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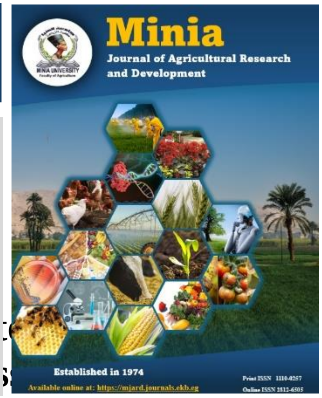

Seeds treatment with proline improves host resistance to *Macrophomina phaseolina* in soybean seedlings under salinity stress conditions.

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ABSTRACT

Soybean seeds of two tested cultivars were soaked for 2 hr in 1 mM and 5 mM proline (Pr.), then growing seedlings were subjected to salinity stress (40 mM NaCl) and at 12 days after sowing (DAS) and at 19 DAS were inoculated with *M. phaseolina*. Results showed that proline treatment improves vegetative growth parameters (no. of leaves, no. of branches, stem height, root length (cm)). Also, biomass of seedlings (fresh and dry weight) was increased for both cultivars compared with the control. Data also indicated that chlorophyll pigment and carotenoids contents were significantly increased in both tested cultivars compared with the control. Additionally, means relative water content (RWC) and total phenolics in leaves. However, data exhibited that Giza 35 accumalate less Na^+ than that recorded for Giza 111, higher content of K^+ and total protein compared with unstressed seedlings. Results did confirm that proline treatment lead to a significant decrease in Na^+ content and a significant increase in K^+ content in both cultivars compared with untreated ones. However, proline treatment resulted in a decrease in disease severity which reached to about 70– and 67– for Giza 111 and Giza 35, at 5mM proline respectively. In conclusion, this study explored the dual effect of exogenous proline treatment to cope the adverse effect of both biotic and abiotic stress in soybean plants.

Keywords: Exogenous proline, seed treatment, salinity stress, *Macrophomina* infection, soybean seedling.

1. INTRODUCTION

Worldwide, soil salinity is one of the major factors limiting an expected sustainable agricultural production, especially in arid and semi-arid regions, including Egypt. In these areas, low rainfall and the use of low-quality irrigation water with high content of salts contribute to salt accumulation in the soil because of high evaporation and poor drainage **(Hailu and Mehari, 2021)**. Approximately one-third of the irrigated lands worldwide are affected by salinity.

Salinity stress has been shown to adversely affect various physiological and metabolic processes, ultimately reducing

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plant growth and productivity **(Munns, 2005; Rozema *et al.*, 2008; Rahman *et al.*, 2010; James *et al.*, 2011)**. It also increases plant vulnerability to pathogen infection **(El-Abyad *et al.*, 1988; Snapp *et al.*, 1991; You *et al.*, 2011a; Stetina 2013; Kumar *et al.*, 2024;)**. Salinity-induced osmotic stress inhibits growth, followed by ion toxicity and hypotonic stress in the root system, which reduces water uptake. Concurrently, increased water loss through leaves contributes to salt accumulation **(Snapp and Shennan, 1994; You *et al.*, 2011b)**. Osmotic stress leads to drastic changes in physiological processes, such as membrane dysfunction, nutrient imbalance, reduced capacity to detoxify reactive oxygen species (ROS), altered antioxidant enzyme activity, and decreased stomatal conductance **(Munns *et al.*, 2008)**. One of the most adverse effects of salinity is the accumulation of Na^{+} and Cl^{-}, which may cause various physiological disorders and interfere with K^{+} uptake **(Apel and Hirt, 2004)**.

Salinity stress, also, triggers the formation of ROS, including singlet oxygen, superoxide, hydroxyl radical, and hydrogen peroxide, which cause oxidative damage to proteins, lipids, and DNA, interrupting essential cellular functions **(Mahajan and Tuteja,2005; Ahmed,2010; Ahmad and Umar,2011; Ahmad *et al.*, 2012).**

Proline is a multifunctional amino acid biosynthesized via glutamate or ornithine pathways in higher plants **(El Moukhtari *et al.*, 2020)**. It plays a key role in plant defense under abiotic stress, including salinity. Salinity stress induces proline biosynthesis genes, resulting in endogenous accumulation of proline, which acts as a signaling molecule that activates defense pathways **(Armenqued, 2004;Kim and Nam, 2013 ;Nguyen *et al.*, 2013).**

Exogenous application of proline under salinity stress improves plant biomass and vegetative growth **(Wu *et al.*, 2017; de Freitas *et al.*, 2019)**. It positively influences photosynthesis by increasing chlorophyll a and b levels, stomatal conductance, and relative water content **(Ben Ahmed *et al.*, 2011; Hayat *et al.*, 2012; Wani *et al.*, 2016a; Mansour and Ali, 2017).**

Proline helps maintain osmotic balance, especially in leaves, thus supporting water retention **(Ben Ahmed *et al.*, 2014; Zheng *et al.*, 2015a)**. It acts as a scavenger for ROS and enhances antioxidant defense systems **(Huang *et al.*, 2006).**

Oxidative stress is commonly both abiotic and biotic stresses. Reactive oxygen species (ROS) accumulation is one of the first responses of host plants to pathogen attack, leading to the activation of antioxidant mechanisms. Exogenous proline application under biotic stress has been reported to increase tolerance by stimulating phenolic compound production and activating enzymatic antioxidants such as POD, CAT, and SOD **(Gao *et al.*,2023).**

Recent studies confirmed that proline also influences soil microflora, reducing pathogen infection, such as *Verticillium* wilt **(WeiSong *et al.*,2024).** Also, it has been reported proline treatment was used to manage the infection in pepper with *Phytophthra capsicistress* **(Koç, 2022)** and the infection of pear millet with *Scelerospora graminicola* **(Raj *et al.*,2004)**.

Soybean (*Glycine max* L.) is an important legume crop for its high protein and oil content, essential in both human nutrition and animal feed. In Egypt, the demand for soybean continues to rise. Though moderately salt-tolerant **(Phang *et al.*, 2012)**, soybean yield declines when soil salinity exceeds 5 dS/m **(Chang *et al.*, 1994).**

High salt concentrations negatively impact soybean seed germination **(Essa, 2002)**. With seedlings being more sensitive than seeds

Macrophomina phaseolina (Tassi) Goid., the causal agent of charcoal rot, is a destructive soil-borne fungus affecting over 500 plant species. It significantly reduces soybean yields, especially under drought stress, with losses reaching 30–50% (**Elez *et al.*, 2023).**

This study aimed to investigate the dual role of proline in mitigating the adverse effects of salinity and *Macrophomina* infection in soybean seedlings. The objectives include assessing its impact on growth parameters, ion content, relative water content, chlorophyll, phenolic compounds and lesion length caused by infection.

2. MATERIALS AND METHODS

2.1. Plant Materials

Seeds of both soybean cultivars Giza 35 and Giza 111 were purchased from local markets, El-Minia Goveronate .

2.2. Source of the fungal pathogen .

The tested was isolate from naturally infected soybean seedlings collected from soybean fields in El- Minia Goveronate , Egypt. Pathogenicity tests were conducted against the soybean cultivar Giza 111(Unpublished data). Identification of the isolate was confirmed by Molecular Biology Research Unit, Assiut University using patho-gene-spin DNA/RNA extraction kit (Intron Biotechnology company , Korea) under accession number (PV48988).

2.3. Experimental condition , desine and treatments

All pot experiments were carried out in the greenhouse of Plant- Pathology Department, faculty of Agriculture , El-Minia University , El- Minia , during the growing season 2023. Surface disinfected seeds (treated with 40mM sodium hypochlorite) were sown in sterilized pots (30 cm).

Containing sand loamy soil (salinity level was 40%). Pots were arranged in completely randomized Block Design with four treatments 1. (0proline (pr.) +0 (Nacl) +(-) *Macrophomina phaseolina*(Mp) 2.(0proline (pr.) + 40mM (Nacl) +Mp) 3. (1mM proline (pr.) + 40mM (Nacl) + Mp) 4. (5proline(pr.) +40mM (Nacl) + Mp). Each treatment seedlings at age 12day after sowing(DAS) 5 pots each pot grown with 4 seeds were subjected to salt stress (SS) (40mMNacl solution) used to irrigate seedlings at 2d intervals for a week. *Macrophomina.* inoculation was carried out at age 21(Das) length of blighted lesion measured one week after inoculation. A modified method described by (**Elez *et al.*, 2023).**

2.4. Determination of vegetative Growth Parameters and Biomass .

The samples were collected 30 days. Seedlings were aborted and brought the lab for determination of various vegetable growth .Three Soybean plants were randomly selected from each replicate for study. The leaf number per plant, The branches number per plant, number of secondary roots were recorded, main shoot length (cm), main root length (cm) , shoot fresh weight(g) and root fresh(g) weight were immediately determined using lab balance were determined by left the samples to dry in oven at 70°c for 24 hour**.(Kumar *et al.*,2009).**

2.5. Measurement of Chlorophyll Pigment in leaves

For chlorophyll a, b and carotene analysis, the method described by **(Lichtenthaler 1987; Tantawy *et al.*, 2020)** was used. A sample of 10 mg (fresh weight) of seedling leaves of soybean plants was added to 200 mL of 80% acetone, mixed well, and incubated overnight at 5°C in darkness. After incubation absorbance were recorded at wavelengths of 662, 645, and 470 nm, respectively against 80% acetone blank using a spectrophotometer. The amount of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were calculated in mg/g fresh weight.

Chlorophyll a = 11.75 A662 – 2.350 A645 Chlorophyll b = 18.61 A645 – 3.960 A662 Carotene = Cx + c = 1000 A440 – 2.270 Ca – 81.4 Cb / 227 Ca = Chlorophyll a; Cb = Chlorophyll b; Cx + c = Total carotene

2.6. Determination of relative Water Content in leaves (RWC)

The process was try replicated for each treatment relation water contant was carried out according to the method described by **(Dionisio-Sese and Tobita 1998)**. Ten deskes were obtund room fresh seedling leaves were immediately weighted . And then were shocked deionized distilled water for our, then weighted . After that deskes were left to dry in oven at 70°c for 24 hour expressed the turger weight. The relation water contant (postage) was determind using the formula

$$\mathbf{RWC(\%)}=\frac{\mathbf{Dry\ weight-Fresh\ weight}}{\mathbf{Turger\ weight}}\mathbf{X100.}$$

2.7. Determination of levels of Na+, K+.

Na+, K+ levels were determined by methodology of **(Amirijani, 2010)**. Plant samples were left to dry in oven at 70°C for 24 h and were ground using a pestle and mortar for determination of mineral composition. Ash of plant samples was dissolved in 5.1% HNO_3 and used to determine of Na^+ , K^+ and contents using an atomic absorption spectrophotometer

2.8. Determination of soluble carbohydrates, starch and protein content

The contents of glucose and fructose were estimated following the method described by **(Hahloul and Kheriberg, 1972)**. A fresh leaf sample (0.1 g) was extracted using 2 mL of 80% (v/v) ethanol. After evaporation of the alcohol, the supernatant was transferred to an Erlenmeyer flask and diluted to 100 mL with distilled water. For quantification, 1 mL of the extract was mixed with 2 mL of anthrone reagent. To determine glucose content, the mixture was incubated in a water bath at 95°C for 15 minutes. For fructose, the mixture was incubated at 40°C for 30 minutes, followed by cooling in an ice bath. The absorbance was measured at 620 nm, and the concentrations were expressed in ppm per gram of fresh weight (FW) using glucose (Merck K13654437) and fructose (Merck K04317907) as standards.

Total soluble proteins were extracted following **(Kurkela *et al.*, 1988**). A 0.1 g fresh leaf sample was homogenized in 1.5 mL of 50 mM Tris-HCl buffer (pH 6.8) containing 1% (w/v) β-mercaptoethanol (Sigma-Aldrich M6250) and 50 mg L^{-1} phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich P7626). Protein concentration was determined by the Bradford method **(Bradford, 1976)** using bovine serum albumin (BSA) (Sigma-Aldrich B4287) as the standard.

2.9. Determination of accumulated phenolic Components

The Folin-Ciocalteu reagent was followed to calculate the extract's total phenolic content. The total phenolic content was determined in milligrams of Gallic Acid Equivalents (GAE) per gram of extract. All tests were run in triplicate. Standard solutions containing concentrations of 0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml of gallic acid were made in methanol in order to establish the calibration curve.

Then, a methanol-based solution containing 1 mg/ml of the plant extract was made. In test tubes, 0.5 ml of the plant extract solution, 2.5 ml of a Folin-Ciocalteu reagent diluted 10 times, and 2 ml of a 7.5% sodium carbonate solution were combined for the analysis. Tubes were left for 1 hour reaped with parafilm and Following by using spectrophotometry was used to determine the solution's absorbance at 750 nm **(Maurya and Singh, 2010).**

2.10. Assessment of *Macrophomina phaseolina* infection.

This assessment was carried out by measuring the length of blighted lesion on stems of inoculated seedlings.

2.11. Statistical Analysis

All the data were analysed using Excel 2010 (Microsoft) and SPSS v. 20.0 (IBM, Inc.). Significant differences between the treatments were evaluated using a one-factor analysis of variance (ANOVA) followed by a least significant difference (LSD) test at the level of $p < 0.05$. The data shown in the tables and figures are presented as the mean ± standard error (SE).

3. RESULTS:

3.1.Effects of proline treatment on vegetative growth and biomass of soybeans seedlings.

Table (1) summarises the analysis of recorded data of various vegetative growth parameters (i.e., no. of leaves, length of shoot, no. of branches, secondary roots, and shoot + root length) per each seedling of collected samples of unstressed and stressed seedlings of both tested cultivars Giza 35 and Giza 111.

Recorded data reflected the visual observation of stressed seedlings which show a stunted growth, with low number of leaves, branches and secondary roots. However, application of proline improved vegetative growth parameters, especially with 5 mM level of proline, where a significant difference was observed between treated and untreated seedlings. Moreover, fresh weight of both roots and shoots was also Biomass of seedlings was also observed in both cultivars due to proline treatment. For example, fresh weight of shoot and fresh root recorded for Giza 111 and Giza 35 were 6.4, 5.44 and 4.1, 3.71 grams, respectively, compared with the untreated seedlings (control), which were 4.0, 4.9 and 3.39, 2.96 grams for both tested cultivars at the same arrangement.

Table (1) Effect of proline (Pr.) seed treatment on some vegetative growth parameters and biomass on soybeans seedlings subjected to salinity stress (40mM Nacl), 12days after sowing(DAS) and myclial inoculation with *Macrophomina phaseolina* (MP) ,19 (DAS).

	Leaves No.		Branches No.		Shoot length		Root length		Secondry roots		Shoot fresh weight (g/plant)		Root fresh weight (g/plant)		Shoot dry weight (g/plant		Root dry weight (g/plant)	
Cultivars / Treatment	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35
0 (Pr.) +0(Nacl) +(-)Mp	10bc	6.8cd	2.6b.d	1.6cd	10.5bc	9.8bc	9ab	9a	11.4cd	9.6ab	5.14c	5.06c	3.47cd	3.38cd	2.57b	2.53c	1.73cd	1.55cd
0(Pr.) +40mM (Nacl) +Mp	9.8bc	6.2d	2.4cd	1.4d	9.8c	9.3c	8.4b	8.4a	9.2d	8.8b	5.03c	4.90c	3.39cd	2.96d	2.51a	2.45c	1.69cd	1.48d
1mM(Pr.) +40mM (Nacl) +Mp	8.4c	8cd	2d	2cd	11.1a.c	11.1ab	10.74ab	10.5a	13.8b.d	11.6ab	5.51bc	5.25bc	3.78cd	3.39cd	2.75b	2.63bc	1.89cd	1.69c
5mM(pr.) +40mM (Nacl) +Mp	12.4ab	11.6ab	3.6a.c	3.2ab	11.92a.c	11.7a	13.4ab	10.54a	17ab	13.8ab	6.4ab	5.44bc	4.1bc	3.75cd	3.20c	2.72bc	2.05bc	1.88cd
mean	11.2	9.1	3.07	2.37	11.4	11.11	11.20	10.17	14.1	11.8	5.89	5.51	4.22	3.71	2.94	2.76	2.11	1.83
LSD (0.05%)	3.33	2.86	1.37	0.95	2.21	1.70	5.04	3.89	4.93	4.83	1.21		1.01		0.60		0.56	

Mean values with the same letter(s) having no significant differences

3.2. Effects of proline treatment on chlorophyll pigment in leaves of soybean seedlings.

Table (2) Results in Table show the recorded means of chlorophyll pigments (i.e., chlorophyll a, chlorophyll b, and carotenoids) expressed as mg/g fresh weight in leaves of soybean seedlings of both tested cultivars (i.e., Giza 111 and Giza 35) under different treatments.Data indicated that stressed seedlings showed a significant decrease in measured three chlorophyll pigments compared with unstressed seedlings. However, application of proline increases average means of the chlorophyll pigments with different levels of significance. For example, application of proline at 5 mM increase in chlorophyll a content, but it induced a significant increase in chlorophyll b content with mean of 1.42, 1.65 for chlorophyll a and 1.33, 1.55 for chlorophyll b in leaves of Giza 111 and Giza 35, respectively, compared with the control 1.17, 1.41 for chlorophyll a and 0.29, 0.32 for chlorophyll b.

Additionally, proline treatment had not induced a significant increase in carotene content in leaves of Giza 35.

Table (2) Effect of proline (Pr.) seed treatment on chlorophyll pigments(mg/g FW) in leaves of soybean seedlings subjected to salinity stress(ss) (40mM Nacl) , 12days after sowing(DAS) and myclial inoculation with *Macrophomina phaseolina* (Mp) , 19(DAS).

Treatment	Chl. A		Chl. B		Carotens	
	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35
0 (Pr.)+0(Nacl)+(-)Mp	1.30a	1.54a	0.93ab	0.83b.c	2.23ab	2.32a
0 (Pr.)+40mM(Nacl)+Mp	1.27a	1.41ab	0.92ab	0.32c	1.62b	2.23a
1mM(Pr.)+40mM(Nacl)+Mp	1.41a	1.59a	1.12ab	1.41ab	2.25ab	2.43a
5mM(pr.)+40mM(Nacl)+Mp	1.42a	1.65a	1.33a	1.55a	2.27ab	2.49a
mean	1.35	1.55	1.08	1.03	2.09	2.37
LSD(0.05%)	**0.33**	**0.54**	**0.65**	**0.89**	**0.73**	**0.54**

Mean values with the same letter(s) having no significant differences.

3.3. Effects of proline treatment on water contant in leaves of soybean seedlings.

Table (3) To investigate the effects of salinity stress on soybean water relations; and determination of relative water content in leaves in both tested cultivars i.e. Giza 111 and Giza 35 were carried out. Data in Table (4) exhibited that effects of proline applications on relative water content are shown in Table (3). Results indicated that there was a decrease in stressed seedling due to salinity stress compared with unstressed seedling in both cultivars. However, application of proline led to an increase of water content in leaves of both cultivars (Giza 111 and Giza 35). At both tested levels of proline (i.e. 1 mM and 5 mM). Moreover, increase in water content in leaves of stressed seedlings due to application of proline at 5 mM, reached about 26.6% and 46.9% for Giza 111 and Giza 35, respectively.

Table (3) Effect of proline (Pr.) seed treatment on relative water content (RWC %) on soybeans seedlings subjected to salinity stress (ss) (40mM Nacl) , 12days after sowing(DAS) and myclial inoculation with *Macrophomina phaseolina* (Mp) , 19(DAS) .

	Water contant	
Cultivars / Treatment	Giza 111	Giza 35
0 (Pr.)+0(Nacl)+(-)Mp	32c	34c
0 (Pr.)+40mM(Nacl)+Mp	30d	31d
1mM(Pr.)+40mM(Nacl)+Mp	34b	38b
5mM(pr.)+40mM(Nacl)+Mp	38a	45a
mean	33.5	37
LSD(0.05%)	**1.997**	**1.88**

Mean values with the same letter(s) having no significant differences.

3.4. Effects of proline treatment on Na^+, K^+ (mg/kg) , soulable carbohydrates(%) and total protein(%) on soybean seedlings.

Table (4) Data analysis of Na^+ , K^+ , total soluble carbohydrate and total protein exhibited in Table (4). Results indicated that salinity stress increased Na^+ content in plant tissues of both cultivars (i.e. Giza 111 and Giza 35). However, it was noticed that Giza 35 retains much more Na^+ compared with Giza 111. (with average mean = 622.15 vs. 535.12 Na^+ mg/g dry weight) This was also associated with an increase in levels of K^+ .Also, results indicated that there was a slight increase in soluble carbohydrate at non-significant level in stressed seedlings compared with unstressed seedlings in both tested cultivars.There was a considerable increase in total protein in stressed seedlings compared with unstressed ones. Results in Table (7) also indicated that application of proline resulted in a significant decrease in Na^+ content associated with a highly significant increase in K^+ content in both cultivars at 5 mM level of proline.Proline application induces slight increase in carbohydrate but at not significant level. Also, total protein was slightly increased due to proline application.

Table (4) Effect of proline (Pr.) seed treatment on Na^+, K^+ (mg/kg) , soulable carbohydrates(%) and total protein(%) in soybean seedlings subjected to salinity stress(ss) (40mM Nacl) , 12days after sowing (DAS) and myclial inoculation with *Macrophomina phaseolina* (Mp) , 19(DAS).

Treatment	Protein %		Total Carbohydrates %		K (mg/kg)		Na (mg/kg)	
	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35	Giza 111	Giza 35
0 (Pr.)+0(Nacl) +(-)Mp	29.17c	24.06c	6.88bc	5.37a	4628.88c	5718.31c	4472.25a	3678.37b
0 (Pr.)+40mM(Nacl) +Mp	27.29c	21.06d	5.16c	5.24a	1136.64d	3522.37d	5904.12a	6243.65a
1mM(Pr.)+40mM (Nacl)+Mp	31.68b	26.81b	8.89b	6.03a	31193.15b	11820.74b	1367.13b	1126.03c
5mM(pr.)+40mM (Nacl)+Mp	43.09a	45.94a	13.05a	6.8a	33680.30a	27759.28a	604.57b	222.30d
mean	32.81	29.47	8.50	5.86	17659.74	12205.18	3087.02	2817.59
LSD(0.05%)	**1.984**	**2.016**	**2.011**	**2.107**	**1.945**	**1.895**	**1.902**	**2.049**

Mean values with the same letter(s) having no significant differences.

3.5. Effects of proline treatment on on phenolic compound content on soybean seedlings.

Table (5) Levels of phenolic compounds in soybean seedlings as affected by application of proline treatment are recorded in Table (5).Obtained data show a highly significant rise in levels of total phenolic compounds in seedlings of both cultivars (i.e. Giza 111 and Giza 35) at both levels of proline (i.e. 1 mM and 5 mM).Results indicated there were an increase of phenolic compounds content by over 50% and 100% for Giza 111 and Giza 35, respectively, for application of proline at 5 mM.

3.6. Effects of proline treatment on severity on soybean seedlings.

Table (6) Effects of proline on severity of stem blight on soybean seedlings of both tested cultivars i.e. Giza 111 and Giza 35 are presented in Table (6). Results indicated that salinity stress (40 mM NaCl) caused an increase in the severity of the disease (expressed in length of stem lesions) compared with the control.Recorded data indicated that proline seed treatment resulted in a decrease in the disease severity at the two tested levels (1 mM and 5 mM). However, application of proline at 5 mM significantly decreased seedling blight in both tested cultivars. Average means of stem blight recorded were 0.8 and 0.8 cm in treated seedlings compared with the untreated seedlings which were 2.7 and 2.46 cm in Giza 111 and Giza 35, respectively.(2) Table (6) Cont.In other words, percentage of decrease in stem blight reached 70.03% and 66.6% in Giza 111 and Giza 35 respectively due to proline at 5 mM compared with stressed seedlings.

Table (5) Effect of proline (Pr.) seed treatment on phenolic compound content in soybeans seedlings subjected to salinity stress(ss) (40mM Nacl) , 12days after sowily(DAS) and myclial with *Macrophomina phaseolina* (Mp), 19(DAS).

Phenolic compounds (mg/g) dry weight plant tissue		
Treatment / **Cultivars**	**Giza 111**	**Giza 35**
0 (Pr.)+40mM(Nacl)+Mp	13.15b	21.46a
1mM(Pr.)+40mM(Nacl)+Mp	17.90ab	28.86ab
5mM(pr.)+40mM(Nacl)+Mp	20.85a	43.45a
Mean	17.30	31.25
LSD(0.05%)	**6.14**	**7.49**

Mean values with the same letter(s) having no significant differences.

Table(6) Effect of proline(pr.) seed treatment on stem blight disease (cm) in soybean seedling subjected to salinity stress(ss) (40mM Nacl) , 12days after sowing (DAS) and myclial inoculation with *Macrophomina phaseolina* (Mp) , 19(DAS).

Diseases Severity		
Treatment / **Cultivars**	**Giza 111**	**Giza 35**
0 (Pr.)+0(Nacl)+(-)Mp	0d	0c
0 (Pr.)+0(Nacl)+ Mp	2.43ab	2bc
0 (Pr.)+40mM(Nacl)+Mp	2.7a	2.46a
1mM(Pr.)+40mM(Nacl)+Mp	1.8b	1.7a
5mM(pr.)+40mM(Nacl)+Mp	0.8c	0.8b
Mean	1.33	1.24
LSD(0.05%)	**0.65**	**0.73**

Mean values with the same letter(s) having no signification differences**.**

4. DISCUSSION

The co- occurrence of abiotic stress and biotic during the growing season of a certain crop is considered a complex problem , in which the plants undergo a high levels of stress that may result in a great decrease in growth and productivity . for example , exposure of plants to salinity stress (abiotic stress) and pathogen infection (biotic stress) lead to negative effects on growth and disruption in physiological process due to the effect of salts ; but , also , salinity stress predispose plants to infection by the pathogen infection. Consequently, management of both stresses to more extent will be difficult. In this study, the potential effects of using proline as seed treatment to suppress the adverse effects of salinity stress and *Macrophomina phaseolina* were evaluated in seedlings of two soybean cultivars Giza 35 and Giza 111.

Results showed that stressed seedlings were stunted, less vigrious , and with low number of leaves and branches compared with the control due to the effect of salinity stress . However, treated plants with proline showed an increase of all vegetative growth parameters (length of roots , length of shoot , number of leaves and branches). Moreover, treatment with proline resulted in an increase in tissues biomass (fresh and dry weight). These findings were in agreement with reported by **(Amirjani, 2010; El-shawa, 2020; Dawood *et al.*, 2021; Gao *et al.*, 2023)** who found the same trend data.

Promoted growth and increased in bio-mass is mainly contributed photosynthesis in which resulted in the formation of organic matters and releasing of oxygen **(flaxes *et al.*, 2019).** Moreover , chlorophyll it in the an essential pigment for SD-8 resulted photosynthesis in plants **(wong *et al.*, 2021).**

The accumulation of ions in the saline soil will result in incidence of osmotic stress. In this case, the water uptake by roots will be retarded, and also, intracellular water will be loosed**(Yan *et al.*, 2013).**

Under this condition, plants adjust this disorder through accumulation of osmolytic molecules such as proline or glycine beaten. These compounds are able to balance osmotic stress through triggering of accumulation of organic substances especially soluble carbohydrates that can retain water **(Flowers *et al.*, 1977; Meggio *et al.*, 2002; Demiral and Turkan, 2004; Yan *et al.*, 2013).**

The results of this study indicated there were a reduction in the contents of chlorophyll pigment in the leaves of stressed plants compared with the control. However, proline treatment increased the total content and carotenoids in the leaves of soybean seedlings. These findings agreed with those reported by **(Dawood *et al.*, 2021 and Gao *et al.*, 2023).**

In this study, the impact of exogenous proline application in soybean- water relations under salinity stress was evaluated in two soybean cultivars. Both tested levels of proline as seed treatments obviously increased levels of relative water content in leaves of both cultivars. However, proline applied at 5 mM significantly increased water content compared with the control plants. These findings are strongly supported with those reported by **(Huong *et al.*, 2009; Wu *et al.*, 2017 and Ferguson *et al.*, 2019)** Several studies explored the possibility of proline to triggen the accumulation of different organic substance that have the potential to retain water (**Khan *et al.*, 2014; Zheng *et al.*, 2015b; Wani et al., 2016b)**

Oxidative stress resulted from the generation of different elements of reactive oxygen species (ROS). ROS are commonly generated under abiotic and biotic stress due to the incomplete reduction of oxygen (O_2) including singlet oxygen ($^1O_2$), superoxide anion (O_2^-), and radical (OH) respect **(Yan *et al.*, 2013).** ROS is commonly considered as a devastating agent that can damage different cellular organelles.

Generation of ROS leads plants to increase biosynthesis of organic compounds like phenolic compounds and flavonoids Which have capacity to eliminate ROS.

Chemical analysis of tissues of soybean seedlings leaves to determine both Na^+ and K^+ revealed that there was an increase of Na^+ content and a decrease in K^+ content in stressed seedlings compared with unstressed seedlings.These results were agreed with those reported by **(Amirjani, 2010)**who found an increase in Na^+ in soybean seedlings at the expanse of Ca^{++} , K^+ , and Mg^{++} in soybean seedlings subjected to different levels of NaCl salt. Several studies confirmed the occurrence of ionic imbalance specially with K^+ , Ca^{++} and Mg^{++} when plants subjected to salinity stress (**Lynch and Lauchli, 1985; Chandrashekhar *et al.*, 1996).**

The decrease in K^+ , Ca^{++} , and Mg^{++} in plants had an adverse effect on growth and developmental of processes plants **(Amirjani, 2010).** However, it has been documented that accumulation of Na^+ and Cl^- in plant tissues has a toxic effect on plant cells . Moreover, it has been reported that there is a correlation between increased Cl^- and chlorosis in leaves of soybean **(Essa, 2002).**

Results of this study showed that Giza 35 cv. accumulated less content of Na+ compared with that recorded in

Giza 111 cv. According to the findings reported by **(Liu *et al.,* 2006)**, who stated that a tolerant soybean cultivar must maintain less amount of Na^+ and high content of K^+ . So, according to the present data, it has been confirmed that Giza 35 cv. is much more tolerant to salinity stress at 40 mM NaCl than Giza 111 cv.

Results, also, indicated, under salt stress, there were an increases in both soluble carbohydrates and total protein compared with the control plants. This could be explained by the tendency of plants under stress to accumulate endogenous organic molecules to cope with that condition including amino acids, sugars, and phyto-hormones, antioxidants **(Phang *et al.*,2012)**

Application of exogenous proline as seed treatment positively affected resulted in a significant decrease in Na^+ and significant increase in K^+ in stressed seedlings compared with unstressed ones. It was observed that application of 5mM proline was much more effective than 1mM proline level. However, results concerning total protein and soluble carbohydrates were not significant. Those findings were supported with reported by **(Ahmad and Umar,2011**; **Alam *et al.,* 2016 and Yan *et al.,* 2017;**) and who showed the same trend of data.

In this study , effect of application of exogenous proline on the phenolic content in leaves of soybean seedlings of Giza 35 and Giza 111 under salinity stress was estimated . Data indicated that there was a rise in the mean value of phenolic content with both tested levels of phenolic compounds. However , proline at 5mM was significantly increased the mean value of value of phenolic content compared with the control plants in both cultivars. These findings were in agreement with reported by **(Gao *et al.,* 2023)** who found application of exogenous, proline increased levels of phenolic compounds in seedlings leaves , and with these reported by **(Kusvuran *et al.,* 2021 and xie *et al.*,2021) .** in leaves of pepper and *Brussels spell* and leaves of celery **(Gao et al.,** 2023).

Concerning of results related assessment of stem blight of soybean seedling caused by *M. phaseolina*, data indicated that stressed seedlings were vulernable to infection compared with unstressed ones. However, seedlings of cv. Giza 111 were to some extent much more susceptible compared with those of Giza 35 cv.

However, application of proline as seed treatment led to a significant decrease in disease severity. For example, application of proline at 5 mM resulted in a reduction in infection reached to 70 and 67 in seedlings of Giza 111 cv. and Giza 35 cv., respectively.These findings were strongly supported by those reported by **(WeiSong,2024)** , who applied L-proline at 100, 200 and 300 mM against *Verticillium* wilt in cotton. Application of these rates of L-proline reduced disease index to 22.23, 60, 23 and 64.23%, respectively. Also, **(Koç, 2022)** applied proline as seed treatment at 1 and 10 mM against pepper blight caused by *Phytophthora capsici*. Results of that study indicated application of proline improve the resistance of the susceptible cultivar SD-8. Moreover, **(Raj *et al.,* 2004)** , found that application of proline gave more than 50% protection in pearl millet against *Sclerospora graminicola* the causal pathogen of downy mildew. In conclusion, our study explore the dual effects of proline to combat the adverse effects of biotic and abiotic complex of stress in soybean.

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الملخص العربي:

معامله البذور بالبرولين تحسن مقاومه العائل للمكروفومينا فاصولينا في بادرات فول الصويا تحت ظروف الاجهاد الملحي.

تمت معامله بذور فول الصويا لصنفين مختلفين بالنقع لمده ساعتين في محلول برولين بتركيز (١و٥ ملليمول) ،ثم تعرضت البادرات الناميه لاجهاد الملحي (٤٠ ملليمول كلوريد صوديوم)، كما تم تلقيحها بفطر ماكروفومينا فاصولينا في اليوم الثاني عشر واليوم التاسع عشر بعد الزراعه.

واظهرت النتائج ان المعامله بالبرولين ادت الي تحسين الصفات الخضريه للنباتات، مثل عدد الاوراق ، وعدد الافرع ، وطول الساق ، وطول الجذر. كما زادت الكتله الحيويه للبادرات (الوزن الطازج والجاف) في كلا الصنفين مقارنه بالكنترول غير المعامل . واظهرت النتائج ايضا زياده في محتوي الصبغات (الكلوروفيل والكاروتين) ، بالاضافه الي زياده المحتوي النسبي للماء واجمالي الفينولات في اوراق كلا الصنفين. كما اظهرت النتائج ايضا ان الصنف جيزه ٣٥ راكم كميه اقل من الصوديوم مقارنه بصنف جيزه ١١١، بينما سجل محتوي اعلي من ايونات البوتاسيوم والبروتين الكلي مقارنه بالبادرات الغير معرضه للاجهاد. كما أكدت النتائج أن معاملة البرولين ادت الي خفض محتوى الصوديوم وزيادة محتوى البوتاسيوم في كلا الصنفين مقارنة بالنباتات غير المعاملة. ومن ناحيه اخري، ساهمت معاملة البرولين في خفض ملحوظ في شدة الإصابة المرضية، إذ بلغت ٧٠% في جيزة ١١١ و٦٧% في جيزة ٣٥ عند تركيز ٥ ملليمول برولين .

الخلاصة

تُبرز هذه الدراسة الدور المزدوج لمعاملة البرولين الخارجي في تعزيز قدرة نباتات فول الصويا على مواجهة التأثيرات السلبية لكل من الإجهادات الحيوية واللاحيويه