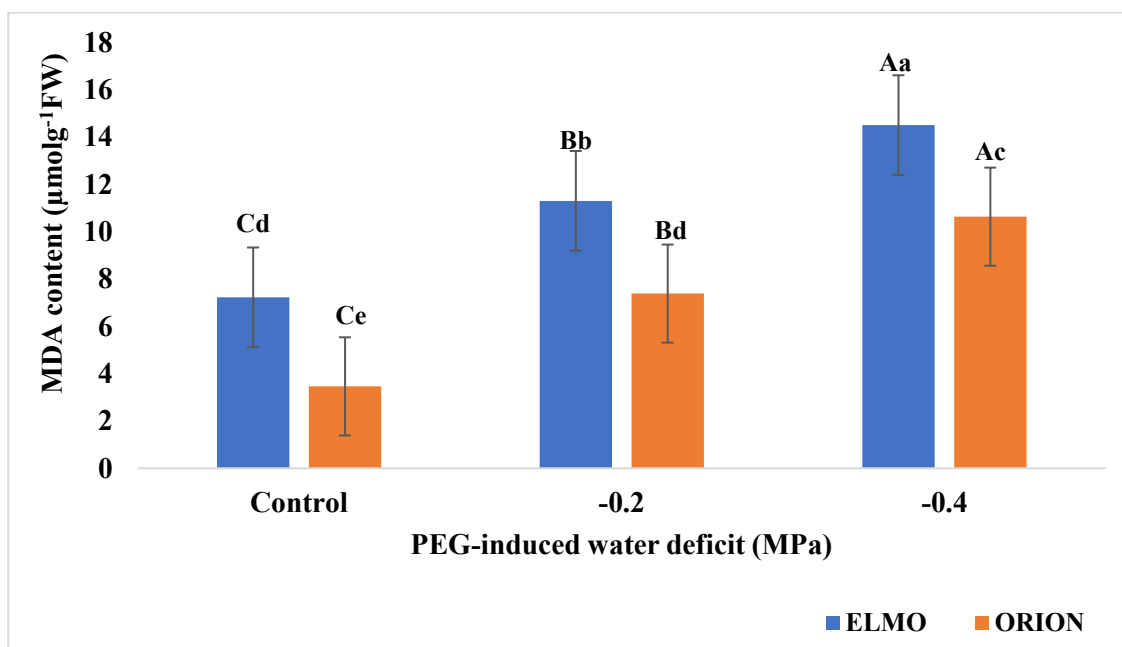


Fig. 27. Effect of PEG-induced water deficit on MDA ($\mu\text{mol g}^{-1}$ FW) of two chickpea genotypes under hydroponic conditions ($n=3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

In soybean, the maximum MDA content was observed in POCA (14.86 $\mu\text{mol g}^{-1}$ FW) at -0.6 MPa, while the minimum was in YAKARTA (4.85 $\mu\text{mol g}^{-1}$ FW) under control conditions. On average, POCA exhibited significantly higher MDA levels (10.14 $\mu\text{mol g}^{-1}$ FW) than YAKARTA (8.17 $\mu\text{mol g}^{-1}$ FW), indicating that YAKARTA maintained lower lipid peroxidation under water stress. (Fig. 28). Overall, ORION (chickpea) and YAKARTA (soybean) displayed lower MDA accumulation under stress compared to

their counterparts, suggesting relatively greater tolerance to oxidative damage induced by water deficit.

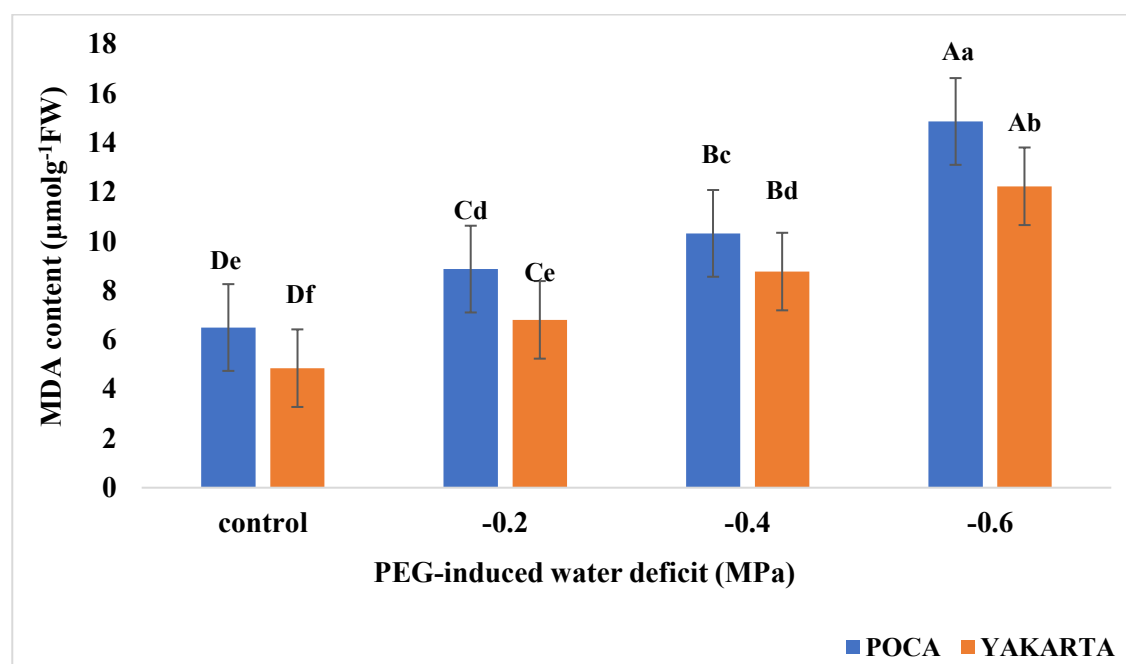


Fig. 28. Effect of PEG-induced water deficit on MDA ($\mu\text{mol g}^{-1}$ FW) of two soybean genotypes under hydroponic conditions ($n=3$, $\pm$ s.e.). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

Malondialdehyde (MDA) is a well-established indicator of oxidative membrane damage, with its accumulation directly reflecting the severity of lipid peroxidation triggered by environmental stress (Ahmad et al., 2018). In the present study, MDA content significantly increased with increasing PEG-induced water deficit in both chickpea and soybean genotypes, highlighting that drought stress triggered oxidative damage and membrane lipid peroxidation.

In chickpea, the genotype ELMO accumulated significantly higher MDA across stress levels, with a maximum of $14.50 \mu\text{mol g}^{-1}$ FW at -0.4 MPa, compared to ORION, which showed consistently lower values ($7.157 \mu\text{mol g}^{-1}$ FW). This suggests that ORION experiences less oxidative membrane damage under drought stress, which may be linked to more effective protective mechanisms, possibly involving antioxidant enzymes or osmolyte accumulation. Similar genotype-dependent differences in MDA accumulation have been reported in other legumes, where tolerant genotypes maintained lower lipid peroxidation under water stress (Ahmad et al., 2010; Smolik et al., 2013). Likewise, Wang et al. (2022) observed that MDA content in soybean genotypes steadily increased as soil moisture decreased, with peak values occurring under prolonged stress, further supporting the link between water deficit and enhanced lipid peroxidation.

The differential MDA accumulation between genotypes can be explained by variations in oxidative stress tolerance. POCA and ELMO, which accumulated more MDA, also exhibited higher solute leakage compared to YAKARTA and ORION, suggesting weaker membrane stability. Increased solute leakage is strongly associated with elevated lipid peroxidation and greater membrane damage (Ahmad et al., 2018). In contrast, ORION and YAKARTA maintained lower MDA accumulation, implying greater efficiency in protecting membrane integrity under drought conditions. This protective effect may be linked to enhanced antioxidant defense systems, as genotypes with higher activity of enzymatic antioxidants or elevated levels of non-enzymatic antioxidants, such as ascorbic acid, often exhibit lower lipid peroxidation (Ahmad et al., 2018; Fardus et al., 2017). In general, oxidative stress affects all cellular components, but membrane lipids are particularly susceptible to peroxidation (Ahmad et al., 2018). The current findings confirm that MDA is a reliable indicator of drought-induced oxidative damage, and genotypic differences in its accumulation reflect contrasting abilities to maintain membrane integrity. Therefore, ORION (chickpea) and YAKARTA (soybean) appear to be relatively more tolerant to water deficit compared to ELMO and POCA, owing to their lower MDA accumulation and presumably stronger antioxidant defense mechanisms.

4.11.3 Solute leakage (membrane integrity)

The results of the statistical analysis revealed significant differences ($p \leq 0.05$) in solute leakage (SL) between PEG-induced water deficit levels, genotypes, and their interaction in both chickpea and soybean.

In chickpea, solute leakage increased progressively with increasing water deficit stress, reaching the highest value at -0.4 MPa (83.66%), while it was significantly lower under control conditions (30.33%). Across genotypes, the ELMO genotype exhibited significantly higher SL (60.67%) compared to ORION (47.78%). At -0.4 MPa, ELMO recorded the maximum leakage (90.00%), whereas ORION exhibited 77.33%. Under control conditions, ELMO showed higher SL (34.67%) than ORION (26.00%) (Table 39).

Table 39. Effect of PEG-induced water deficit on Membrane Integrity (Solute Leakage) (%) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	ELMO	ORION	Average
control	34.67 ^d	26.00 ^e	30.33 ^C
-0.2	57.33 ^c	40.00 ^d	48.66 ^B
-0.4	90.00 ^a	77.33 ^b	83.66 ^A
Average	60.67 ^A	47.78 ^B	-
Variable	Genotypes	PEG levels	Interaction
LSD (0.05)	4.153	3.800	7.194
CV (%)	8.00		

In soybean, similar trends were observed. Solute leakage was significantly higher at the maximum stress level (−0.6 MPa; 83.83%), while it was significantly lower in the control (37.83%), representing an increase of 122% under severe stress compared to the control. Among genotypes, POCA displayed significantly higher SL (71.67%) compared to YAKARTA (42.75%). At −0.6 MPa, Poca reached the highest leakage value (92.33%), while YAKARTA remained lower at 75.33%. In contrast, under control conditions, YAKARTA exhibited the lowest SL (20.00%) compared with POCA (55.67%) (Table 40). Overall, these findings confirm that solute leakage increased significantly with the severity of water deficit stress in both chickpea and soybean, with ELMO and POCA genotypes showing greater membrane instability compared to ORION and YAKARTA, respectively.

Table 40. Effect of PEG-induced water deficit on Membrane Integrity (Solute Leakage) (%) of two soybean (YAKARTA and POCA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	POCA	YAKARTA	Average
control	55.67 ^b	20.00 ^g	37.83 ^D
-0.2	60.67 ^c	32.33 ^f	46.50 ^C
-0.4	78.00 ^b	43.33 ^e	60.67 ^B
-0.6	92.33 ^a	75.33 ^b	83.83 ^A
Average	71.67 ^A	42.75 ^B	-
Variable	Genotypes	Salinity levels	Interaction
LSD (0.05)	2.257	3.328	4.515
CV (%)	4.19		

The stability of cell membranes under stress is commonly assessed through solute leakage (SL), which reflects damage from oxidative and water deficit conditions (Li and Nong, 2018; Wang et al., 2019). Our results show that SL rose progressively with the intensity of PEG-induced water deficit in both species, confirming heightened membrane permeability. This effect is directly associated with ROS-driven lipid peroxidation, which disrupts membrane structure and facilitates the leakage of intracellular solutes (Zhang et al., 2019). Moreover, the variation in SL between genotypes may be associated with differences in proline accumulation, as proline contributes to protein stabilization and membrane protection under stress (Jogaiah et al., 2013). Genotypes with higher proline content, such as ORION and YAKARTA, exhibited lower SL, indicating that osmotic adjustment plays a key role in maintaining membrane stability under water deficit. Overall, these findings confirm that solute leakage is a sensitive indicator of cellular membrane integrity under water deficit. Genotypic differences in SL highlight the potential of using this parameter to identify drought-tolerant cultivars, with ORION (chickpea) and YAKARTA (soybean) demonstrating superior ability to maintain membrane stability compared to ELMO and POCA, respectively.

4.11.4 H_2O_2 levels

Both chickpea and soybean exhibited significant increases in leaf H_2O_2 content with increasing PEG-induced water deficit, indicating enhanced oxidative stress under water-limited conditions. In chickpea, H_2O_2 content increased from 609.3 $\mu\text{mol g}^{-1}$ FW in the control to 1144.3 $\mu\text{mol g}^{-1}$ FW at -0.4 MPa, with the sensitive genotype ELMO showing consistently higher levels (995.9 $\mu\text{mol g}^{-1}$ FW) than ORION (725.8 $\mu\text{mol g}^{-1}$ FW) (Table 41).

Table 41. Effect of PEG-induced water deficit on H_2O_2 Content ($\mu\text{mol g}^{-1}$) of two chickpea (ELMO and ORION) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	ELMO	ORION	Average
control	723.3 ^c	495.3 ^d	609.3 ^C
-0.2	899.3 ^b	758.7 ^c	829.0 ^B
-0.4	1365.3 ^a	923.3 ^b	1144.3 ^A
Average	995.9 ^A	725.8 ^B	-
Variable	Genotypes	PEG levels	Interaction
LSD (0.05)	66.3	54.2	93.9
CV (%)	3.53		

Similarly, in soybean, H₂O₂ content rose sharply from 102 $\mu\text{mol g}^{-1}$ FW in the control to 986 $\mu\text{mol g}^{-1}$ FW at -0.6 MPa, with the sensitive genotype POCA accumulating more H₂O₂ (620 $\mu\text{mol g}^{-1}$ FW) than Yakarta (334 $\mu\text{mol g}^{-1}$ FW) (Table 42). These results indicate that water deficit triggers reactive oxygen species (ROS) accumulation, with water deficit-sensitive genotypes exhibiting higher H₂O₂ levels, reflecting a greater oxidative challenge and potential membrane damage under stress.

Table 42. Effect of PEG-induced water deficit on H₂O₂ Content ($\mu\text{mol g}^{-1}$) of two soybean (POCA and YAKARTA) genotypes under hydroponic conditions. Different lowercase letters indicate significant differences at $p \leq 0.05$ between salinity levels within the same genotype. Different uppercase letters indicate significant differences among the general means of salinity treatments and genotypes.

PEG-induced stress (MPa)	POCA	YAKARTA	Average
control	128 ^g	76 ^g	102 ^D
-0.2	467 ^d	236 ^f	352 ^C
-0.4	627 ^c	310 ^e	469 ^B
-0.6	1259 ^a	714 ^b	986 ^A
Average	620 ^A	334 ^B	-
Variable	Genotypes	PEG levels	Interaction
LSD (0.05)	129.1	91.3	64.6
CV (%)	12.77%		

Hydrogen peroxide (H₂O₂) is a key reactive oxygen species (ROS) that accumulates in plant cells under abiotic stress, including water deficit, and serves as both a signaling molecule and an indicator of oxidative stress (Mittler, 2017). In the present study, leaf H₂O₂ content increased significantly with increasing PEG-induced water deficit in both chickpea and soybean. In chickpea, the sensitive genotype ELMO accumulated significantly higher H₂O₂ compared to ORION, while in soybean, POCA accumulated higher H₂O₂ than the tolerant YAKARTA, particularly under severe water stress. These results indicate that water deficit-sensitive genotypes experience greater oxidative stress under water deficit, which is consistent with their higher malondialdehyde (MDA) content and increased solute leakage observed in this study. The strong correlation between H₂O₂ accumulation and MDA content supports the notion that excessive ROS production leads to lipid peroxidation and cytoplasmic membrane damage (Ahmad et al., 2018; Li and Nong, 2018). Moreover, the sensitive genotypes exhibited lower relative water content and proline accumulation compared to tolerant genotypes, suggesting that insufficient osmotic adjustment further exacerbates ROS-induced damage.

The differential accumulation of H₂O₂ between genotypes highlights the importance of antioxidant defense mechanisms in mitigating oxidative stress. Tolerant genotypes such as ORION and YAKARTA maintained lower H₂O₂ levels, reflecting more effective ROS scavenging through enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as reported by Wu et al. (2019). This protective capacity helps preserve membrane integrity, maintain cellular homeostasis, and support physiological processes under water deficit conditions. Overall, H₂O₂ accumulation serves as a reliable indicator of oxidative stress severity and genotypic tolerance under water deficit conditions. The combination of high H₂O₂, elevated MDA, and increased solute leakage in sensitive genotypes underscores their vulnerability, while lower H₂O₂ coupled with higher proline and relative water content in tolerant genotypes reflects adaptive mechanisms to maintain cellular stability and mitigate stress-induced damage (Jogaiah et al., 2013; Zhang et al., 2019).

4.12 Ascorbic acid accumulation under PEG-induced water deficit

The statistical analysis revealed significant differences ($p \leq 0.05$) in leaf ascorbic acid (AsA) content between water deficit levels, genotypes, and their interaction in both chickpea and soybean. In chickpea, the average AsA content increased progressively with water deficit. ORION exhibited significantly higher AsA (165.33 $\mu\text{mol g}^{-1}$ FW) compared to ELMO (130.78 $\mu\text{mol g}^{-1}$ FW) across PEG treatments. The highest PEG-induced water deficit (-0.4 MPa) caused the largest increase, with ORION reaching 361.33 $\mu\text{mol g}^{-1}$ FW and ELMO 237.33 $\mu\text{mol g}^{-1}$ FW, while the control recorded the lowest values (ORION 58.33 $\mu\text{mol g}^{-1}$ FW, ELMO 66.33 $\mu\text{mol g}^{-1}$ FW.) (Fig. 29).

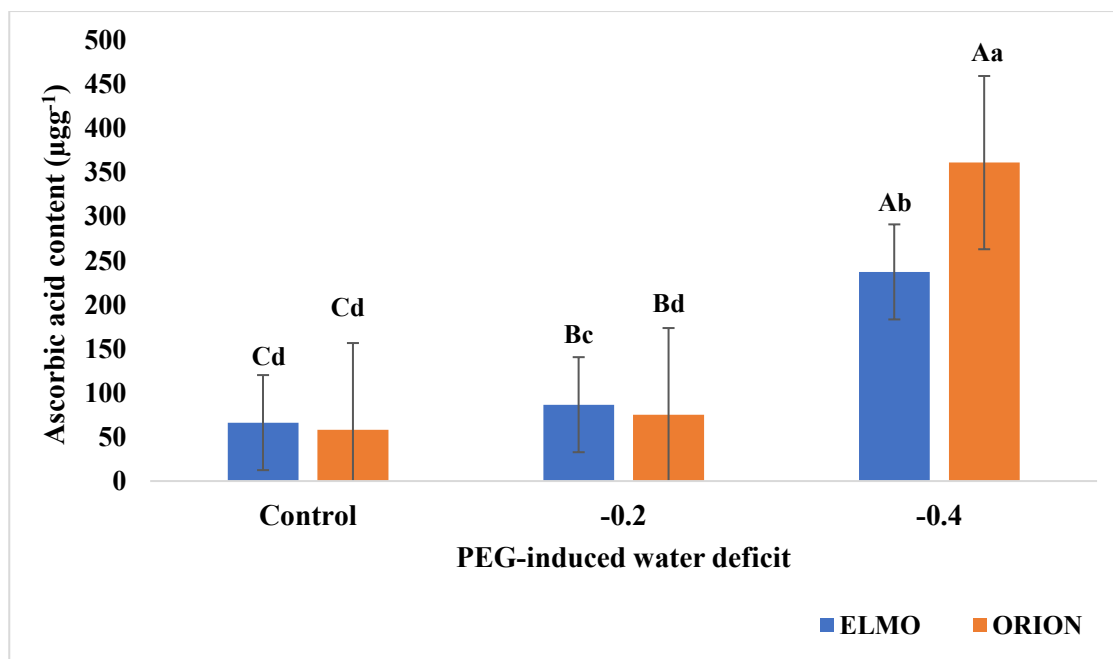


Fig. 29. Effect of PEG-induced water deficit on ascorbic acid ($\mu\text{g g}^{-1}$) of two chickpea genotypes (ELMO AND ORION) under hydroponic conditions ($n = 3, \pm \text{s.e.}$). Different capital letters among salinity levels indicate significant differences ($p \leq 0.05$). Different small letters at each salinity level between genotypes indicate significant differences ($p \leq 0.05$).

In soybean, AsA content similarly increased under water deficit, with POCA accumulating more AsA ($201.25 \mu\text{mol g}^{-1} \text{FW}$) than YAKARTA ($172.17 \mu\text{mol g}^{-1} \text{FW}$) on average. At the highest PEG-induced stress (-0.6 MPa), POCA reached $394.33 \mu\text{mol g}^{-1} \text{FW}$, while YAKARTA recorded $368.00 \mu\text{mol g}^{-1} \text{FW}$. In the control, AsA content was lowest (POCA $41.33 \mu\text{mol g}^{-1} \text{FW}$, YAKARTA $33.33 \mu\text{mol g}^{-1} \text{FW}$) (Fig. 30).

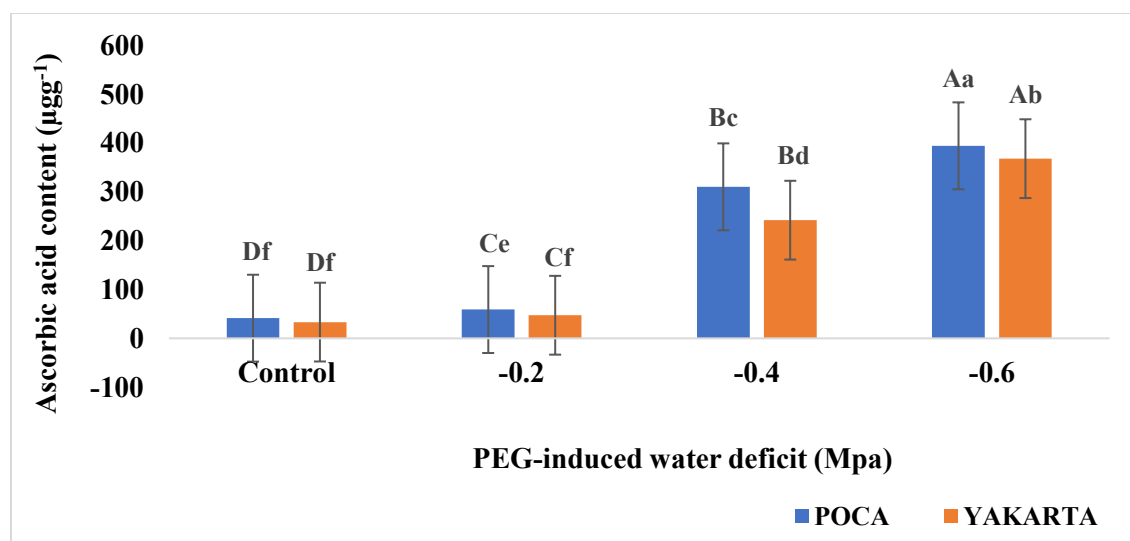


Fig. 30. Effect of PEG-induced water deficit on ascorbic acid ($\mu\text{g g}^{-1}$) content of two soybean genotypes ($n=3, \pm \text{s.e.}$). Different small letters indicate significant differences ($p \leq 0.05$) for the interactions between salt levels and genotypes, while the capital letters indicate significant differences ($p \leq 0.05$) for the general means of salt treatments and genotypes.

The scavenging of reactive oxygen species (ROS) under water deficit is a primary function of ascorbic acid (AsA), a crucial non-enzymatic antioxidant that mitigates oxidative cellular damage (Noctor and Foyer, 1998). In both chickpea and soybean, PEG-induced water deficit significantly increased AsA content, reflecting an upregulated antioxidant defense in response to drought-induced oxidative stress. In chickpea, ORION accumulated significantly higher AsA than ELMO, particularly at the highest water deficit (-0.4 MPa) (Fig. 31). This indicates a stronger antioxidant capacity, helping ORION maintain cellular redox balance and mitigate oxidative damage. In soybean, although POCA exhibited higher AsA content than YAKARTA under severe stress (-0.6 MPa), the elevated AsA in POCA likely reflects a stress-induced overaccumulation in response to severe ROS generation rather than effective stress tolerance. In contrast, YAKARTA, a more tolerant genotype, maintained relatively lower AsA levels while preserving better cellular integrity, lower H₂O₂ and MDA accumulation, and less solute leakage, indicating more efficient antioxidant and osmotic regulation under stress. These results align with previous research indicating that oxidative stress triggers a substantial accumulation of AsA in sensitive genotypes, often as a compensatory response to severe damage. In contrast, tolerant genotypes typically exhibit a more modulated increase, reflecting a preemptive and efficient ROS management system that mitigates the need for a drastic antioxidant response (Shalata and Neumann, 2001; Akram et al., 2017). Thus, in water deficit-sensitive genotypes like POCA, elevated AsA may serve as an emergency defense response, whereas in tolerant genotypes such as YAKARTA, controlled AsA accumulation supports sustained physiological function under water deficit. Overall, AsA content is a key indicator of oxidative stress and plant response to drought, but its interpretation should consider genotypic tolerance, as high AsA in sensitive genotypes may indicate stress severity rather than effective adaptation.



Soybean (POCA)



Soybean (YAKARTA)



Chickpea (ELMO)



Chickpea (ORION)

Figure 31. Effect of PEG-induced water deficit (0.0, - 0.2, -0.4, and – 0.6 MPa PEG) on the performance of different soybean and chickpea genotypes under controlled conditions.

4.13 Correlation among physio-biochemical traits under PEG-induced water deficit

4.13.1 Chickpea correlations among the investigated traits

The present study highlights the complex interplay between water status, photosynthetic pigments, oxidative stress, and antioxidant responses under the investigated conditions. The correlation analysis provides insights into plant physiological responses and adaptive mechanisms. Solutes leakage, an indicator of membrane damage, showed strong negative correlations with relative water content (RWC) ($r = -0.969^{**}$) and chlorophyll content (Chlorophyll-a: $r = -0.963^{**}$; SPAD: $r = -0.966^{**}$), and positive correlations with

oxidative stress markers such as MDA ($r = 0.932^{**}$) and H_2O_2 ($r = 0.926^{**}$) (Fig. 32). These relationships suggest that water deficit or stress conditions compromise membrane integrity, leading to increased solute leakage, reduced chlorophyll content, and accumulation of reactive oxygen species (ROS), consistent with previous reports in water deficit-stressed plants (Zhang et al., 2020). The negative association between RWC and solute leakage further supports the critical role of water retention in maintaining cellular stability.

Chlorophyll-a showed strong positive correlations with carotenoids ($r = 0.943^{**}$) and SPAD values ($r = 0.891^*$), and negative correlations with MDA ($r = -0.859^*$) and H_2O_2 ($r = -0.916^*$). Chlorophyll-b exhibited similar but slightly weaker trends (Fig. 32). These findings indicate that photosynthetic pigments are highly sensitive to oxidative stress, and reductions in chlorophyll content may be a direct consequence of lipid peroxidation and ROS accumulation. Carotenoids, as accessory pigments, likely play a protective role against oxidative damage, corroborating their role in photoprotection and ROS scavenging. MDA and H_2O_2 , classical indicators of lipid peroxidation and oxidative stress, showed strong positive correlations with solute leakage and negative correlations with RWC and chlorophyll content (Fig. 32). The results demonstrate that water deficit induces oxidative stress, which subsequently causes membrane damage and a decline in photosynthetic efficiency. The highly significant positive correlation between MDA and H_2O_2 ($r = 0.961^{**}$) further substantiates that ROS accumulation is a primary driver of this stress-induced injury.

Proline and ascorbic acid (AsA) exhibited moderate positive correlations with each other ($r = 0.835^*$) and with solute leakage (Proline: $r = 0.553^{NS}$; AsA: $r = 0.832^*$) (Fig. 32) implying their roles in osmotic adjustment and antioxidant defense under stress conditions. Although their correlations with primary stress markers were weaker than those of RWC or chlorophyll, these metabolites may contribute to mitigating oxidative damage, stabilizing membranes, and maintaining cellular homeostasis, in line with previous findings (Szabados and Saviouré, 2010).

Overall, the correlation patterns observed in this study highlight that water status, membrane integrity, photosynthetic efficiency, and oxidative stress are tightly interconnected under stress conditions. Maintaining higher RWC appears crucial for preserving photosynthetic pigments and limiting ROS accumulation, while antioxidants and osmolytes provide additional protective mechanisms. These findings suggest that

integrated assessment of physiological, biochemical, and oxidative stress indicators can provide a comprehensive understanding of plant stress tolerance.

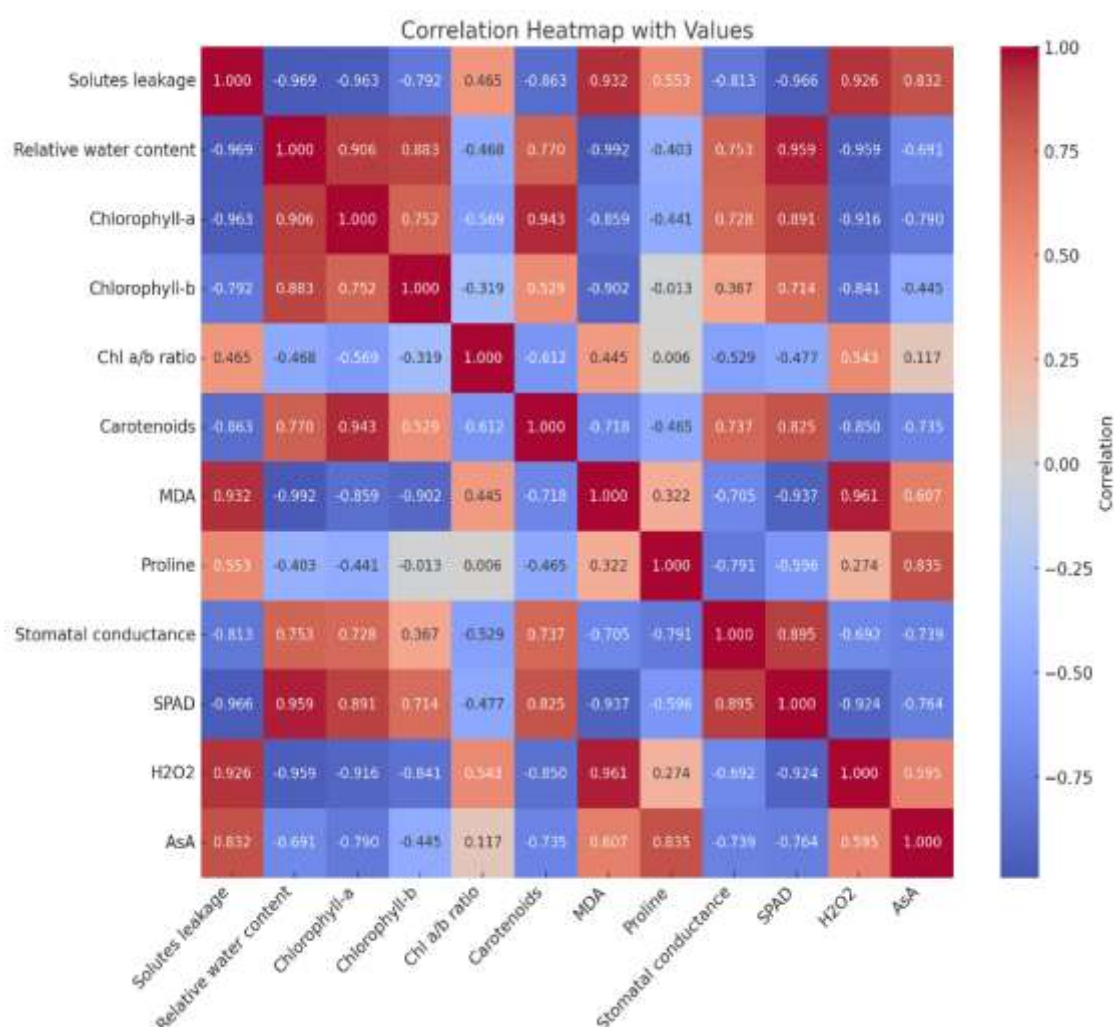


Figure 32. Correlations among physio-biochemical traits under PEG-induced water deficit. Significant positive correlations are shown in red, negative in blue; *, ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

4.13.2 Soybean correlations among the investigated traits

The correlation heatmap of soybean under PEG-induced osmotic stress clearly shows two opposing clusters of traits: oxidative stress indicators (solute leakage, MDA, H₂O₂) were strongly and positively correlated with each other, but negatively correlated with RWC, photosynthetic pigments (chlorophyll-a, carotenoids, SPAD), and stomatal conductance (Fig. 33), confirming that PEG stress enhances membrane injury and ROS accumulation while reducing water status and photosynthetic efficiency (Alzahrani et al. 2024). Chlorophyll a clustered tightly with carotenoids and SPAD, suggesting coordinated regulation of pigment content, while its strong negative correlation with MDA and H₂O₂

highlights the vulnerability of pigments to oxidative damage (Hossain et al. 2024). Stomatal conductance also aligned with chlorophyll and carotenoids, supporting its role in maintaining photosynthetic function, whereas proline and AsA showed weaker but positive associations with oxidative markers, indicating their contribution to osmotic adjustment and antioxidant defense (Mahmud et al. 2023). Overall, the heatmap reveals that PEG stress triggers a coordinated decline in water status, stomatal function, and pigment stability, while osmolyte and antioxidant accumulation represent compensatory protective mechanisms (Fig. 33).

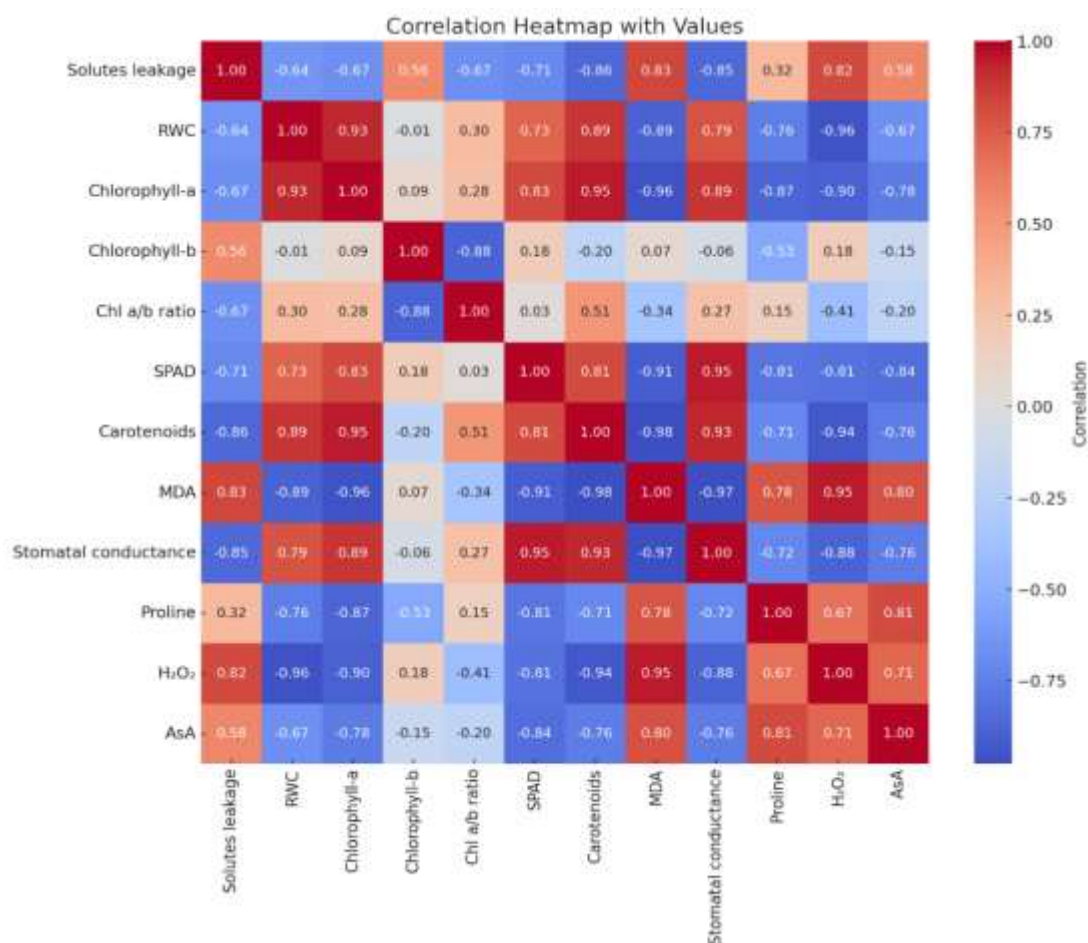


Figure 33. Correlations among physio-biochemical traits under PEG-induced water deficit. Significant positive correlations are shown in red, negative in blue; *, ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively.

4.14 Comparative tolerance between species and genotypes

To summarize genotypic responses to PEG-induced water deficit, comparative tables (43 and 44) and a schematic figure (34) highlight the contrasting performance of tolerant genotypes (ORION in chickpea and YAKARTA in soybean) versus sensitive genotypes (ELMO in chickpea and POCA in soybean) across key morpho-physiological and biochemical parameters. This visual overview underscores the traits underlying water

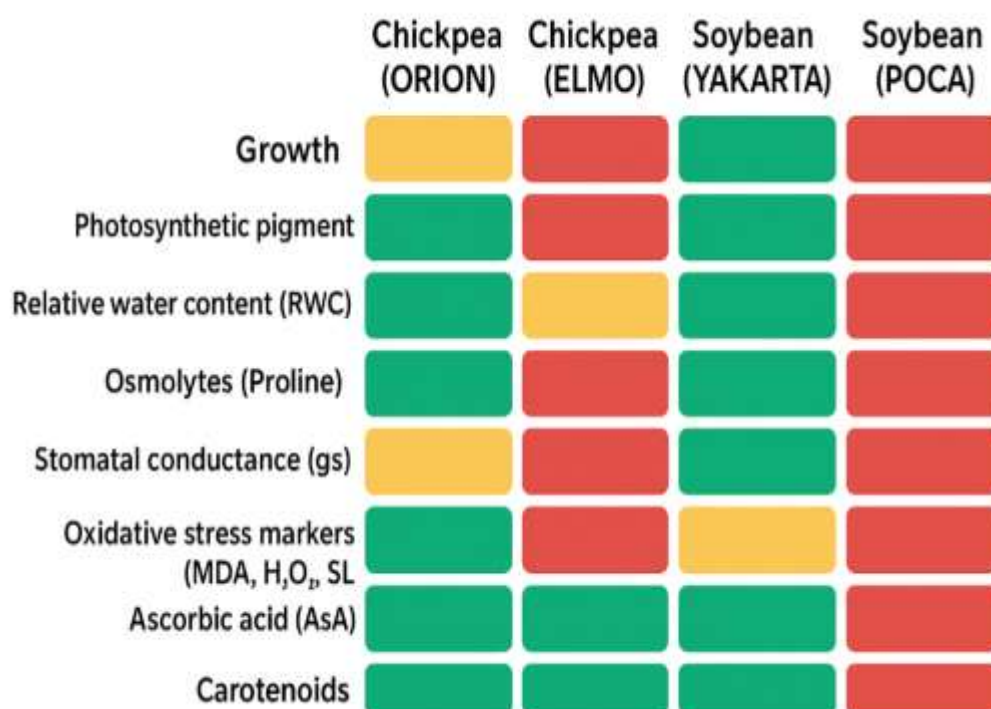
deficit tolerance and provides a framework for interpreting the detailed data presented in the following tables (43 and 44) and figure (34).

Table 43. Comparative responses of chickpea and soybean genotypes to PEG-induced water deficit across morpho-physiological and biochemical parameters.

Parameter	Chickpea (ORION)	Chickpea (ELMO)	Soybean (YAKARTA)	Soybean (POCA)	Comparative Insight
Growth	Moderate reduction; better shoot and root maintenance	Strong reduction, highly sensitive	Higher growth retention; extensive root proliferation	Strong reduction, limited root expansion	Soybean > Chickpea; tolerant genotypes (ORION, YAKARTA) outperform sensitive ones
Photosynthetic pigments	Maintained higher chlorophyll and carotenoids	Sharp decline	Higher pigment retention under stress	Significant decline	YAKARTA > ORION > POCA $\approx$ ELMO
Relative water content (RWC)	Higher maintenance under stress	Lower under stress	Maintains higher leaf hydration	Declines sharply	Tolerant genotypes sustain leaf hydration
Osmolytes (Proline)	High accumulation ($8.49 \mu\text{mol g}^{-1}$ FW), supporting osmotic adjustment	Moderate accumulation ($4.23 \mu\text{mol g}^{-1}$ FW)	High accumulation ($5.47 \mu\text{mol g}^{-1}$ FW)	Low accumulation ($4.17 \mu\text{mol g}^{-1}$ FW)	Tolerant genotypes enhance osmotic adjustment and water uptake
Stomatal conductance (g_s)	Moderate; supports growth and conservative water use	Greater relative reduction	Moderate; balances photosynthesis and water conservation	Low g_s , conservative water use	Controlled g_s contributes to drought resilience
Oxidative stress markers (MDA, H_2O_2 , SL)	Lower accumulation; membrane integrity maintained	Higher accumulation; more oxidative damage	Lower accumulation; effective ROS scavenging	Higher accumulation; weak antioxidant defense	Tolerant genotypes show reduced oxidative stress and better membrane stability
Ascorbic acid (AsA)	Higher maintenance under stress ($165.33 \mu\text{mol g}^{-1}$ FW)	Lower levels ($130.78 \mu\text{mol g}^{-1}$ FW)	Moderate levels with maintained cellular integrity	Higher under stress ($201.25 \mu\text{mol g}^{-1}$ FW) but stress-induced	Antioxidant defense is stronger in tolerant genotypes; excessive AsA in sensitive genotypes reflects stress damage
Carotenoids	Maintained higher content under stress	Declined sharply	Maintains better under severe stress	Sharp decline under severe stress	Tolerant genotypes preserve photoprotection
Genotypic variation	ORION is clearly more tolerant than ELMO	–	YAKARTA is clearly more tolerant than POCA	–	Intraspecific variation strongly influences drought tolerance
Overall tolerance ranking	Moderate tolerance	Sensitive	High tolerance	Sensitive	YAKARTA > ORION > POCA $\approx$ ELMO

Table 44. Comparative table of PEG-induced water deficit tolerance mechanisms in chickpea and soybean genotypes

Trait / Parameter	Tolerant Genotypes (ORION – Chickpea, YAKARTA – Soybean)	Sensitive Genotypes (ELMO – Chickpea, POCA – Soybean)
Growth performance	Better maintenance of shoot and root biomass; higher growth indices	Severe growth inhibition at moderate to high water deficit
Chlorophyll stability	Higher retention of chlorophyll a, b, and carotenoids; photosynthesis is preserved	Rapid decline in chlorophyll pigments; photosynthetic activity reduced
Proline accumulation	Strong induction of proline, supporting osmotic adjustment and leaf hydration	Limited proline accumulation; weaker osmotic regulation
Stomatal conductance	Balanced g_s to sustain photosynthesis while conserving water	Excessive closure or insufficient g_s restricts carbon assimilation
Oxidative stress markers (MDA, H_2O_2 , SL)	Lower levels, better membrane and cellular protection	Higher levels: increased lipid peroxidation and membrane damage
Ascorbic acid (AsA)	Maintains moderate levels to efficiently detoxify ROS	High stress-induced AsA reflects oxidative damage rather than tolerance
Carotenoid content	Maintains higher content under severe stress; photoprotection is preserved	Declines sharply under stress; lower photoprotective capacity
Integrated water deficit response	Efficient osmotic adjustment + antioxidant defense + root-to-shoot redistribution → water deficit-tolerant	Poor osmotic adjustment + weak antioxidant system + shoot growth restriction → water deficit-sensitive



Yellow color stands for moderate response; Green color stands for tolerance response; Red color stands for sensitive response.

Fig. 34. Comparative response of chickpea and soybean genotypes to PEG-induced water deficit.

4.15 Comparative responses of soybean and chickpea to salinity and water deficit

Under both NaCl-induced salinity and PEG-induced drought stress, soybean and chickpea exhibited distinct adaptive strategies reflecting species- and genotype-specific

tolerance. Soybean, particularly the YAKARTA genotype, displayed greater resilience through active osmotic adjustment, root proliferation, and effective ion homeostasis, which maintained water status, photosynthetic pigments, and reduced oxidative damage. In contrast, chickpea, especially ORION, relied on conservative growth allocation, shoot-water conservation, and enhanced antioxidant defense, allowing moderate tolerance under drought but showing vulnerability under high salinity due to weaker ion regulations. Sensitive genotypes (POCA in soybean and ELMO in chickpea) suffered severe reductions in growth, biomass, and membrane stability under both stresses. Overall, these findings highlight that while soybean generally outperforms chickpea across both stress types, significant intra-species variation provides opportunities for targeted breeding of stress-resilient legume varieties (Table 45).

Table 45. Comparative physiological and biochemical responses of soybean and chickpea genotypes under salinity and water deficit stress.

Species/ Genotype	Stress Type	Key Adaptive Traits	Osmolytes and Water Status	Ion Homeostasis (K ⁺ /Na ⁺)	Oxidative Stress (MDA, H ₂ O ₂)	Notes on Tolerance
Soybean (YAKARTA)	Salinity	Root proliferation, osmotic adjustment, and antioxidant activity	High proline, high RWC	High K ⁺ /Na ⁺	Low MDA, controlled H ₂ O ₂	Highly tolerant
Soybean (POCA)	Salinity	Limited osmotic adjustment, weak ROS defense	Low proline, low RWC	Low K ⁺ /Na ⁺	High MDA, high H ₂ O ₂	Sensitive
Chickpea (ORION)	Salinity	Water conservation, moderate antioxidant activity	Moderate proline, moderate RWC	Moderate K ⁺ /Na ⁺	Low-moderate MDA	Tolerant but less than YAKARTA
Chickpea (ELMO)	Salinity	Poor ion regulation, weak antioxidant defense	Low proline, low RWC	Low K ⁺ /Na ⁺	High MDA	Highly sensitive
Soybean (YAKARTA)	Water deficit	Root proliferation, osmotic adjustment, antioxidant defense	High proline, high RWC	Not applicable	Low MDA, controlled H ₂ O ₂	Highly tolerant

Soybean (POCA)	Water deficit	Weak osmotic and antioxidant responses	Low proline, low RWC	Not applicable	High MDA	Sensitive
Chickpea (ORION)	Water deficit	Shoot-water conservation, antioxidant activity	High proline, high RWC	Not applicable	Low-moderate MDA	Tolerant
Chickpea (ELMO)	Water deficit	Weak osmotic and antioxidant response	Low proline, low RWC	Not applicable	High MDA	Sensitive

4.16 Comparative severity of salinity and water deficit on soybean and chickpea

The analysis showed that stress impacts are highly species- and genotype-dependent. Salinity severely affected soybean, especially the sensitive genotype POCA, reducing growth, biomass, and pigments due to ionic toxicity and disrupted K^+/Na^+ balance. Drought had milder effects in hydroponics. The tolerant genotype YAKARTA performed better under both stresses but remained slightly more sensitive to salinity.

Chickpea, in contrast, was more vulnerable to drought, particularly the sensitive genotype ELMO, which showed sharp declines in water content, biomass, and chlorophyll stability. Salinity affected chickpea less severely, thanks to osmotic adjustment and antioxidants, especially in the tolerant genotype ORION. However, ORION's limited ion regulation made it less resilient under salinity.

Overall, salinity is more challenging for soybean due to ionic regulation needs, while drought critically limits chickpea, where water retention is key. These findings guide targeted breeding and highlight the importance of integrating multiple traits to assess stress tolerance.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Response of soybean and chickpea genotypes to NaCl-induced salinity stress

The results demonstrated clear genotypic variability in salinity tolerance between the two soybean (YAKARTA, POCA) and two chickpea (ORION, ELMO) genotypes studied. The tolerant genotypes, YAKARTA and ORION, maintained higher growth performance, better pigment stability, and more efficient antioxidant defenses compared to their sensitive counterparts. YAKARTA exhibited superior ionic homeostasis, reflected in a markedly higher K^+/Na^+ ratio (61.8) relative to the sensitive genotype POCA (32.2). ORION displayed moderate tolerance through improved osmotic adjustment and antioxidant activity. In contrast, the sensitive genotypes, POCA and ELMO, suffered severe growth inhibition, drastic pigment loss, and pronounced oxidative damage.

Recommendations:

- YAKARTA (soybean) and ORION (chickpea) are promising parental lines for breeding programs targeting salinity resilience.
- Future research should emphasize field trials to validate hydroponic findings under natural soil salinity conditions.
- Key screening criteria for salinity tolerance in legumes should include ionic homeostasis (K^+/Na^+ ratio), chlorophyll stability, and antioxidant capacity.
- Based on the contrasting responses observed in this study, traits such as the maintenance of relative water content, K^+/Na^+ ratio, proline accumulation, and malondialdehyde (MDA) levels have been identified as promising candidates for further evaluation as screening biomarkers for salinity tolerance. It is explicitly acknowledged that these results are derived from a limited set of genotypes under controlled hydroponic conditions. Their reliability and practical utility for breeding programs must therefore be confirmed through validation across a wider genetic pool and in diverse field environments. Despite this limitation, the research establishes a mechanistic framework that prioritizes these physiological and biochemical traits as key targets for future efforts to enhance salinity tolerance in soybean.
- To advance this understanding, future molecular studies are recommended. Investigations should focus on the genetic and physiological mechanisms underlying sodium (Na^+) exclusion in soybean and the regulation of antioxidant-

related genes in chickpea. Such work would elucidate the specific strategies these crops employ to cope with ionic and oxidative stress under saline conditions.

5.2 Response of soybean and chickpea genotypes to PEG-induced water deficit

PEG-induced water deficit revealed distinct adaptive mechanisms among the investigated genotypes. YAKARTA displayed strong tolerance by sustaining root proliferation, maintaining leaf hydration, and accumulating proline, which collectively preserved biomass and photosynthetic pigments. ORION exhibited moderate tolerance by conserving shoot water and enhancing antioxidant defense. Sensitive genotypes, POCA and ELMO, recorded sharp declines in relative water content (RWC), pigment concentration, and membrane stability. Correlation analysis indicated that RWC was strongly associated with chlorophyll retention and membrane integrity, underscoring its importance as a physiological determinant of drought tolerance.

Recommendations:

- YAKARTA (soybean) and ORION (chickpea) should be prioritized for incorporation into breeding programs targeting drought resilience.
- Screening protocols for drought tolerance should emphasize traits such as RWC, proline accumulation, stomatal conductance, and oxidative stress markers.
- Field-based studies are essential to confirm the hydroponic PEG-based results under natural drought conditions.
- Genomic studies focusing on osmolyte biosynthesis and antioxidant regulation may provide molecular markers for improving drought tolerance in legumes.

5.3 Comparative tolerance of soybean and chickpea under salinity and water deficit

The comparative analysis revealed that soybean, particularly the genotype YAKARTA, consistently outperformed chickpea under both salinity and drought conditions. YAKARTA combined osmotic adjustment, robust antioxidant defense, and highly efficient ion regulation to withstand abiotic stresses. ORION, while moderately tolerant, relied primarily on shoot-water conservation and antioxidant mechanisms, which were effective under drought but less successful under salinity stress due to limited ion regulation. Sensitive genotypes, POCA (soybean) and ELMO (chickpea), performed poorly under both stress conditions, showing substantial reductions in biomass, water status, and pigment content.

Recommendations:

- Breeding programs should prioritize soybean YAKARTA as a model tolerant genotype and ORION as a moderately tolerant chickpea line.
- Crossbreeding or molecular introgression strategies may combine the ion-regulation efficiency of soybean with the antioxidant-based tolerance of chickpea to develop more resilient cultivars.
- For practical utilization, salinity-prone areas should favor soybean YAKARTA, while drought-prone but non-saline environments could benefit from chickpea ORION.
- Integrated management strategies combining tolerant genotypes with sustainable agronomic practices (e.g., optimized irrigation and soil amendments) are recommended to maximize productivity under abiotic stresses.

5.4 Comparative impact of salinity and water deficit on soybean and chickpea

The results indicate that both NaCl-induced salinity and PEG-induced water deficit imposed significant constraints on growth, physiology, and biochemical traits; however, the magnitude and nature of the stress effects differed between the two legumes and across genotypes. Overall, salinity stress posed a greater challenge to soybean, particularly in sensitive genotypes, due to ionic toxicity and impaired ion homeostasis, whereas water deficit was relatively more detrimental to chickpea, as shoot-water conservation and osmotic adjustment only partially mitigated growth loss. Across all genotypes, sensitive lines (POCA and ELMO) suffered the strongest negative effects under salinity, while tolerant lines (YAKARTA and ORION) showed more resilience, yet still exhibited stress-specific vulnerabilities.

- **Soybean:** Sensitive genotype POCA experienced more severe reductions under salinity stress, with drastic ionic imbalance (low K^+/Na^+ ratio), pigment loss, and increased oxidative stress. YAKARTA, while tolerant to both stresses, showed slightly greater reductions in growth and photosynthetic parameters under high salinity than under drought.
- **Chickpea:** Sensitive genotype ELMO was highly affected under both stresses, but drought stress caused slightly stronger impacts on relative water content, membrane stability, and pigment retention. ORION maintained moderate tolerance under drought due to effective shoot-water conservation and antioxidant

defenses but showed limitations under salinity stress because of weaker ionic regulation.

Recommendations:

- Breeding strategies should consider stress-specific tolerance mechanisms: improving ion regulation for salinity tolerance in soybean and enhancing osmotic adjustment and antioxidant capacity for drought tolerance in chickpea.
- Comparative assessment under combined field conditions of salinity and water limitation is advised to guide cultivar deployment in stress-prone environments.

6. NEW SCIENTIFIC RESULTS

1. ***Salinity Tolerance is Defined by Genotype-Specific Coordination of Growth, Ionic Balance, and Oxidative Stress Management:*** Under NaCl stress, tolerant soybean YAKARTA outperformed sensitive POCA with 49.6% greater shoot length (19.9 vs. 13.3 cm) and 283% higher shoot fresh weight (3.41 vs. 0.89 g), linked to preserved pigments (Chl-a 15.39, Chl-b 6.83, carotenoids 3.58 mg·g⁻¹ FW) and a high shoot K⁺/Na⁺ ratio (61.8). This minimized oxidative damage, as seen by lower MDA (111.8 vs. 122.0 μmol·g⁻¹ FW) and adaptive H₂O₂ levels (883.80 μmol g⁻¹ FW). Similarly, chickpea ORION showed analogous tolerance over sensitive ELMO. Notably, despite POCA having longer roots (32.3 cm) than YAKARTA (21.9 cm), its poor shoot performance confirms that overall tolerance relies on integrated ionic, photosynthetic, and oxidative management, not single traits.
2. ***Salinity Tolerance is Associated with Enhanced Membrane Stability and a Functionally Integrated Oxidative Stress Response:*** Under salinity, tolerant soybean YAKARTA and chickpea ORION showed reduced lipid peroxidation (MDA: 255.0 vs. 270.9 μmol g⁻¹ FW in sensitive POCA; 268.4 vs. 292.3 in ELMO) and greater membrane stability, while sensitive genotypes exceeded 90% solute leakage. Key mechanistic insight: YAKARTA's controlled H₂O₂ accumulation (833.80 μmol g⁻¹ FW) acts as an adaptive stress signal, whereas sensitive POCA's lower but dysregulated ROS burst (720.20) reflects metabolic breakdown and oxidative injury. This explains how moderate H₂O₂ levels can coexist with superior performance when ROS metabolism is functionally integrated.
3. ***Water deficit Tolerance is Characterized by Maintained Growth, Superior Water Status, and Photosynthetic Pigment Retention:*** Under PEG-induced water deficit, tolerant chickpea ORION and soybean YAKARTA showed smaller growth reductions than sensitive ELMO and POCA (e.g., ELMO shoot length -46.3% vs. ORION -38.0%; POCA shoot length -38.2% vs. YAKARTA -40.4%, though YAKARTA retained higher pigments). Chlorophyll-a dropped markedly (~59-63% in severe stress), but tolerant lines maintained higher Chl-a, Chl-b, and relative water content (RWC: ORION 76.2% vs. ELMO 65.7%; YAKARTA 78.5% vs. POCA 71.3%), underpinning their drought resilience.

4. ***Osmotic Adjustment and Oxidative Stress Mitigation Differentiate Drought Tolerance:*** Proline accumulation increased substantially under water deficit, reaching $13.91 \mu\text{mol g}^{-1}$ FW in ORION and $9.66 \mu\text{mol g}^{-1}$ FW in YAKARTA, significantly higher than in sensitive genotypes. Oxidative damage was more pronounced in POCA and ELMO, with MDA contents of 14.86 and $14.50 \mu\text{mol g}^{-1}$ FW, respectively, compared to $8.17 \mu\text{mol g}^{-1}$ FW in YAKARTA and $7.16 \mu\text{mol g}^{-1}$ FW in ORION, demonstrating enhanced antioxidant protection in tolerant genotypes.
5. ***Comparative Stress Responses Highlight Species-Specific Vulnerabilities:*** Soybean is more affected by salinity (ionic stress), while chickpea is more vulnerable to water deficit (reliance on water retention and osmotic adjustment). Tolerant genotypes show coordinated responses, sustained growth, osmotic adjustment, and antioxidant defense, whereas sensitive genotypes exhibit concurrent declines in biomass, pigments, and membrane stability. This highlights the need for stress-specific selection in legume breeding programs.
6. ***Key Traits for Screening and Breeding:*** Key salinity tolerance indicators include a high K^+/Na^+ ratio; drought tolerance links to higher RWC, proline, and sustained stomatal conductance. General resilience involves pigment retention and antioxidant activity (ascorbic acid, MDA, H_2O_2). However, these findings come from limited genotypes under controlled conditions and need validation across broader populations and field environments. Nevertheless, they provide a mechanistic framework and identify high-priority targets for breeding salinity- and drought-resilient soybean and chickpea.
7. ***Correlation Analysis Identifies Relative Water Content as a Central Integrative Trait:*** Relative water content (RWC) strongly correlates with chlorophyll-a ($r = 0.963$), stomatal conductance ($r = 0.985$), and K^+/Na^+ ratio, while showing strong negative correlations with MDA and H_2O_2 (r up to 0.961), positioning RWC as a central trait integrating growth, photosynthesis, and oxidative stress under both salinity and water deficit. These findings offer a framework for targeted breeding of soybean and chickpea with enhanced dual tolerance for stress-prone environments.

7. PRACTICAL UTILIZATION OF RESULTS

1. ***Genotype-Specific Selection for Stress-Prone Environments:*** Hydroponic trials showed that tolerant soybean YAKARTA maintains superior growth, pigments, and ionic balance under salinity and drought, making it suitable for stressed soils, while sensitive POCA is not. Tolerant chickpea ORION exhibits effective osmotic adjustment and antioxidant activity for drought-prone areas, whereas sensitive ELMO should be avoided under such conditions.
2. ***Screening and Breeding Implications:*** Key salinity tolerance markers include ion homeostasis (K^+/Na^+ ratio), chlorophyll stability, and antioxidant capacity. Drought resilience markers are high relative water content (RWC), proline accumulation, regulated stomatal conductance, and low oxidative stress. Integrating these physiological and biochemical traits into breeding pipelines enables targeted development of more resilient soybean and chickpea cultivars.
3. ***Field Validation and Agronomic Optimization:*** Hydroponic insights require field validation under natural conditions. Tailoring irrigation, soil amendments, and planting strategies to each genotype maximizes stress tolerance and yield. For example, YAKARTA soybean benefits from salinity management practices, while ORION chickpea pairs best with water-conservation techniques in drought-prone regions.
4. ***Integration into Sustainable Agriculture:*** Plant tolerant genotypes like YAKARTA soybean and ORION chickpea in stress-prone areas to reduce crop losses and enhance food security. Combining this genotype selection with agronomic practices (organic amendments, mulching, partial irrigation) further boosts resilience and productivity.
5. ***Monitoring and Technology-Assisted Management:*** Farmers can use SPAD meters and spectrophotometry to monitor chlorophyll and pigment status, then adjust irrigation or nutrient management based on plant physiological responses. This enables precision agriculture for legumes under stress, optimizing growth while minimizing resource use.
6. ***Comparative Stress Impact for Practical Decisions:*** Under salinity, chickpea (especially ELMO) is severely affected, while YAKARTA soybean remains resilient due to efficient ion regulation. Under drought, both species suffer, but ORION chickpea maintains better water status and antioxidant defenses than POCA soybean. Therefore, deploy YAKARTA soybean on saline soils and ORION chickpea in drought-prone areas for targeted genotype use.

8. SUMMARY

Global agricultural systems face mounting pressure from climate change, which is amplifying abiotic stressors like drought and soil salinity. These constraints severely restrict crop development and yield, with particularly detrimental effects during sensitive phenological stages. Soybean (*Glycine max* L.) and chickpea (*Cicer arietinum* L.) are essential legumes, providing protein, oil, and micronutrients, while supporting sustainable agriculture through nitrogen fixation. However, their cultivation is constrained by early-stage drought and salinity, which can severely reduce biomass and yield. In Hungary, the frequency and severity of droughts have increased, and soil salinity is emerging as a growing threat, highlighting the need for stress-tolerant legume genotypes.

This investigation was conducted to assess the morpho-physiological and biochemical reactions of selected soybean (YAKARTA and POCA) and chickpea (ORION and ELMO) genotypes to early vegetative-stage salinity (NaCl-induced) and water deficit (PEG-induced) stress. A hydroponic system was employed to ensure a controlled and uniform experimental environment. The assessment focused on three categories of traits: morphological (root/shoot length, biomass, growth indices), physiological (chlorophyll content, stomatal conductance, relative water content), and biochemical (proline, MDA, H₂O₂, ascorbic acid, and Na⁺/K⁺ ratio), providing a comprehensive profile of stress adaptation, oxidative damage, and ion regulation.

Under salinity stress, soybean YAKARTA exhibited superior tolerance, maintaining shoot and root growth, pigment stability, and efficient ion regulation (K⁺/Na⁺ ratio of 61.8), while the sensitive POCA showed reduced growth and higher oxidative stress. In chickpea, ORION displayed moderate salinity tolerance through antioxidant activity and preserved growth, whereas ELMO suffered severe biomass loss and membrane damage. These findings emphasize the role of ionic regulation and antioxidant defenses in salinity resilience.

Water deficit responses revealed distinct drought-adaptive mechanisms. YAKARTA sustained root proliferation, leaf hydration, and proline accumulation, preserving biomass and photosynthetic pigments. ORION relied on shoot-water conservation and antioxidant activity for moderate tolerance. Sensitive genotypes POCA and ELMO experienced marked declines in RWC, pigment retention, and membrane stability. Correlation analyses highlighted RWC as a key determinant of drought resilience.

Comparative analysis indicated species-specific vulnerabilities: soybean was more affected by salinity due to ionic stress, while chickpea was more sensitive to water deficit, relying on osmotic adjustment and water conservation. Tolerant genotypes exhibited coordinated adaptive responses, whereas sensitive genotypes showed simultaneous declines in growth, pigments, and membrane integrity under both stresses.

Key traits for breeding and screening emerged: efficient ion regulation (K^+/Na^+ ratio) and chlorophyll stability for salinity tolerance, RWC, proline accumulation, and stomatal conductance for water deficit tolerance, and antioxidant activity (AsA, MDA, H_2O_2) for overall stress resilience. These indicators facilitate early-stage genotype selection for developing climate-resilient legumes.

Practically, YAKARTA soybean is suitable for saline and water-limited soils, while ORION chickpea is recommended for drought-prone areas. Sensitive genotypes should be avoided in stress-prone regions. Integrating tolerant genotypes with sustainable practices—optimized irrigation, soil amendments, and physiological monitoring using SPAD meters—can enhance productivity under adverse conditions. Field validation is essential to confirm hydroponic findings and optimize genotype-specific management strategies.

From a breeding perspective, YAKARTA and ORION are valuable parental lines for improving stress tolerance. Molecular studies on Na^+ exclusion in soybean and antioxidant-related gene regulation in chickpea may provide genetic markers for resilience. Combining genotypic selection with physiological trait monitoring provides a framework for developing legumes capable of sustaining productivity under climate variability.

Overall, this study clarifies genotype- and species-specific tolerance mechanisms to salinity and water deficit, identifies reliable screening traits for breeding, and provides practical strategies for cultivating legumes under stress-prone environments. These results advance the development of more resilient legume varieties, supporting efforts to ensure food security and reduce the adverse effects of climate change on global agricultural systems.

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10. PUBLICATION LIST



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List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (2)

1. **Sobh, M.**, Basal, O., Ayman, S. A. O., Veres, S.: Assessing the impact of salinity stress on some morpho-physiological traits of two chickpea genotypes under hydroponic conditions.
Acta agrar. Debr. 2, 47-53, 2024. ISSN: 2416-1640.
DOI: <http://dx.doi.org/10.34101/ACTAAGRAR/2/14277>
2. **Sobh, M.**, Zargar, T. B., Basal, O., Veres, S.: Evaluation of chickpea (*Cicer arietinum* L.) in response to salinity stress.
Agrártud. közl. 2023 (1), 105-110, 2023. ISSN: 1587-1282.
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Foreign language scientific articles in international journals (4)

3. **Sobh, M.**, Zargar, T. B., Basal, O., Ayman, S. A. O., Veres, S.: Evaluating salt tolerance of chickpea genotypes based on morphophysiological and biochemical parameters.
Notulae Botanicae. 53 (2), 1-15, 2025. ISSN: 0255-965X.
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4. **Sobh, M.**, Zargar, T. B., Basal, O., Ayman, S. A. O., Veres, S.: Evaluation of salinity tolerance potentials of two contrasting soybean genotypes based on physiological and biochemical responses.
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8. Basal, O., **Sobh, M.**, Veres, S.: A csicseriborsó élettani válasza a késői vetési időre: jobb felhasználás lehetősége vetésforgóban. Physiological response of chickpea to late sowing date: possibility of better exploitation in crop rotations.
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9. **Sobh, M.**, Basal, O., Veres, S.: Quinoa termesztése öntözött körülmények között Magyarországon: előzetes kutatás élettani alapokon.
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In: 59th Croatian & 19th International Symposium on Agriculture : Book of Abstracts / Klaudija Carović-Stanko, Kristina Kljak, University of Zagreb Faculty of Agriculture, Zagreb, Croatia, Dubrovnik, 134-134, 2024, (ISSN 2459-5551)
16. **Sobh, M.**, Zargar, T. B., Dilo, A. M., Basal, O., Ayman, S. A. O., Veres, S.: Physiological and biochemical mechanisms of salt tolerance in chickpea genotypes.
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22 January, 2026



11. DECLARATION

STATEMENT

I wrote this thesis in the framework of the University of Debrecen Kerpely Kálmán Doctoral School for the purpose of obtaining a doctoral degree (Ph.D.) at the University of Debrecen.

Debrecen, .../...../2026

.....

signature of the candidate

Mawia Sobh

STATEMENT

I hereby certify that the doctoral candidate Mawia Sobh has carried out her work under my supervision within the framework of the above-mentioned Doctoral School between 2022-2024. The candidate has made a decisive contribution to the results of the thesis through her independent creative work, and the thesis is the candidate's independent work. I recommend that the thesis be accepted.

Debrecen, .../...../2026

.....

signature of the supervisor

Dr. Szilvia Veres