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Research Article

Harnessing the Power of Ellagic Acid: A Natural Shield Against Salt Stress in Wheat and Chickpea

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Abstract
The research investigates how ellagic acid (EA) influences the reduction of salinity stress in wheat and chickpea plants. Salinity is a major challenge for global agriculture as it interferes with vital plant physiological functions, especially photosynthesis, by causing ion imbalances and oxidative damage. This study examines EA, a phenolic compound known for its antioxidant capabilities, and its ability to counteract the detrimental impacts of salt stress. In this experiment, wheat and chickpea plants were grown under controlled conditions and exposed to salinity (100 mM NaCl), both with and without the application of EA (12.5 µM). Various parameters, such as fresh and dry biomass, proline concentration, and gas exchange rates, were recorded. The findings revealed that salt stress drastically lowered both biomass and gas exchange performance in the plants, but the application of EA partially alleviated these negative effects. EA enhanced both fresh and dry weights, minimized electrolyte leakage, and elevated proline levels, particularly in chickpea plants. Additionally, gas exchange parameters, including carbon assimilation (A), stomatal conductance (gs), and transpiration rate (E), improved with the combined EA and salt treatment compared to salt stress alone. The study concludes that EA serves as a protective agent against oxidative damage caused by salinity, enhancing growth parameters and boosting photosynthetic performance. This suggests that EA could be a valuable approach to increasing plant tolerance to salinity in agricultural systems.

1. Introduction

The adverse effects of saline soils on agricultural land have become an increasingly significant global issue. Water scarcity, triggered by global warming and climate change, along with improper irrigation techniques, has led to a rapid rise in soil salinity. Today, approximately 1 billion hectares of land worldwide are affected by salinization, which negatively impacts 20% of global agricultural production and 33% of irrigated agricultural land [1]. Saline soils restrict the growth and development of plants, reduce productivity, and jeopardize food security. Plants exposed to salt stress suffer from severe physiological and biochemical damage [2]. Salt stress in plants disrupts physiological processes by reducing water uptake and causing ion imbalance. One of the most significant effects of this stress is the decline in the photosynthesis process [3]. Studies have shown that salinity reduces chlorophyll content in plants, leading to a decrease in photosynthetic activity. For instance, a study conducted on *Raphanus sativus* L. revealed that salt stress reduced leaf area, impaired photosynthetic performance, and negatively impacted nutrient uptake [4]. Similarly, another study on *Spinacia oleracea* found that salt stress induced oxidative stress and negatively affected chloroplast proteins, resulting in significant reductions in photosynthesis and plant growth [5]. A study on lentil seedlings (*Lens culinaris*) observed that salinity disrupted growth and ion balance while negatively influencing antioxidant enzyme activity. This ultimately compromised the plants' ability to cope with oxidative stress [6]. Salt stress leads to water loss in plants and causes cellular dehydration. This means that plants are unable to maintain water balance, resulting in damage to cellular structures. A study on *Momordica charantia* indicated that salt stress reduced net photosynthetic rate, decreased PSII efficiency, and increased the production of reactive oxygen species (ROS), leading to severe physiological impairments in the plants [7]. One of the most noticeable effects of salt stress is the increase in oxidative stress within plants. Salt induces the accumulation of ROS in plants, which in turn damage plant cells. In a study conducted on *Populus euphratica*, it was found that salinity and alkaline stress disrupted growth and ion balance, causing leaf damage. Male individuals of the

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species displayed higher resistance to salinity compared to females; however, the stress generally led to physiological deterioration [8]. A study on potato plants revealed that even low levels of salinity (1 g/kg NaCl) reduced plant biomass, while higher salinity levels (7 g/kg NaCl) increased lipid peroxidation and negatively impacted enzymatic antioxidants. This demonstrates that salinity severely compromises plant quality [9].

In recent years, the use of phenolic compounds to alleviate the negative effects of salt stress on plants has become a significant area of research. Phenolic compounds are secondary metabolites that play crucial roles in the defense mechanisms of plants and enhance adaptive responses to abiotic stress factors such as salinity [10]. These compounds mitigate the effects of stress by reducing oxidative stress, maintaining ion balance, and improving photosynthetic activity in plants. A study on Morus spp. plants demonstrated that phenolic and flavonoid contents increased when exposed to salt stress. In this study, under high salt concentrations, the highest phenolic content was observed in the M4 plant, while the highest flavonoid content was detected in the M2 plant [11]. Similarly, a study on *Dracocephalum kotschyi* found that salt stress increased total phenolic content, and this increase was further supported by the application of calcium and melatonin [12]. These findings suggest that exogenous applications can stimulate the production of phenolic compounds in plants, playing a protective role against salt stress. The mechanism by which phenolic compounds alleviate salt stress typically involves enhancing antioxidant activity and combating oxidative stress. In a study on the halophyte plants *Spergularia marina* and *Glaux maritima*, it was observed that phenolic compound levels increased under salt stress, leading to improvements in antioxidant activities [13]. Similarly, a study on *Mentha piperita* revealed that salt stress increased the accumulation of phenolic compounds, which participated in the defense process against oxidative stress [14]. In recent years, the effect of exogenously applied phenolic compounds in mitigating salt stress has been emphasized in numerous studies. These compounds strengthen the antioxidant defense system in plants, reducing oxidative damage and enhancing photosynthetic efficiency. Phenolic compounds play a crucial role in managing salt stress by regulating ion homeostasis in plants. For instance, the exogenously applied phenolic compound coumarin played a significant role in the defense mechanism against salt stress in tomato plants. Coumarin reduced salt-induced oxidative stress and toxicity by enhancing the antioxidant defense system, the glyoxalase system, and ion homeostasis. This application also significantly improved photosynthesis and growth performance in plants under salt stress [15]. In another example, exogenously applied salicylic acid (SA) alleviated salt stress in St. John's Wort plants and increased antioxidant enzyme activity. This treatment improved photosynthetic activity while reducing abscisic acid levels under stress conditions [16]. These exogenously applied phenolic compounds play an essential role in enhancing the biochemical and physiological resilience of plants against salt stress. Similar mitigating effects on salt-induced osmotic stress have been recorded for ellagic acid (EA), a dimeric product of gallic acid, which contains four hydroxyl groups and a dilactone structure [17]. The biological activities of ellagic acid have been studied since the late 1990s [18]. The hydroxyl groups and lactone structures in ellagic acid are rich in hydrogen bonds and can act as electron acceptors and hydrogen donors, making it a potent antioxidant agent due to its ability to accept electrons from various substrates [18]. Additionally, EA functions as both a primary antioxidant (as a free radical scavenger) and a secondary antioxidant, making it a multifunctional antioxidant [19]. EA is a natural phenolic antioxidant and allelopathic compound found in many fruits and vegetables. Most of the ellagic acid content in plant cells is stored in the vacuoles as water-soluble ellagitannins, which are thought to play a role in plant defense against pathogen attacks. EA has garnered significant attention due to its nutritional and pharmacological potential, as it has been reported to possess antioxidant and antiviral activity. In earlier studies across various disciplines, Cozzi, Ricordy [20] demonstrated the beneficial role of EA by scavenging free radicals of ROS in Chinese hamster ovary cells. Furthermore, EA has additional antioxidant roles, such as binding to DNA, inhibiting ROS production, and protecting DNA from alkylating damage [20]. From a plant physiology perspective, the pretreatment of chickpea seeds with EA has been reported to enhance resistance to osmotic stress [17]. Additionally, Khan, Nazar [21] found that EA application to *Brassica napus* (canola) plants exposed to salt stress resulted in increased growth and chlorophyll content, both of which were diminished under stress conditions.

Given this limited data, it is evident that ellagic acid could serve as a highly effective protective agent for plants. However, a review of the literature reveals that the physiological and biochemical changes induced by ellagic acid under salt stress conditions in monocot (wheat) and dicot (chickpea) plants remain understudied and unclear. In our study, in addition to examining growth parameters, electrolyte leakage, proline content, and gas exchange parameters will be analyzed to reveal the potential protective mechanisms and functions of ellagic acid under salt stress conditions.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Wheat (Taner variety) was sourced from the Bahri Dağdaş International Agricultural Research Institute, and chickpeas (Hasanbey variety) were obtained from the Faculty of Agriculture, Department of Soil Science and Plant Nutrition in selcuk university. A preliminary experiment was conducted using ellagic acid at five concentrations (6.25, 12.5, 25, 50, and 100 µM) to identify the optimal dosage for further studies. Based on the results, the main experimental design included four groups: control (C), 12.5 µM ellagic acid (EA), 100 mM salt (S), and a combined treatment of 12.5 µM ellagic acid with 100 mM salt (EA+S). After germination, the wheat and chickpea seedlings were grown in hydroponic culture under controlled conditions in a climate chamber. The necessary controls for temperature, humidity, light, and sterilization were maintained. In the climate chamber, seed germination and subsequent growth of young seedlings were carried out under conditions of 45-55% humidity, a 16-hour light/8-hour dark photoperiod, 22±1°C temperature, and approximately 10,000 lux/day light intensity. Full, healthy, and relatively uniform seeds of wheat and chickpea were selected, and surface sterilization was performed prior to use. Seeds were treated with 5% sodium hypochlorite for 15 minutes and then rinsed with sterile deionized water. The sterilized seeds were grouped and placed in petri dishes, then allowed to germinate at 24±1°C under a 16-hour light/8-hour dark photoperiod in the climate chamber. Germinated seeds were grown under controlled conditions with a light period of 16 hours (at a light intensity of 350 µmol $m^{-2}s^{-1}$, 23°C temperature, and 45-55% humidity) and an 8-hour dark period (22°C temperature and 45-55% humidity). The seedlings were fed with Hoagland nutrient solution, and the solution was refreshed every 5 days during the cultivation period in the climate chamber.

2.2. Analysis of Physiological Parameters

After harvesting, the roots of the plants were separated, and both fresh and dry weights of the leaf regions were measured. The dry weights were determined after the samples were dried in an oven at 70°C for 72 hours. Cell membrane permeability was assessed by measuring electrolyte leakage according to the method of Dionisio-Sese and Tobita (1998). For this procedure, 100 mg of leaf samples were cut into 5 mm segments and transferred to test tubes containing 10 mL of deionized water. The tubes were sealed with plastic caps and placed in a water bath at 32°C for 2 hours. The electrical conductivity of the solution (EC1) was measured using a conductivity meter. The samples were then autoclaved at 121°C for 20 minutes to kill all tissues and release the remaining electrolytes. After cooling to 25°C, the final conductivity (EC2) was measured. Electrolyte leakage was calculated using the formula: EL (%) = (EC1/EC2) × 100. The carbon assimilation rate (A), stomatal conductance (gs), intercellular CO_2 concentration (Ci), stomatal limitation (Ls), and transpiration rate (E) were measured using a portable gas exchange system (LCpro+; ADC, Hoddesdon, UK). Gas exchange parameters were recorded for 6 replicates by selecting leaves of similar size from each treatment group at the end of the experiment. Stomatal limitation (Ls) was calculated using the formula Ls = 1 – Ci/Ca.

2.3. Measurement of Proline Content

The determination of proline content was carried out according to the method of Bates, Waldren and Teare [22]. Leaf samples (0.1 g) from each group were homogenized in 3% sulfosalicylic acid. The filtered homogenate was mixed with acid ninhydrin and glacial acetic acid and then incubated in a water bath at 100°C for 1 hour. To stop the reaction, the mixture was transferred to an ice bath. After cooling, toluene was added for extraction, and the toluene fraction was aspirated from the liquid phase. The absorbance of the toluene fraction was measured at 520 nm using a spectrophotometer.

2.4. Measurement of Hydroxyl Radical ($OH^{\bullet}$) Scavenging Activity.

Hydroxyl radical scavenging activity in leaf tissues will be measured using the method of Kim and Minamikawa [23]. Freshly prepared reagents will be used, and $OH^{\bullet}$ scavenging will be assessed by measuring deoxyribose competition with OH• from the Fe3+/ascorbate/EDTA/H_2O_2 system in the sample. The reaction mixture, including buffer, deoxyribose, $FeSO_4$, EDTA, H_2O_2, water, and sample, will be incubated at 37°C for 2 hours. After incubation, TCA and TBA will be added, boiled for 20 minutes, and absorbance will be measured at 520 nm.

2.5. Statistical Analyses

The experiments were conducted in a **completely randomized design (CRD)** to minimize variability and ensure reliability of the results. Each experiment consisted of at least 3 replicates, and each finding, except for growth parameters (n=10), was based on 2 replicates (n=6). The obtained data were analyzed using one-way analysis of variance (ANOVA), and differences between the means were compared using the Least Significant Difference (LSD) test. Values with $P<0.05$ were considered statistically significant. Statistical analyses were performed using the SPSS software (standard version 13.0).

3. Results

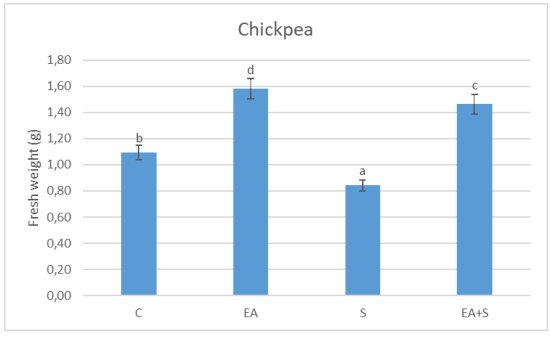
The variance analysis revealed significant effects of treatments (T), variety (V), and their interaction (T×V) on all the studied parameters, which include fresh weight (FW), dry weight (DW), photosynthetic rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci), stomatal conductance (gs), stomatal limitation (Ls), hydroxyl radical (OH), electrical conductivity (EC), and proline content. Notably, treatments had a highly significant effect ($P<0.01$) on FW, DW, A, gs, and proline content, indicating that the applied treatments strongly influenced plant growth and stress tolerance. The interaction between variety and treatment (T×V) also had significant effects on parameters such as FW, A, and proline, highlighting the differential response of the varieties under different treatment conditions. These findings suggest a complex interplay between variety and treatment in mitigating salt stress effects in the studied plants (Table 1).

Table 1. Variance analysis for the effects of Treatments (T), Variety (V), and their interaction (T x V) on Fresh Weight (FW), Dry Weight (DW), Photosynthetic Rate (A), Stomatal Conductance (gs), Intercellular CO_2 Concentration (Ci), Stomatal Limitation (Ls), Transpiration Rate (E), Hydroxyl Radical (OH), Electrical Conductivity (EC), and Proline. (*****P < 0.05 - ******P < 0.01).

Source of Variation	SD	FW	DW	A	E	Ci	gs	Ls	OH	EC	Proline
General	23	5.6083**	0.1135**	202.68 **	13.15 **	54205 **	37316 **	0.4040 **	376.73**	2695.19**	1484.02**
Treatment (T)	3	3.7929 **	0.0505 **	179.39 **	5.23 **	39562 **	16760 **	0.3519 **	137.59 **	1642.19 *	189.65 **
Variety (V)	1	0.0144 **	0.0410 **	10.15 **	5.31 **	206.37 *	13120 **	0.0015 *	152.56 **	0.0135*	190.56 **
T x V	3	1.7829 **	0.0211 **	13.29 **	2.59 **	13383 **	7395.2 **	0.0473 **	60.31 **	1037.18 *	1091.03 **
Error	16	0.0179	0.0018	0.8406	0.0140	1053.19	31.728	0.0032	26.26	15.79	12.76

Salinity stress significantly affected the growth parameters of wheat and chickpea compared to the non-stress group. The 100 mM NaCl (S) treatment resulted in a reduction of fresh weight (FW) in both plants, with the greatest reduction observed in wheat, by 61%. In contrast, the exogenous application of ellagic acid (EA) alone led to an increase in FW by 23% in wheat and 45% in chickpea (Figure 1 a-b). This increase was also observed in the EA+S group, where EA mitigated the negative effects of salinity stress. In wheat, EA application resulted in a 30% increase in dry weight (DW), while salinity stress alone caused a 54% decrease (Figure 1 c-d). However, the EA+S treatment showed an 11% increase, partially mitigating the negative effects of salinity. For chickpea, EA application led to a 44% increase in DW, while salinity stress caused a 20% decrease. The EA+S treatment improved DW by 20%, indicating a mitigating effect against salinity-induced reductions. Electrical conductivity (EC) reflects the impact of salinity stress on plant metabolism. In the present study, the application of EA alone did not significantly affect the EC levels in plants. However, under salinity stress, EC levels increased in all treated plants, with the highest increase observed in chickpea, showing a 4.9-fold rise. In the EA+S group, the EC levels were reduced in both plants, with an 18% reduction in wheat and a 42% reduction in chickpea.

(**a**)



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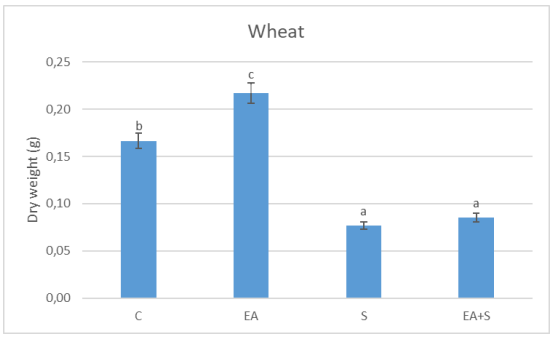

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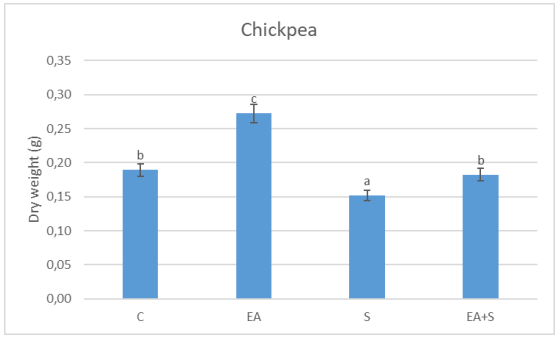
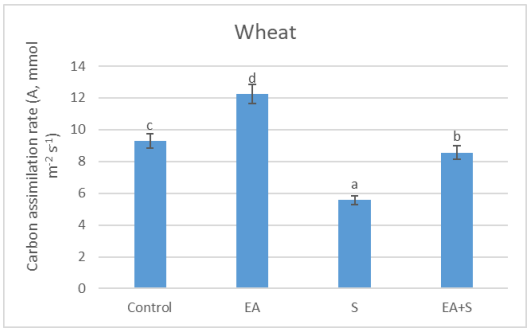


(**d**)

Figure 1. (a) Fresh weight (FW) of wheat; (b) FW of chickpea; (c) Dry weight (DW) of wheat; (d) DW of chickpea under salinity stress (S) (100 mM) and with or without ellagic acid (EA) (12.5 µM)

Gas exchange parameters, such as carbon assimilation rate (A), transpiration rate (E), intercellular CO_2 concantration (C_i), stomatal conductance (g_s) and stomatal limitation (L_s) are important in how plants respond to salt stress. Exogenous application of EA resulted in an increase in the content of A in wheat, while no significant change was observed in chickpea (Figure 2). However, under salinity stress, a substantial decrease in A content was noted in both plants. With the EA+S treatment, significant increases in A content were detected in both wheat and chickpea, with increases of 53% and 62%, respectively. The content of E in wheat increased 2.2-fold with the application of EA, while, interestingly, a decrease was observed in chickpea. On the other hand, salt application did not lead to a significant reduction in wheat; however, in chickpea, a substantial 80% decrease was detected. With the EA+S treatment, wheat showed a 37% increase compared to the salt-only group, whereas chickpea exhibited a significant 3-fold increase. In the study, C_i content did not exhibit significant differences across treatments, with the most notable change observed in wheat, where EA application alone resulted in a 40% increase compared to the control group. Although salt stress caused a reduction in C_i content in both wheat and chickpea, the highest increase was detected in chickpea. Under the EA+S treatment, a statistically significant change was observed only in chickpea, showing a 23% increase compared to the salt-only treatment. Exogenous EA application resulted in a 2.3-fold increase in g_s content in wheat, while, conversely, it led to a 20% decrease in chickpea (Figure 2). Under salt stress, both plants showed a reduction in g_s content, with the highest decrease of 85% observed in chickpea. However, with the combined application of EA and salt stress, an increase in g_s content was observed in both plants, with the most notable increase detected in chickpea, showing a 3.7-fold rise compared to the salt-only treatment. The L_s content did not exhibit statistically significant changes with the application of EA alone. However, a substantial increase in L_s content was observed under salt stress, with a 1.5-fold increase in wheat and a 2-fold increase in chickpea. While the EA+S treatment did not cause a significant change in wheat, it led to a 44% reduction in L_s content in chickpea compared to the salt-only treatment (Figure 2).



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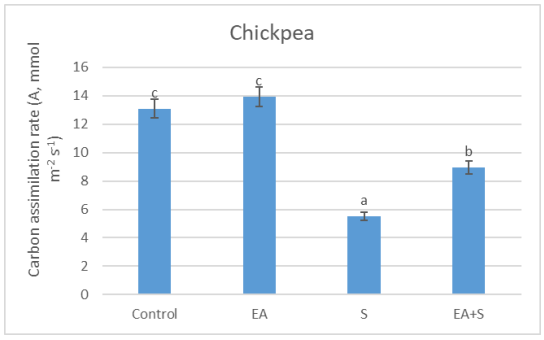

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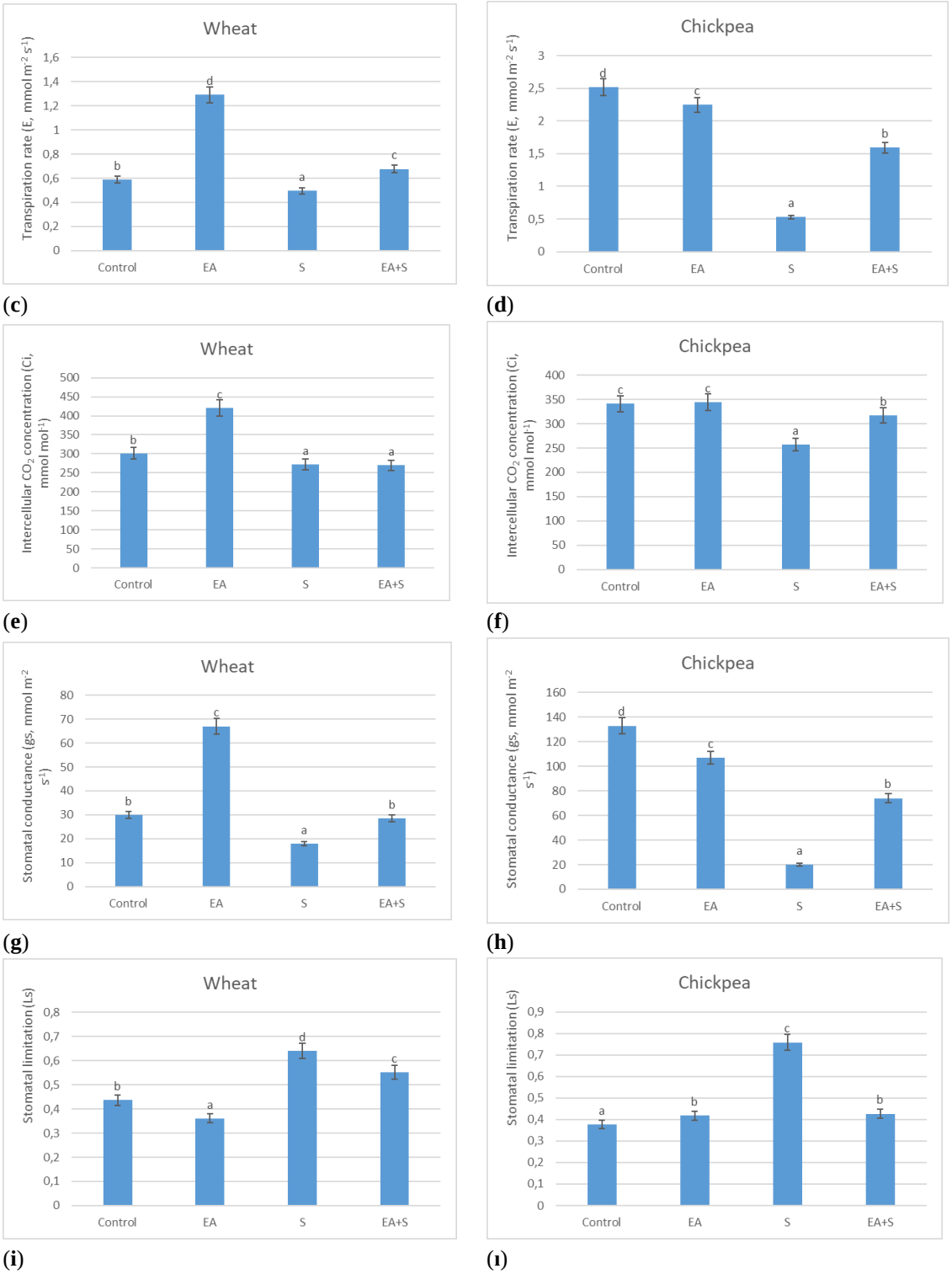

Figure 2. Gas exchange parameters: carbon assimilation rate (A) (a-b), transpiration rate (E) (c-d), intercellular CO_2 concentration (Ci) (e-f), stomatal conductance (gs) (g-h), and stomatal limitation (Ls) (i-ı) of chickpea and wheat plants under salinity stress (S) (100 mM) and with or without ellagic acid (EA) (12.5 µM).

The exogenous application of EA had no significant effect on the hydroxyl radical (OH•) scavenging activity in wheat plants; however, interestingly, it exhibited a negative impact on this activity in chickpea. Under salinity stress, OH• radical levels increased in all treated plants, with a 5% rise observed in wheat and a 7% rise in chickpea (Figure3 c-d). Nevertheless, the combined treatment of EA and salinity stress (EA+S) mitigated the adverse effects of salinity, leading to a reduction in OH• radical levels in both plant species. Proline (Pro) is a crucial osmolyte that plays a significant role in plant stress tolerance systems. In the current study, the application of EA alone resulted in an increase in proline content in all treated plants, with the highest increase observed in wheat, showing a 53% increment (Figure3 e-f). Under salinity stress conditions, Pro content decreased in wheat, whereas, interestingly, a 3.1-fold increase was observed in chickpea

when compared to the control group. In the EA+S treatment, the Pro content in wheat continued to decrease, similar to the trend observed under stress conditions alone. However, in chickpea, the Pro content continued to rise, with a two-fold increase detected compared to the control.

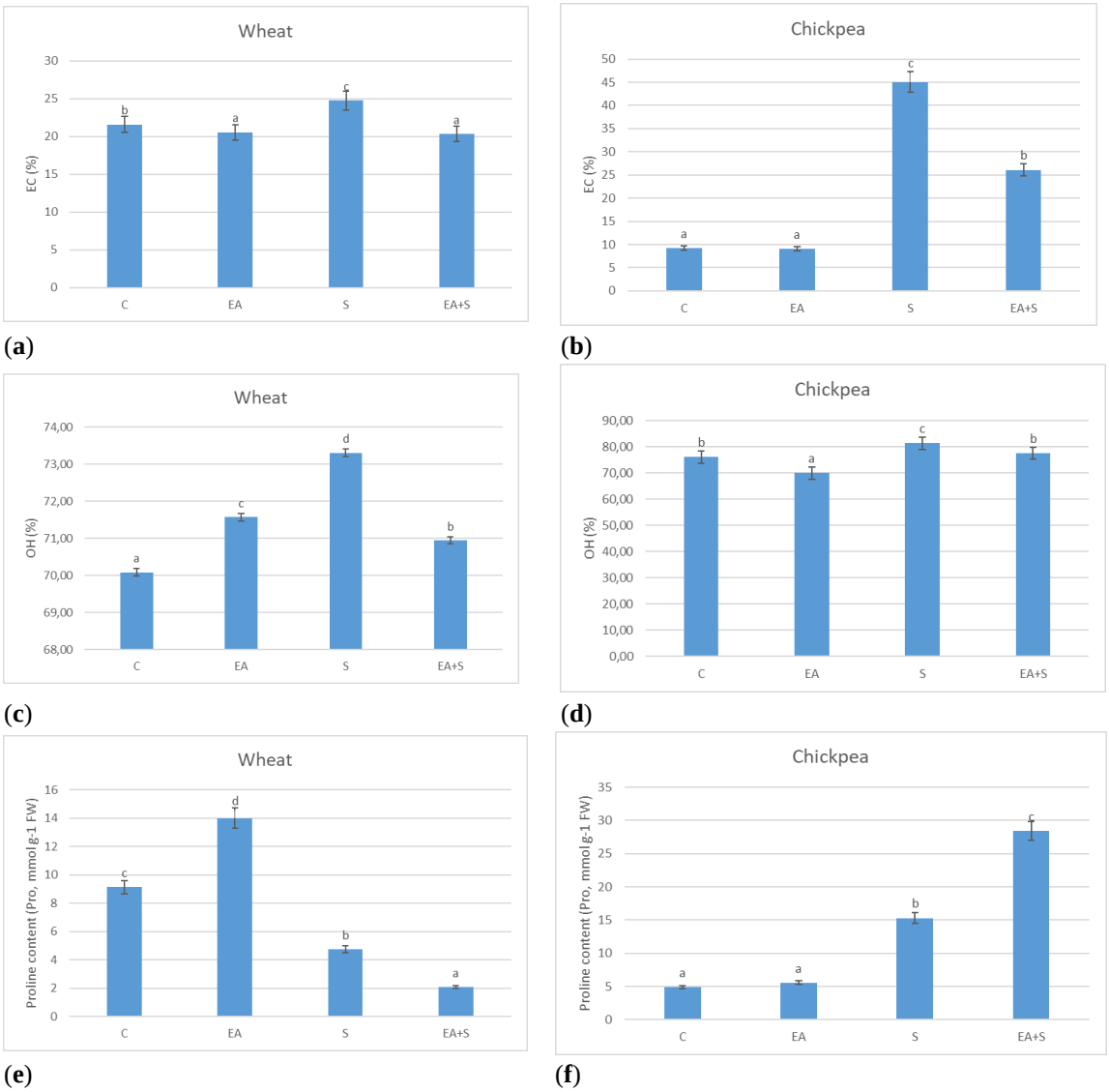

Figure 3. Electrical conductivity (%) (a-b), hydroxyl radical (OH•) (c-d), and proline (Pro) (e-f) of chickpea and wheat plants under salinity stress (S) (100 mM) and with or without ellagic acid (EA) (12.5 µM).

As shown in Figure 4, the correlation matrix reveals key interactions among various physiological and biochemical parameters under stress conditions, with particular emphasis on the relationships between FW, DW, and Pro accumulation. These parameters show strong positive correlations, indicating that higher proline levels are linked to increased biomass. In contrast, EC, a marker of stress, exhibits negative correlations with both FW and DW, suggesting that as salinity rises, biomass production decreases a typical response to salt stress. OH• correlate positively with EC and negatively with proline, suggesting that oxidative stress escalates with increasing salinity, which may lead to a reduction in proline accumulation. Furthermore, gas exchange parameters, including A, g_s, E, and C_i, display negative correlations with stress indicators like EC and OH•. This indicates that as stress levels rise, both photosynthesis and gas exchange processes are impaired. On the other hand, the parameter "L_s" shows strong positive correlations with gas exchange parameters and negative correlations with stress markers, implying that it may play a protective role in preserving physiological function under stress conditions.

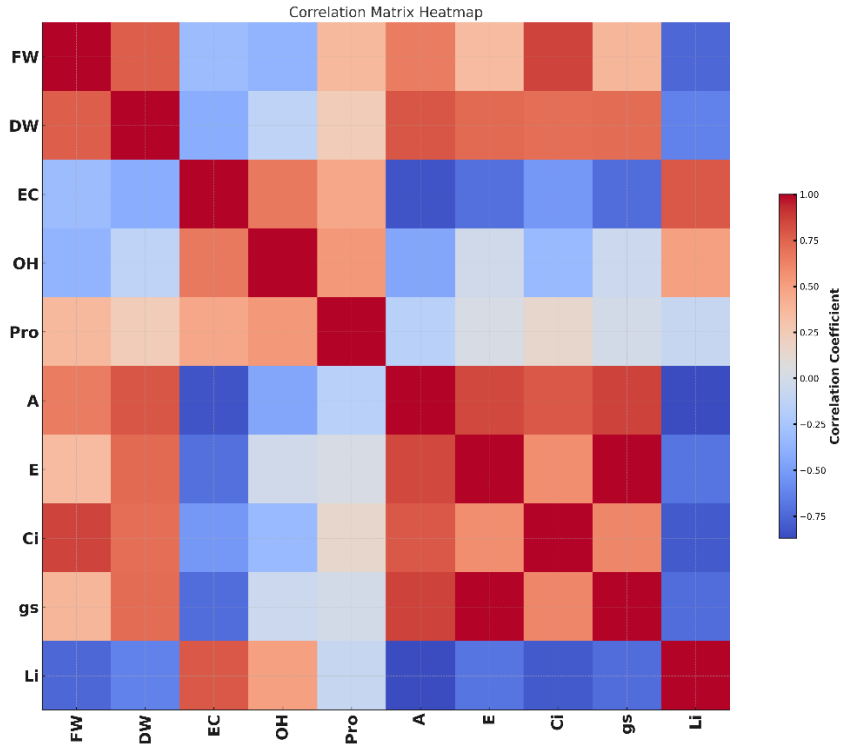

Figure 4. Correlation matrix heat map showing the relationships between various physiological and biochemical parameters, including fresh weight (FW), dry weight (DW), proline (Pro), electrical conductivity (EC), hydroxyl radical (OH•), and gas exchange parameters such as carbon assimilation rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci), stomatal conductance (gs), and stomatal limitation (Ls) in chickpea and wheat plants under salinity stress (S) (100 mM) and with or without ellagic acid (EA) (12.5 µM). Positive and negative correlations are indicated by color intensity.

4. Discussion

Salinity stress, one of the most significant environmental stress factors, can adversely affect the normal functioning of physiological and biochemical pathways in plants. Long-term or high concentrations of salinity can influence plant biomass, directly affecting agricultural productivity. In our study, it was found that salinity stress significantly reduced biomass levels in both wheat and chickpea plants. In a previous study, higher concentrations of 100 mM NaCl caused significant growth reductions due to ion toxicity and oxidative damage in *Moringa oleifera* plants [24]. Similarly, a study on *Rosmarinus officinalis* demonstrated that increasing salinity levels resulted in reduced plant height and total dry weight, indicating a direct inhibitory effect of salt on growth processes [25]. Additionally, in strawberries, elevated salinity stress reduced growth parameters such as plant height and biomass, with variations observed between cultivars in terms of tolerance levels [26]. The exogenous application of secondary metabolites to mitigate the negative effects of salt stress on plant growth has become an important research focus in recent years. Specifically, the regulatory effects of phenolic compounds on plant physiology under abiotic stresses such as salinity have been extensively studied. It has been reported that the antioxidant properties of phenolic compounds protect plants from oxidative stress, improving their growth performance [27]. In our study, the application of ellagic acid (EA), a potent phenolic compound, suppressed the salt stress-induced reduction in growth, and increases were observed in the EA+S groups compared to the salt-only treatment. When comparing the plants, the highest increase was detected in chickpea, which corresponded with the electrical conductivity (EC) values, suggesting that EA was more effective in enhancing tolerance in chickpea. In line with previous studies, the exogenous application of salicylic acid (SA), another phenolic compound, under salt stress conditions has been shown to reduce the growth inhibition caused by salinity and increase plant biomass in leguminous species and cotton [28]. Furthermore, it has been reported that other phenolic compounds, such as gallic acid (GA), contribute to the preservation of total biomass and the improvement of chlorophyll content in salt-stressed plants when applied as a foliar spray [29] These findings suggest that phenolic compounds not only enhance antioxidant activity under abiotic stress but also promote growth by regulating ion balance and water relations [30].

Excessive accumulation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl radicals ($OH^{\bullet-}$), triggered by salinity stress, is known to cause the degradation of chlorophyll pigments [31].

This degradation is a clear indicator of oxidative damage in plants, as the breakdown of these pigments can impair photosynthesis and overall plant health. In our study, both wheat and chickpea plants subjected to salinity stress exhibited higher OH^- levels compared to the control group. This increase in hydroxyl radical concentration was found to be correlated with the plants' EC values, suggesting a direct relationship between ion imbalance due to salinity and oxidative damage. Specifically, the rise in EC, which is indicative of salt stress, aligns with the increase in ROS levels, particularly $OH^{\bullet-}$, thus exacerbating oxidative stress. The positive correlation between $OH^{\bullet-}$ levels and EC highlights the impact of ionic stress on ROS production. As ROS accumulation intensifies, it can lead to oxidative damage, including lipid peroxidation, protein denaturation, and DNA damage, all of which can significantly hinder plant growth and productivity [32]. Many other research has shown that exogenously application of phenolic compounds can upregulate key antioxidant enzymes and related pathways, including phenylpropanoid biosynthesis, which directly contributes to the reduction of harmful ROS, such as hydroxyl radicals. For instance, flavonoids are reported to enhance metal chelation processes, which helps mitigate hydroxyl radical damage in salt-stressed plants. Additionally, polyphenols such as tannins and lignins play a significant role in scavenging ROS, including hydroxyl radicals, thereby protecting plants from oxidative damage [33]. In our study, similarly, it was observed that the application of EA led to reductions in ROS accumulation caused by salt stress in both plants, with a comparable decrease detected in each species. Controlling leaf gas exchange parameters plays a crucial role in enhancing the resilience of crops to a range of biotic and abiotic stress factors [34]. Our results indicate a negative impact of salt stress on gas exchange parameters, with A, E, Ci, and gs significantly decreasing under salt stress across all treatment plants. Salt-induced reductions in leaf gas exchange parameters, such as Ci, A and gs, have been documented in various plant species. For example, studies on *B. juncea* L. [35] and *V. radiata* L. [36] revealed significant declines in these key physiological parameters under saline conditions. These reductions suggest that salt stress disrupts essential processes related to photosynthesis and transpiration, affecting the overall gas exchange efficiency in both species. Such consistent findings across different plants underscore the widespread negative impact of salinity on fundamental physiological functions. In our study, it was observed that with the EA+S treatment, the previously reduced gas exchange parameters showed an increase. This suggests that EA enhanced plant tolerance by reducing oxidative stress and improving these parameters associated with photosynthesis. Similarly, previous studies demonstrated that in tomato plants, foliar application of flavonoids improved gas exchange parameters, such as net photosynthesis (Pn) and gs under salt stress. Treated plants exhibited higher CO_2 fixation rates and better water uptake, even under saline conditions, due to increased stomatal aperture, which directly promoted plant growth [37]. This improvement in gas exchange was linked to the upregulation of aquaporins and phenolic metabolism, helping plants manage stress more effectively. In another study, salicylic acid (SA) application under salt stress also led to improvements in gas exchange parameters, which were suggested to be associated with reduced ROS content and an enhanced antioxidant enzyme system [38]. The application of exogenous phenolic compounds significantly boosts proline levels in plants, enhancing their resilience to environmental stresses by improving photosynthesis, antioxidant defense, and cellular protection mechanisms [39, 40]. Similarly, exogenous applications combined with salinity stress (EA+S) have been found to mitigate the harmful effects of salinity by reducing hydroxyl radical ($OH^{\bullet}$) levels in plants. Proline, a vital osmolyte, plays a central role in plant stress adaptation. For instance, in transgenic tobacco and olive trees, phenolic compounds increased total phenolic content and reduced oxidative stress under water deficit and salinity conditions, highlighting their role in environmental stress mitigation [41, 42]. Furthermore, under stress, proline levels showed varied trends: while chickpea exhibited a 3.1-fold increase under salinity, wheat's proline content decreased. Interestingly, when EA was applied alongside salinity stress, chickpea proline levels doubled compared to the control, while wheat continued to show a decline. These findings underscore the importance of exogenous compounds in enhancing stress tolerance and promoting agricultural productivity.

5. Conclusion

In summary, this research highlights that ellagic acid (EA) plays a crucial role in alleviating the harmful impacts of salt stress on wheat and chickpea plants by improving key physiological and biochemical traits. The application of EA led to increased plant biomass, enhanced gas exchange rates, and elevated proline content, underscoring its effectiveness as a potent antioxidant in countering oxidative stress. Additionally, the observed reductions in electrolyte leakage and reactive oxygen species (ROS) levels further confirm EA's role in preserving cellular integrity under saline stress. These results point to the potential of EA as a promising biostimulant for enhancing crop resilience to salinity, particularly in arid and semi-arid regions where soil salinization is a major concern. Future investigations should focus on the long-term impacts of EA across a broader range of crop species and assess its potential incorporation into sustainable farming systems to boost food security. Moreover, exploring the molecular mechanisms underpinning EA’s effects could offer valuable insights for optimizing its use under diverse environmental stressors.

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References

[1] Singh, A. **(2022).** *Soil salinity: A global threat to sustainable development*. Soil Use and Management 38(1): 39-67. DOI: 10.1111/sum.12772

[2] Hao, S.,Y. Wang,Y. Yan,Y. Liu,J. Wang.,S. Chen. **(2021).** *A review on plant responses to salt stress and their mechanisms of salt resistance*. Horticulturae 7(6): 132. DOI: 10.3390/horticulturae7060132

[3] Zahra, N.,M.S. Al Hinai,M.B. Hafeez,A. Rehman,A. Wahid,K.H. Siddique.,M. Farooq. **(2022).** *Regulation of photosynthesis under salt stress and associated tolerance mechanisms*. Plant Physiology and Biochemistry 178: 55-69. DOI: 10.1016/j.plaphy.2022.03.003

[4] Mahawar, L.,M. Živčák,M. Barboricova,M. Kovár,A. Filaček,J. Ferencova,D.M. Vysoká.,M. Brestič. **(2024).** *Effect of copper oxide and zinc oxide nanoparticles on photosynthesis and physiology of Raphanus sativus L. under salinity stress*. Plant Physiology and Biochemistry 206: 108281. DOI: 10.1016/j.plaphy.2023.108281

[5] Vajjiravel, P.,D. Nagarajan,V. Pugazhenthi,A. Suresh,M.K. Sivalingam,A. Venkat,P.P. Mahapatra,K. Razi,M. Al Murad.,D.W. Bae. **(2024).** *Circadian-based approach for improving physiological, phytochemical and chloroplast proteome in Spinacia oleracea under salinity stress and light emitting diodes*. Plant Physiology and Biochemistry 207: 108350. DOI: 10.1016/j.plaphy.2024.108350

[6] Sarkar, M.M.,P. Rudra,P. Paul,T.K. Dua.,S. Roy. **(2024).** *Enhanced adaptation to salinity stress in lentil seedlings through the use of trehalose-functionalized silica nanoparticles (TSiNPs): Exploring silica-sugar absorption and oxidative balance*. Plant Physiology and Biochemistry 206. DOI: 108309 10.1016/j.plaphy.2023.108309

[7] Sheikhalipour, M.,G. Gohari,B. Esmaielpour,M. Behnamian,M.T. Giglou,M.H. Milani,M.K. Bahrami,M. Kulak,A. Ioannou.,V. Fotopoulos. **(2023).** *Effect of melatonin foliar sprays on morphophysiological attributes, fruit yield and quality of Momordica charantia L. under salinity stress*. Plant Physiology and Biochemistry 205: 108194. DOI: 108309 10.1016/j.plaphy.2023.108194

[8] Yu, L.,S. Tang,C. Guo,H. Korpelainen.,C. Li. **(2023).** *Differences in ecophysiological responses of Populus euphratica females and males exposed to salinity and alkali stress*. Plant Physiology and Biochemistry 198: 107707. DOI: 10.1016/j.plaphy.2023.107707

[9] Wang, H.,J. Li,H. Liu,S. Chen,Q. uz Zaman,M. Rehman,K. El-Kahtany,S. Fahad,G. Deng.,J. Yang. **(2023).** *Variability in morpho-biochemical, photosynthetic pigmentation, enzymatic and quality attributes of potato for salinity stress tolerance*. Plant Physiology and Biochemistry 203: 108036. DOI: 10.1016/j.plaphy.2023.108036

[10] Ahlawat, Y.K.,M. Singh,K. Manorama,N. Lakra,A. Zaid.,F. Zulfiqar. **(2024).** *Plant phenolics: neglected secondary metabolites in plant stress tolerance*. Brazilian Journal of Botany 47(3): 703-721. DOI: 10.1007/s40415-023-00949-x

[11] Wulandari, Y.,Y. Sulistyaningsih,A. Suprayogi,M. Rahminiwati.,T. Triadiati. *Effect of salinity on glandular trichomes density, phenolic and flavonoid contents of Morus spp. accessions leaves from Indonesia*. in *IOP Conference Series: Earth and Environmental Science*. 2023. IOP Publishing. DOI: 10.1088/1755-1315/1271/1/012037

[12] Vafadar, F..,P. Ehsanzadeh. **(2023).** *Synergistic effects of calcium and melatonin on physiological and phytochemical attributes of Dracocephalum kotschyi genotypes under salinity stress*. Physiologia Plantarum 175(3): e13912. DOI: 10.1111/ppl.13912

[13] Pungin, A.,L. Lartseva,V. Loskutnikova,V. Shakhov,E. Popova,L. Skrypnik.,O. Krol. **(2023).** *Effect of salinity stress on phenolic compounds and antioxidant activity in halophytes Spergularia marina (L.) Griseb. and Glaux maritima L. cultured in vitro*. Plants 12(9): 1905. DOI: 10.3390/plants12091905

[14] Gholamnia, A.,A. Mosleh Arani,H. Sodaeizadeh,S. Tarkesh Esfahani.,S. Ghasemi. **(2022).** *Expression profiling of rosmarinic acid biosynthetic genes and some physiological responses from Mentha piperita L. under salinity and heat stress*. Physiology and Molecular Biology of Plants 28(3): 545-557. DOI: 10.1007/s12298-022-01159-7

[15] Parvin, K.,M. Hasanuzzaman,S. Mohsin,K. Nahar.,M. Fujita. **(2021).** *Coumarin improves tomato plant tolerance to salinity by enhancing antioxidant defence, glyoxalase system and ion homeostasis*. Plant Biology 23: 181-192. DOI: 10.1111/plb.13208

[16] Kwon, E.-H.,A. Adhikari,M. Imran,D.-S. Lee,C.-Y. Lee,S.-M. Kang.,I.-J. Lee. **(2023).** *Exogenous SA Applications Alleviate Salinity Stress via Physiological and Biochemical changes in St John's Wort Plants*. Plants 12(2): 310. DOI: 10.3390/plants12020310

[17] El-Soud, W.A.,M.M. Hegab,H. AbdElgawad,G. Zinta.,H. Asard. **(2013).** *Ability of ellagic acid to alleviate osmotic stress on chickpea seedlings*. Plant Physiology and Biochemistry 71: 173-183. DOI: 10.1016/j.plaphy.2013.07.007

[18] Ríos, J.-L.,R.M. Giner,M. Marín.,M.C. Recio. **(2018).** *A pharmacological update of ellagic acid*. Planta medica 84(15): 1068-1093. DOI: 10.1055/a-0633-9492

[19] Alfei, S.,B. Marengo.,G. Zuccari. **(2020).** *Oxidative stress, antioxidant capabilities, and bioavailability: Ellagic acid or urolithins?* Antioxidants 9(8): 707. DOI: 10.3390/antiox9080707

[20] Cozzi, R.,R. Ricordy,F. Bartolini,L. Ramadori,P. Perticone.,R. De Salvia. **(1995).** *Taurine and ellagic acid: Two differently-acting natural antioxidants*. Environmental and Molecular Mutagenesis 26(3): 248-254. DOI: 10.1002/em.2850260310

[21] Khan, A.,S. Nazar,I. Lang,H. Nawaz.,M.A. Hussain. **(2017).** *Effect of ellagic acid on growth and physiology of canola (Brassica napus L.) under saline conditions*. Journal of Plant Interactions 12(1): 520-525. DOI: 10.1080/17429145.2017.1400122

[22] Bates, L.S.,R. Waldren.,I. Teare. **(1973).** *Rapid determination of free proline for water-stress studies*. Plant and soil 39: 205-207. DOI: 10.1007/BF00018060

[23] Kim, J.W..,T. Minamikawa. **(1997).** *Hydroxyl radical-scavenging effects of spices and scavengers from brown mustard (Brassica nigra)*. Bioscience, biotechnology, and biochemistry 61(1): 118-123. DOI: 10.1271/bbb.61.118

[24] Azeem, M.,K. Pirjan,M. Qasim,A. Mahmood,T. Javed,H. Muhammad,S. Yang,R. Dong,B. Ali.,M. Rahimi. **(2023).** *Salinity stress improves antioxidant potential by modulating physio-biochemical responses in Moringa oleifera Lam*. Scientific Reports 13(1): 2895. DOI: 10.1038/s41598-023-29954-6

[25] Al-Fraihat, A.H.,S.Y. Al-Dalain,A.A. Zatimeh.,M.A. Haddad. **(2023).** *Enhancing rosemary (Rosmarinus officinalis, L.) growth and volatile oil constituents grown under soil salinity stress by some amino acids*. Horticulturae 9(2): 252. DOI: 10.3390/horticulturae9020252

[26] Denaxa, N.-K.,A. Nomikou,N. Malamos,E. Liveri,P.A. Roussos.,V. Papasotiropoulos. **(2022).** *Salinity effect on plant growth parameters and fruit bioactive compounds of two strawberry cultivars, coupled with environmental conditions monitoring*. Agronomy 12(10): 2279. DOI: 10.3390/agronomy12102279

[27] Kumar, K.,P. Debnath,S. Singh.,N. Kumar. **(2023).** *An overview of plant phenolics and their involvement in abiotic stress tolerance*. Stresses 3(3): 570-585. DOI: 10.3390/stresses3030040

[28] Sharma, A.,S.K. Kohli,K. Khanna,M. Ramakrishnan,V. Kumar,R. Bhardwaj,M. Brestic,M. Skalicky,M. Landi.,B. Zheng. **(2023).** *Salicylic acid: a phenolic molecule with multiple roles in salt-stressed plants*. Journal of Plant Growth Regulation 42(8): 4581-4605. DOI: 10.1007/s00344-022-10902-z

[29] Babaei, M.,L. Shabani.,S. Hashemi-Shahraki. **(2022).** *Improving the effects of salt stress by β-carotene and gallic acid using increasing antioxidant activity and regulating ion uptake in Lepidium sativum L*. Botanical Studies 63(1): 22. DOI: 10.1186/s40529-022-00352-x

[30] Mishra, N.,P. Dey,R.K. Singhal,C. Sahu,D. Jena,S. Nanda.,J. Chauhan. **(2023)**. *Plant phenolics: as antioxidants and potent compounds under multiple stresses*, in *Plant phenolics in abiotic stress management*., Springer. p. 215-234. DOI: 10.1007/978-981-19-6426-8_11

[31] Bano, A.,A. Gupta,S. Rai,T. Fatima,S. Sharma.,N. Pathak. **(2021).** *Mechanistic role of reactive oxygen species and its regulation via the antioxidant system under environmental stress*. Plant stress physiology—perspectives in agriculture: 1-18. DOI: 10.5772/intechopen.10104

[32] Sachdev, S.,S.A. Ansari,M.I. Ansari,M. Fujita.,M. Hasanuzzaman. **(2021).** *Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms*. Antioxidants 10(2): 277. DOI: 10.3390/antiox10020277

[33] Ahmad, R.,S. Hussain,M.A. Anjum,M.F. Khalid,M. Saqib,I. Zakir,A. Hassan,S. Fahad.,S. Ahmad. **(2019).** *Oxidative stress and antioxidant defense mechanisms in plants under salt stress*. Plant abiotic stress tolerance: Agronomic, molecular and biotechnological approaches: 191-205. DOI: 10.1007/978-3-030-06118-0_8

[34] Mariani, L..,A. Ferrante. **(2017).** *Agronomic management for enhancing plant tolerance to abiotic stresses—drought, salinity, hypoxia, and lodging*. Horticulturae 3(4): 52. DOI: 10.3390/horticulturae3040052

[35] Yusuf, M.,S.A. Hasan,B. Ali,S. Hayat,Q. Fariduddin.,A. Ahmad. **(2008).** *Effect of salicylic acid on salinity-induced changes in Brassica juncea*. Journal of integrative plant biology 50(9): 1096-1102. DOI: 10.1111/j.1744-7909.2008.00697.x

[36] Hayat, S.,S.A. Hasan,M. Yusuf,Q. Hayat.,A. Ahmad. **(2010).** *Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in Vigna radiata*. Environmental and Experimental Botany 69(2): 105-112. DOI: 10.1016/j.envexpbot.2010.03.004

[37] Albaladejo-Marico, L.,L. Yepes-Molina.,M. Carvajal. **(2024).** *Alteration of nutrient uptake and secondary metabolism connection by foliar application of citrus flavonoids to broccoli plants*. Plant Growth Regulation: 1-13. DOI: 10.1007/s10725-024-01204-3

[38] Maqsood, M.F.,M. Shahbaz,U. Zulfiqar,R.U. Saman,A. Rehman,N. Naz,M. Akram.,F.U. Haider. **(2023).** *Enhancing wheat growth and yield through salicylic acid-mediated regulation of gas exchange, antioxidant defense, and osmoprotection under salt stress*. Stresses 3(1): 372-386. DOI: 10.3390/stresses3010027

[39] Ben Ahmed, C.,B. Ben Rouina,S. Sensoy,M. Boukhriss.,F. Ben Abdullah. **(2010).** *Exogenous proline effects on photosynthetic performance and antioxidant defense system of young olive tree*. Journal of Agricultural and Food Chemistry 58(7): 4216-4222. DOI: 10.1021/jf9041479

[40] Zouari, M.,C.B. Ahmed,N. Elloumi,K. Bellassoued,D. Delmail,P. Labrousse,F.B. Abdallah.,B.B. Rouina. **(2016).** *Impact of proline application on cadmium accumulation, mineral nutrition and enzymatic antioxidant defense system of Olea europaea L. cv Chemlali exposed to cadmium stress*. Ecotoxicology and environmental safety 128: 195-205. DOI: 10.1016/j.ecoenv.2016.02.024

[41] Silva, F.L.B.,L.G.E. Vieira,A.F. Ribas,A.L. Moro,D.M. Neris.,A.C. Pacheco. **(2018).** *Proline accumulation induces the production of total phenolics in transgenic tobacco plants under water deficit without increasing the G6PDH activity*. Theoretical and Experimental Plant Physiology 30: 251-260. DOI: 10.1007/s40626-018-0119-0

[42] Rady, M.M.,A. Kuşvuran,H.F. Alharby,Y. Alzahrani.,S. Kuşvuran. **(2019).** *Pretreatment with proline or an organic bio-stimulant induces salt tolerance in wheat plants by improving antioxidant redox state and enzymatic activities and reducing the oxidative stress*. Journal of Plant Growth Regulation 38: 449-462. DOI: 10.1007/s00344-018-9860-5