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Effects of Slag Applications and Salinity Stress on Greenhouse Durum Wheat (*Triticum durum* Desf.) Plants

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Abstract

The search for sustainable practices to increase productivity is a fundamental need in current agriculture. Steel slag has been studied for its potential use in agriculture. These substances present a great ability of agricultural applications since they are rich in nutrients. The effect of steel slag-based fertilizer was investigated on greenhouse durum wheat cultivar under salt-stress conditions. Two doses of slag: 10 g slag/ kg soil (D1) and 20 g slag/ kg soil (D2) were evaluated under no salt-stress (0 mM NaCl) and salt-stress conditions (100 mM NaCl) for salinity stress mitigation. Morpho-physiological and biochemical parameters of wheat were measured and compared to the different treatments. Exposure of wheat to salinity decreased its biomass, stomatal conductance, efficiency of photosystem II, and protein content, but it increased total soluble sugars, hydrogen peroxide (H₂O₂), and malondialdehyde (MDA) contents. Amended plants with 10 g slag/ kg soil (D1) led to a significant improvement in biomass with an increase of shoot and root dry weights (133% and 400% respectively), stomatal conductance (22 %), soluble sugars (14 %), and protein content (158%) under saline conditions compared to the control treatment with 0 g slag/ kg soil (C), thus

indicating a positive influence on durum wheat plants. Soil enrichment with 20 g slag/kg soil (D2) decreased plant growth parameters and presented the highest levels of H₂O₂ and MDA contents compared to the control and treatment (D1) after three months of cultivation under salt stress. This study supports the hypothesis of the application of slag at lower dose, which improves productivity of durum wheat and mitigate salinity stress.

Keywords: Slag, fertilizer, salt stress, biomass, salinity tolerance, durum wheat

Introduction

With the increase of the world's population that is estimated to achieve 8.5 billion in 2030, 9.7 billion in 2050, and 10.4 billion in 2100 (UN, 2017), one of the most urgent challenges addressed by the Sustainable Development Goals is responsible consumption and sustainable food production. As an important source of carbohydrate, cereal yield plays a dominant role in global dietary pattern (Seal et al., 2021). With supply and demand closely matched, cereal production now reaches 2,700 million tons annually (FAO, 2021a). A significant staple grain worldwide is wheat. At least 180 countries consumed wheat in 2019 (FAOSTAT, 2022a). In terms of commercial production and human nutrition, durum wheat (*Triticum turgidum* L. var. durum) globally takes the fifth place after soft wheat, rice, corn, and barley as it contributes to 20% of the total dietary calories and proteins (Maccaferri et al., 2003; Shiferaw et al., 2013). Also, it is considered one of the most popular cereal crops which is mostly grown in South European, North African, and West Asian nations. Although durum wheat production areas typically overlap those of common wheat, durum is less commonly cultivated than common wheat. In addition, durum wheat is more suited than common wheat on the dry Mediterranean landscape (Xynias et al., 2020). Due to its suitability on the dry Mediterranean landscape, durum wheat cultivation and production are concentrated in this region (Turki et al., 2023). As a result of climate change, these regions, where durum wheat is cultivated, experience an increase in temperature. Particularly, abiotic stressors in durum wheat have developed quickly due to global warming and have a significant impact on yields (Bouras et al., 2019). One of the primary issues facing modern agriculture and durum wheat cultivation is salinization (Arora, 2019). Soil and water salinization can hamper the growth and productivity of durum wheat. It can also alter photochemical reactions of photosynthesis, especially at the level of PSII (Baraldi et al., 2019; Zahra et al., 2022). This problem is anticipated to get worse due to climate change, which will also cause more severe droughts and sea level rise. FAO (2021a, b) estimates that based on 73% of the land that has been mapped so far, there are 424 million hectares of topsoil (0–30 cm) and

833 million hectares of subsoil (30–100 cm) that are damaged by salt stress. According to other studies, salinity has a negative impact on 1 billion hectares of land, including more than 20% of all irrigated arable land (Negacz et al., 2022). Therefore, there is a need to develop fertilization approaches to mitigate the negative impacts of salinity and soil poverty on crops. On the other hand, more than 567 million tons of steel slag are produced globally during the production of 1.65 billion tons of iron and steel (Radić et al., 2022). Steel slag is increasingly viewed as a valuable resource rather than as waste. It is used in the steel industry and contributes to a circular economy due to growing awareness of environmental protection and economic benefits (Branca et al., 2020). Due to its richness in CaO, P₂O₅, SiO₂, MgO, MnO, and Fe oxides, steel slag can be used successfully in agriculture as fertilizers, besides its primary uses in the construction industry (cement manufacture, road base material, etc.). Wang and Cai (2006) and Das et al. (2019) have assessed the agronomic utility of steel slag as a fertilizer or as a liming material. Various types of steel slag have shown positive effects on crop output, but the effect varied depending on the plant species, type of soil, or climate (Das et al., 2020; Islam et al., 2022). Iron and steel slags were used in pot experiments, which showed an increase in corn dry matter yield and Fe uptake for moderate rates of slag without having any negative phytotoxic consequences (Wang & Cai, 2006). Other experiments showed the benefits of steel slag on the chemical attributes of the soil, such as increase in the content of phosphorus, calcium, and magnesium (Deus et al., 2014; Deus et al., 2018).

Therefore, there is a need to examine and evaluate the effect of steel slag on global saline agriculture. Thus, this study aims to assess the effect of the application of steel slag based-fertilizer for durum wheat production in greenhouse conditions under salt stress. For this purpose, several morphological and physiological parameters were assisted, such as shoot and root lengths, fresh and dry weight yields. Furthermore, stomatal conductance and photosynthetic efficiency, total soluble sugar (TSS), protein contents, malondialdehyde (MDA) levels, and hydrogen peroxide (H₂O₂) were also evaluated as stress markers in order to know the effects caused by salt stress on durum wheat plants. The main objective of this study was to observe the effectiveness of slag as a fertilizer to increase the tolerance of *Triticum durum* to salt stress, including its effect on growth, physiological, and biochemical parameters in order to examine its contribution to the tolerance of wheat crop to salinization.

Materials and Methods

Site Description and Fertilizer Material

The experiment was conducted in a greenhouse at Cadi Ayyad University in Marrakesh, Morocco. The average temperature inside the

greenhouse during the experiment was 25.5 °C, with an average relative humidity of 68.5% and photon flux density of 410 $\mu\text{m}^{-2} \text{s}^{-1}$. The soil sample used in the experiment was characterized by a pH value of 8.10, electrical conductivity of 0.73 mS/cm, organic matter content of 0.86%, available phosphorus content of 7.96 mg/kg, and K₂O content of 168 mg/kg. The soil texture was also determined, with clay content of 16%, fine silt 3%, gross silt 9%, fine sand 38.3%, and gross sand 33.7%.

The slag used in the experiment was obtained from the "Concamine" company in Berrechid, Morocco, and was composed of a variety of chemical elements and oxides. The majority of the slag was made up of CaO and orthophosphates (PO₄³⁻). The elemental analysis of the slag using energy dispersive X-ray (EDX) analysis showed the presence of different chemical elements, including carbon, oxygen, iron, sodium, magnesium, aluminum, silicon, phosphorus, sulfur, calcium, and manganese (Figure 1). The slag products were used in the form of fine powder and their composition is presented in Table 1.

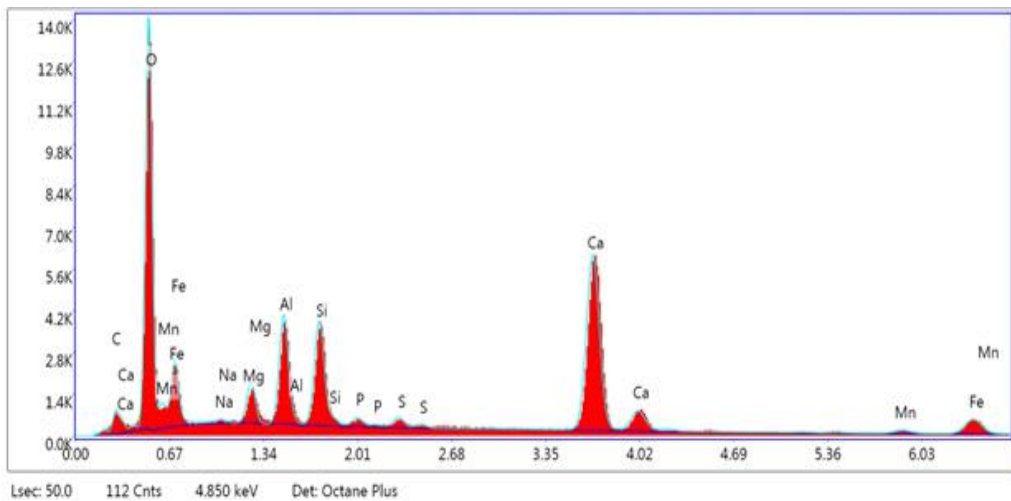


Figure 1. The elemental analysis of the slag sample using energy dispersive X-ray (EDX) analysis

Table 1. Elemental composition of the used slag

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C	0.74	1.57	35.07	13.22	0.0035	1.1869	0.4014	1.0000
O	35.02	55.63	1706.68	8.90	0.1302	1.1247	0.3306	1.0000
Fe	8.28	3.77	174.88	8.39	0.0339	0.8387	0.4878	1.0000
Na	0.06	0.07	4.60	99.99	0.0004	1.0103	0.5818	1.0012
Mg	1.88	1.96	207.92	6.82	0.0139	1.0244	0.7197	1.0021
Al	5.39	5.08	602.22	4.87	0.0432	0.9837	0.8113	1.0033
Si	5.64	5.10	603.10	4.50	0.0489	1.0026	0.8617	1.0045

P	0.41	0.34	35.19	24.34	0.0036	0.9606	0.8942	1.0078
S	0.67	0.53	55.49	9.99	0.0062	0.9770	0.9318	1.0122
Ca	38.22	24.24	1402.04	3.51	0.3562	0.9316	0.9929	1.0072
Mn	3.68	1.70	31.72	20.01	0.0299	0.7981	0.9911	1.0275

Plant Material and Treatments Used

This study was carried out on durum wheat (*Triticum durum* Desf. cv. Carioca). Wheat seeds were sterilized with 12% bleach for 10 minutes, and then rinsed five times with sterile distilled water. Seeds sown on filter paper discs wetted with sterile distilled water in Petri dishes were incubated at 28 °C for 48 h for germination. Wheat seedlings (leaf stage) were transplanted into plastic pots (8 cm / 8 cm / 25 cm) with 2 kg of soil and one seedling per pot. The experiment was designed as a factorial design with three treatments and six replications each under two levels of salinity stress (0 mM NaCl and 100 mM NaCl). The following treatments were tested: (1) C: Control treatment with 0 g slag/kg soil; (2) D1: Plants amended with 10 g slag/kg soil; (3) D2: Plants amended with 20 g slag/kg soil (Table 2).

All treatments were maintained at the same water regime (75% of field capacity) using the procedure described by Meddich et al. (2015).

Table 2. Different treatments applied on durum wheat under salinity stress

Salinity stress level	0 mM NaCl			100 mM NaCl		
Treatments	C	D1	D2	C	D1	D2
Replications	6	6	6	6	6	6

Control: 0 g slag/kg soil, D1: 10 g slag/kg soil, and D2: 20 g slag/kg soil

Plant Growth Parameters Measurements

At harvest (3 months after germination), the root system was separated from the shoot. The biometrical data: shoot height (cm), root length (cm), spike height (cm), fresh biomass, and dry biomass were measured. Shoot and root were dried separately at 75°C for 48 h to record shoot (SDW) and root (RDW) dry weights (g/plant).

Measurement of Physiological and Chlorophyll Content Parameters

After three months of cultivation, the effects of slag amendment on wheat cultivar physiology were evaluated by measuring chlorophyll fluorescence (Fv/Fm) and stomatal conductance (gs). To assess the functional integrity of photosystem II (PS II), chlorophyll fluorescence measurements were made on mature, healthy leaves of plants of the same rank (2nd rank from the apex) using a hand-held fluorometer (Opti-sciences OSI 30p, Hudson, NY, USA) after 20min of adaptation to dark surrounding. The following

parameters were measured: Initial fluorescence (F₀): it is the minimum value of fluorescence when all the electron acceptors of photosystem II (PS II) are completely oxidized. Maximum Fluorescence (F_m): it is the maximum value of fluorescence obtained for the same light intensity. This value is obtained when all the first electron accepting quinones are completely reduced. Quantum efficiency: it is expressed by the ratio $(F_m - F_0) / F_m = F_v / F_m$, where F_v is the variable fluorescence (Hosseinzadeh et al., 2015). The stomatal conductance (g_s) is an indicator of the rate of leaf transpiration and is related to the water state of the plant, thus indicating the opening or closing of the stomata. It was measured on well-developed leaves of the same rank using a porometer system (Leaf Porometer LP1989, Decagon Device, Inc., Washington, USA) from 9:30 to 10:40 am on a sunny day before harvest. Stomatal conductance measurements were taken in the second youngest leaf from six different plants according to each treatment. This parameter is expressed in mmol of H₂O.m⁻². s⁻¹.

Measurement of Total Soluble Sugar (TSS) and Protein Contents

Soluble sugars and proteins are among the metabolites commonly used to assess the degree of response to abiotic stresses (Evelin et al., 2019). The total soluble sugar (TSS) content was measured using an extract of 0.1 g of fresh tissue ground with 4ml of ethanol (80%) according to Dubois et al. (1956). TSS was quantified using 1.25ml of concentrated sulfuric acid and 0.25ml of phenol with the obtained supernatant. TSS content was determined by measuring the absorbance at 485 nm using an UV-3100PC spectrophotometer.

The total soluble protein content was determined according to the technique described by Bradford (1976). Plant material (1 g) was ground with 4ml of 1 M Phosphate buffer (pH 7.2) and centrifuged for 15 min at 18000 g at 4°C. The supernatant was used as the crude protein extract. Total protein content was measured by spectrophotometry at 595 nm.

Determination of Malondialdehyde (MDA) and Hydrogen Peroxide (H₂O₂)

The determination of malondialdehyde (MDA) concentration in plant tissues is among the tools for assessing oxidative stress that reflects the degree of peroxidation of membrane lipids (Batool et al., 2020). The measurement was performed following the method described by Madhava Rao and Sresty (2000). 10 mL of 0.1% trichloroacetic acid (TCA) was used to extract lipid peroxides from 0.05 g of the leaf powder previously frozen. The chromogen was created by combining 1 mL of supernatant with 2.5 mL of thiobarbituric acid (TBA) after centrifugation (18,000 g for 20 min). The mixture was incubated at 95 °C for 30 min, and the reaction was stopped by placing the

tubes in an ice bath. Following measurements of the produced chromogen at wavelengths of 450, 532, and 600 nm, the MDA concentration was estimated using the following formula: $MDA = 6.45 (A_{532} - A_{600}) / 0.56A_{450}$.

Hydrogen peroxide (H_2O_2) contents in leaves were determined according to the method of Velikova et al. (2000). 0.1 g of fresh leaf samples (well developed from the same row for all treatments) were mixed with 5 mL of 10% (w/v) trichloroacetic acid (TCA) in a cold mortar, and the mixture was subsequently centrifuged at 15,000 x g for 15 min at 4 °C. The concentration of H_2O_2 was then determined by recovering the supernatant. A total of 1 mL of iodine potassium (1 M) and 0.5 mL of potassium phosphate buffer (10 mM, pH 7) were added to 0.5 mL of the supernatant. After an hour of incubation in the dark, the absorbance was measured at 390 nm.

Statistical Analysis

The presented data are mean values based on six replicates \pm standard error (S.E.) per treatment. Statistical analysis was carried out with the software package R statistics 10.0 for Windows. All results were subjected to a multivariate analysis of variance (MANOVA) for the main factors (Treatment, condition (stressed, normal), \pm slag) and their interactions. Comparisons between mean were performed using the LSD (Low Significant Difference) test calculated at $P < 0.05$.

Results

Effect of Slag-based Fertilizers on the Growth and Physiology of Durum Wheat

Growth Parameters

Shoot height (SH) of plants was significantly ($P < 0.05$) reduced by the application of salt stress (100 mM NaCl). However, the application of D1 treatment (10 g slag/kg soil) significantly improved ($P < 0.05$) this parameter in the absence and presence of salt stress compared to the control and D2 treatment (Table 3). Root length (RL) of durum wheat grown in the absence and presence of salt stress showed no significant difference in the applied D1 and D2 slag treatments compared to the control (Table 3). Spike height (SH') of durum wheat was significantly ($P < 0.05$) reduced by salinity. The application of 10 g slag/ kg soil treatment (D1) significantly improved this parameter in the absence and presence of salinity stress compared to the control (C) with 0 g slag/ kg soil. On the other hand, the plants treated with 20 g slag/ kg soil treatment (D2) significantly reduced the spike height (SH') of durum wheat compared to the stressed (100 mM NaCl) and unstressed control plants (0 mM NaCl) and D1 treatment (Table 3).

Shoot fresh weight (SFW) was significantly ($P < 0.05$) reduced by salt stress (100 mM NaCl) (Table 3). On the other hand, D1 treatment significantly

improved SFW compared to the control (C) under normal and saline conditions, while D2 treatment recorded a decrease in aboveground fresh biomass under the same conditions. As for root fresh weight (RFW), this parameter was negatively affected by salinity (Table 3). The application of 10 g slag/ kg soil treatment (D1) and 20 g slag/ kg soil treatment (D2) improved this parameter under salinity stress conditions (100 mM NaCl) compared to the control (C).

Salt stress significantly ($P < 0.05$) decreased spike fresh weight (SFW'). The application of 10 g slag/ kg soil treatment (D1) has significantly improved this parameter regardless of the level of salinity applied compared to the control with 0 g slag/ kg soil (C). The largest decrease in SFW' was recorded in D2 treatment (20 g slag/ kg soil) (Table 3).

Table 3. Effect of salinity stress levels (0 mM NaCl and 100 mM NaCl) on growth performance of *Triticum durum* at three different slag rates (C: 0 g slag/kg soil, D1: 10 g slag/kg soil, and D2: 20 g slag/kg soil) after 3 months of cultivation

Salinity stress level	Treatments	SH (cm)	RL (cm)	SH' (cm)	SFW (g)	RFW (g)	SFW' (g)
0 mM NaCl	C	55.00 ± 2.01 b	26.50 ± 3.50 a	11.33 ± 0.58 b	0.45 ± 0.04 b	0.12 ± 0.02 c	0.35 ± 0.03 b
	D1	62.00 ± 2.65 a	24.50 ± 2.18 a	12.50 ± 0.00 a	0.56 ± 0.03 a	0.25 ± 0.02 a	0.45 ± 0.04 a
	D2	52.67 ± 5.03 b	23.00 ± 1.00 a	9.33 ± 0.58 c	0.31 ± 0.05 d	0.09 ± 0.01 c	0.14 ± 0.04 c
100 mM NaCl	C	39.00 ± 5.29 c	24.00 ± 6.08 a	9.67 ± 0.58 c	0.27 ± 0.07 d	0.05 ± 0.02 d	0.16 ± 0.03 c
	D1	56.00 ± 3.00 b	23.50 ± 1.80 a	11.00 ± 0.87 b	0.39 ± 0.03 c	0.19 ± 0.03 b	0.33 ± 0.03 b
	D2	45.00 ± 2.01 c	22.00 ± 4.77 a	7.17 ± 0.29 d	0.18 ± 0.03 e	0.15 ± 0.04 b	0.12 ± 0.01 c

SH: shoot height; RL: root length; SH': spike height; SFW: shoot fresh weight; RFW: root fresh weight; SFW': spike fresh weight. Columns sharing the same letters are not significantly different ($p < 0.05$).

Salt stress induced a significant decrease in the shoot and root dry weights of wheat (Figures 2 and 3). However, the application of slag at D1 treatment as a fertilizer has significantly improved the shoot dry weight of durum wheat plants in the absence (0 mM NaCl) and the presence of salt stress (100 mM NaCl) (Figure 2). In addition, D1 and D2 slag treatments (10 g slag/ kg soil and 20 g slag/ kg soil, respectively) led to a significant increase in root dry weight of wheat under saline conditions (100 mM NaCl) compared to the control (0 g slag/ kg soil) (Figure 3).

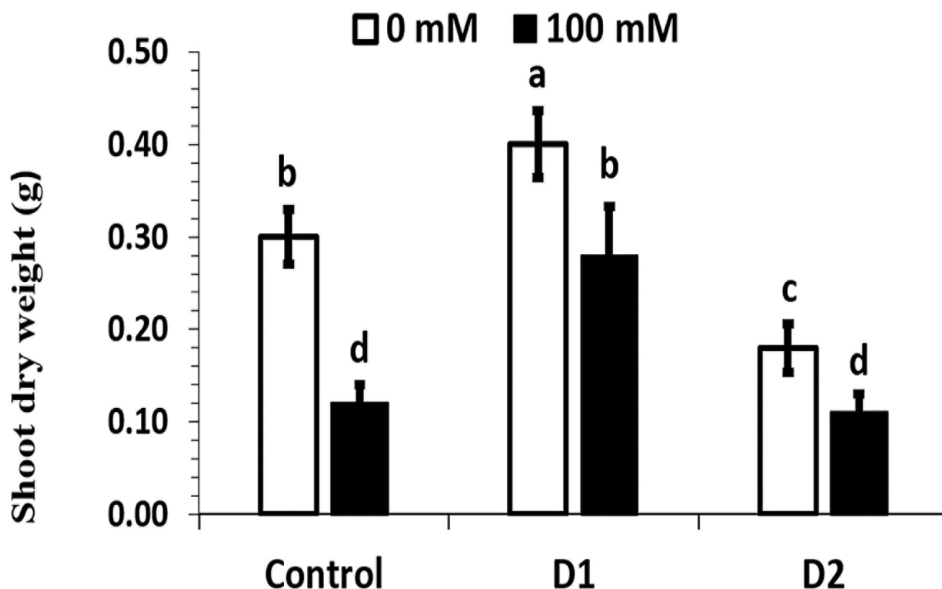


Figure 2. Effect of salinity stress levels (0 mM NaCl and 100 mM NaCl) on shoot dry weight of *Triticum durum* at three different slag rates (C: 0 g slag/kg soil, D1: 10 g slag/kg soil, and D2: 20 g slag/kg soil) after 3 months of cultivation.

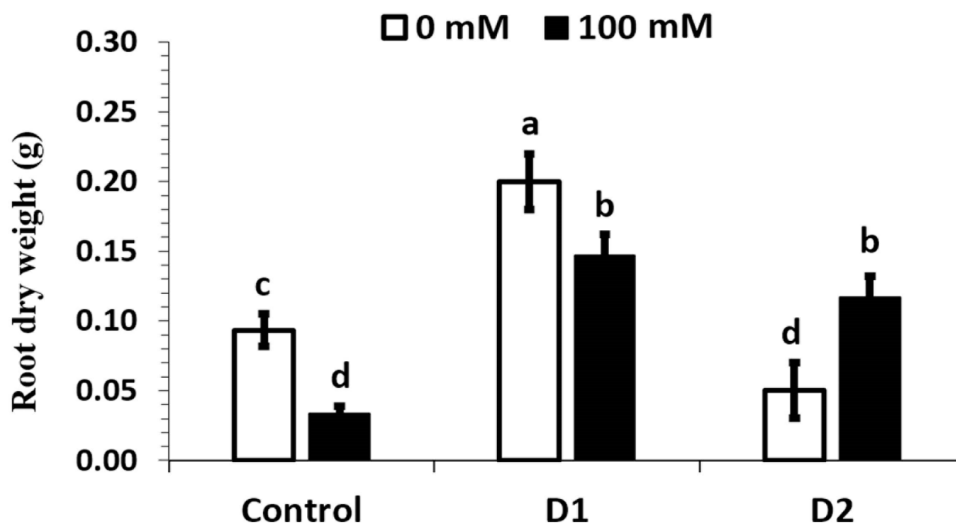


Figure 3. Effect of salinity stress levels (0 mM NaCl and 100 mM NaCl) on root dry weight of *Triticum durum* at three different slag rates (C: 0 g slag/kg soil, D1: 10 g slag/kg soil, and D2: 20 g slag/kg soil) after 3 months of cultivation.

Photosynthetic Parameters

The effect of slag-based fertilizers application on photosynthetic machinery under normal and salinity conditions was evaluated. The stomatal conductance (gs) and quantum efficiency of photosystem II (Fv/Fm) were decreased with the application of salinity stress (100 mM NaCl) compared to normal conditions (0 mM NaCl) (Figures 4 and 5). Stomatal conductance (gs) showed a significant increase after the application of D1 treatment (10 g slag/kg soil) compared to the control (C) under normal and salinity conditions (Figure 4). Fv/Fm significantly increased ($p < 0.05$) by slag application at dose D1 (10 g slag/kg soil) under normal and salt stress conditions after three months of cultivation compared to the control durum wheat plants (0 g slag/kg soil) and D2 treatment (20 g slag/kg soil) (Figure 5). Regardless of the salt level applied to the soil, treatment (D2) decreased both gs and Fv/Fm.

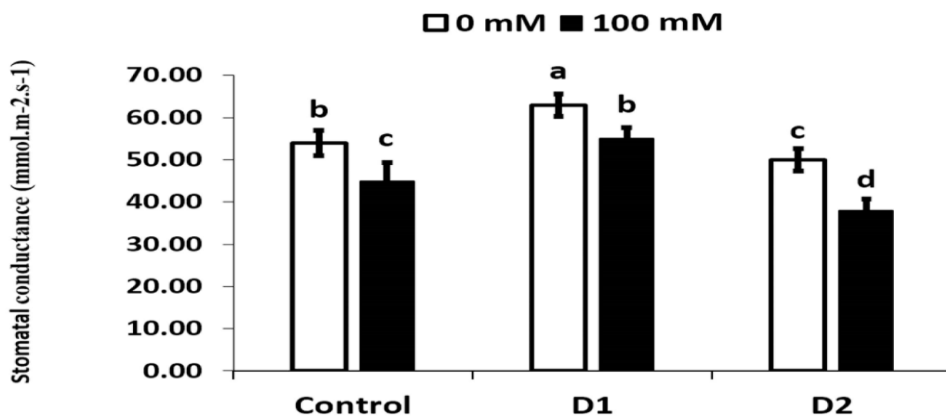


Figure 4. Effect of salinity stress levels (0 mM NaCl and 100 mM NaCl) on stomatal conductance of *Triticum durum* at three different slag rates (C: 0 g slag/kg soil, D1: 10 g slag/kg soil, and D2: 20 g slag/kg soil) after three months of cultivation.

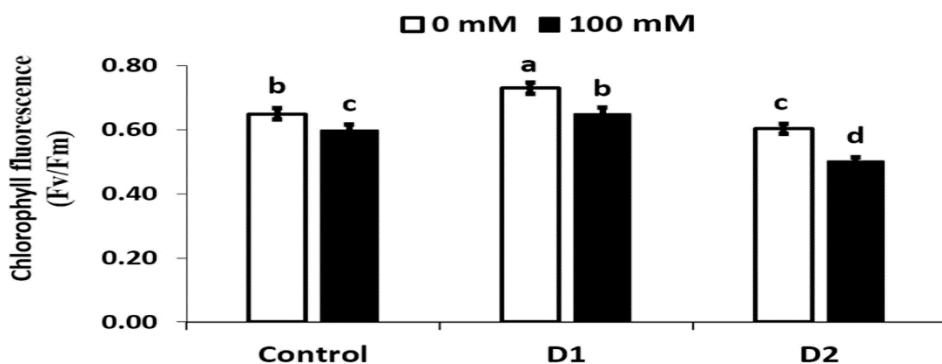


Figure 5. Effect of salinity stress levels (0 mM NaCl and 100 mM NaCl) on chlorophyll fluorescence (Fv/Fm) of *Triticum durum* at three different slag rates (C: 0 g slag/kg soil, D1: 10 g slag/kg soil, and D2: 20 g slag/kg soil) after three months of cultivation.

Effect of Slag on MDA, H₂O₂, Soluble Sugars and Protein Contents of Durum Wheat

The results related to the effect of salt stress on total soluble sugars (TSS) and protein contents, hydrogen peroxide (H₂O₂), and malondialdehyde (MDA) in durum wheat are presented in Table 4. TSS, H₂O₂, and MDA contents in wheat has significantly increased (P <0.05) under salt conditions (100 mM NaCl) compared to the normal conditions (0 mM NaCl). In contrast, salinity stress significantly decreased (P <0.05) protein content in durum wheat. Independently of the presence or absence of salinity stress, the amendment of *T. durum* with 10 g slag/ kg soil and 20 g slag/ kg soil accumulated more soluble sugars and protein content than the control plants (0 g slag/ kg soil). Plants amended with 20 g slag/ kg soil treatment (D2) showed the highest levels of H₂O₂ and MDA contents compared to the control and D1 treatment (0 g slag/ kg soil and 10 g slag/ kg soil, respectively) after three months of durum wheat cultivation under normal and salinity conditions (0 mM NaCl and 100 mM NaCl, respectively).

Table 4. Effect of salinity stress levels (0 Mm NaCl and 100 Mm NaCl) on protein, total soluble sugars (TSS), hydrogen peroxide (H₂O₂), and malondialdehyde (MDA) contents of *Triticum durum* grown at three different slag rates (Control: 0 g slag/kg, D1: 10 g slag/kg, and D2: 20 g slag/kg soil) after three months of cultivation

Salinity stress level	Treatments	Protein (mg g ⁻¹ FM)	TSS (mg g ⁻¹ FM)	H ₂ O ₂ (nmol.g ⁻¹ FM)	MDA (μmol.g ⁻¹ MF)
0 mM NaCl	C	25.00 ± 1.73 c*	28.00 ± 1.00 e	0.04 ± 0.01 d	8.00 ± 0.50 d
	D1	62.33 ± 1.53 a	35.33 ± 1.53 d	0.05 ± 0.01 d	9.33 ± 0.58 d
	D2	63.67 ± 1.53 a	51.00 ± 1.00 b	0.07 ± 0.01 c	20.67 ± 0.58 b
100 mM NaCl	C	20.00 ± 2.00 d	35.33 ± 1.53 d	0.12 ± 0.01 b	16.67 ± 0.29 c
	D1	51.67 ± 1.15 b	40.33 ± 1.53 c	0.12 ± 0.01 b	17.67 ± 0.58 c
	D2	49.67 ± 1.53 b	57.00 ± 1.00 a	0.21 ± 0.02 a	28.00 ± 1.00 a

*Columns sharing the same letters are not significantly different (p < 0.05).

Discussion

Salt stress is one of the most severe limiting factors for crop growth and production (Parihar et al., 2015). During salt stress, photosynthesis is reduced by lowering stomatal conductance (gs) and preventing the synthesis of chloroplast proteins (Liang et al., 2022). Therefore, in order to meet global food demands, it is necessary to discover eco-friendly and sustainable crop-growing methods. The use of steel slag-based fertilizer can mitigate salinity stress on agricultural systems. Subsequently, the effect of applying slag at two concentrations, D1 (10 g slag/ kg soil) and D2 (20 g slag/ kg soil) treatment on growth, physiological and biochemical parameters of durum wheat (*Triticum durum*) plants under salt-stress conditions was evaluated. The interaction between steel slag rich in mineral elements and salt-stress conditions is

essential for improving defense strategies of durum wheat plants. The results indicated that salinity stress negatively affected the growth of durum wheat plants. Hossain et al. (2021) also reported that salinity impaired seedling establishment, stunted plant growth, affected reproductive development, and ultimately reduced yield. In the present study, significant reductions in plant growth parameters such as shoot and root lengths and fresh and dry weight yields of durum wheat plants indicated that the application of salt stress (100 mM NaCl) was toxic within the 90 days of treatment used here. In addition, the application of 10 g slag/ kg soil treatment (D1) increased wheat growth regardless of the salinity level. In accordance with the present study, Wang and Cai (2006) reported that treatment of the soil with steel slag (10 g slag/ kg soil) resulted in significant improvement of the fresh and dry matter of maize as well as Fe uptake by plants. This could be due to the rapid assimilation of the mineral elements contained in the slag at low concentration by the plants in order to ensure their life cycle and promote significant growth (Radić et al., 2022). However, in the present study, the application of 20 g slag/ kg soil treatment (D2) showed a significant decrease in growth parameters compared to the control (C) with 0 g slag/ kg soil under normal and salinity stress conditions. This could be due to excess mineral elements in the D2 concentration of slag (20 g slag/ kg soil), which could become toxic to the development of wheat plants (Wang & Cai, 2006). Other researches showed a significant increase in growth and biomass accumulation for low rates of slag, while increasing application rates of slag did not improve the growth (Cai et al., 2022; Pietrini et al., 2017; Chen et al., 2019; Atland et al., 2015; Wang et al., 2006). Wang et al. (2006) and Islam et al. (2022) showed that the high rates of slag application in the soil led to a huge accumulation of Fe and decreased bacterial biomass in the soil, which could create a poor assimilation of nutrients and become a source of inhibition of plant growth. In contrast, application of steel slag to agricultural land can provide potential benefits for soils in the form of increased availability of Ca and P (Yang et al., 2019). According to the same study, CaO is the major component of slag. Ca also has a positive impact on root strength of plants and facilitates the uptake of K, which is a crucial element for the growth and physiology of plants (Radić et al., 2022). Slag also increases the bioavailability of P, Si, and Ca present in the soil (Radić et al., 2022). In addition, the use of slag has improved soil fertility and consequently improved growth and yield of rice (Ning et al. 2016; Das et al., 2020). The use of slag in acidic soils increases soil pH, soil Ca and Mg levels, decreases soil equivalents of aluminum (Al), manganese (Mn), copper (Cu), and zinc (Zn) in soils, improves yield, and enhances nutrient concentrations in plants (Ghisman et al., 2022; Moraes et al., 2017). The use of steelmaking slag as a fertilizer highlights the importance of considering the physical characteristics of the slag, particularly the particle size distribution,

and its potential effects on soil properties. Although the only requirement for registering as a by-product calcium fertilizer is the alkali content (35 mass% or more) and maximum size (100 % is smaller than 1.7 mm and 85 % is smaller than 600 μm) (Gao et al., 2015), the variation in composition and mineralogical structure of steelmaking slag from different processes can affect the dissolution behaviors of nutrient elements. This variability underscores the need for careful consideration of the physical properties of slag when using it as a fertilizer, as particle size can significantly impact the release of nutrients and the alteration of soil properties. Therefore, it is important to study the performance of different particle size fractions of slag in terms of nutrient release and soil effects to ensure safe and effective use of steelmaking slag as a fertilizer. Measuring physiological characteristics that contribute to the plant's ability to withstand stress could be a way to identify and select wheat varieties that are better able to adapt to salt stress growing conditions (EL Sabagh et al., 2021). Furthermore, the osmotic stress induced by salinity can lead to a reduction of CO_2 assimilation by the plant and consequently result to inhibition of photosynthesis (Ezquer et al., 2020; Yang et al., 2020). In addition, this study showed that the decrease in stomatal conductance was related to a reduction in chlorophyll fluorescence (F_v/F_m) in plants subjected to salt stress. This effect may be associated with the destruction of chloroplasts due to the direct effect of salt stress and the decrease in the activity of photosynthetic pigment synthesis enzymes (Murkute et al., 2018; Zhu et al., 2021).

The decrease in biomass of durum wheat plants under salt stress conditions was accompanied by an increase in H_2O_2 and MDA levels. The results of this study are in agreement with other previous studies on date palm (Anli et al., 2020; Ait-El-Mokhtar et al., 2022), brown mustard (Ahmad et al., 2015), ephedra (Alqarawi et al., 2014), and alfalfa (Ben Laouane et al., 2019). Root tissues exhibited higher oxidative damage than the tissues of the aerial part due to high accumulation of reactive oxygen species (ROS), such as superoxide radical (dioxygen (O_2^-)), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2). This is because the root organs are the first to be affected by excess salt in the soil solution (Batoool et al., 2020; Ait-El-Mokhtar et al., 2022). ROS causes oxidative damage to the different organic and inorganic molecules present in cells, including DNA, proteins, lipids, amino acids, and sugars, thus resulting to lipid peroxidation and protein oxidation (Begum et al., 2020; Khan et al., 2021). The results showed that plants grown in the presence of salt stress (100 mM NaCl) in combination with D2 dose of slag (20 g slag/ kg soil) recorded high concentration of MDA and H_2O_2 compared to untreated stressed durum wheat plants (0 mM NaCl). This suggests that D2 concentration (20 g slag/ kg soil) is an inhibitory dose to plant biological functions that may contribute to high ROS accumulation and membrane

damage. In contrast, plants treated with D1 dose of slag (10 g slag/ kg soil) showed no significant difference compared to the control (0 g slag/ kg soil), regardless of the applied salinity level. These results suggest that the D1 dose of slag (10 g slag/ kg soil) is optimal to maintain osmotic adjustment of wheat even under severe abiotic stress conditions such as salinity.

To reduce the damage caused by abiotic stresses, plants use several mechanisms to adapt and ensure their survival. Osmolytes are organic (sugars, amino acids) or inorganic molecules that intervene to reduce this damage (Siddiqui et al., 2020; Ahanger et al., 2021). In the present study, after 90 days of growth, under salt-stress conditions (100 mM NaCl), plants amended with 10 g slag/ kg soil and 20 g slag/ kg soil (D1 and D2 treatments, respectively) increased soluble sugars and protein contents compared to the control (0 g slag/ kg soil). This is a durum wheat plant defense response to imposed salt stress by maintaining osmotic balance or osmotic adjustment and mitigating free radical damage (Ahanger et al., 2014; Hasanuzzaman et al., 2019). Farooq et al. (2020) and Shafiq et al. (2021) suggested that sugars and proteins are good osmoregulators that can play an important role in osmotic adjustment and adaptation of plants to salinity stress. Osmotic adjustment is an early physiological response of plants to abiotic stress that allows cells to remain turgid at very low water potentials through the active accumulation of solutes. They help in maintaining turgor pressure, which is necessary for cell expansion and synthesis of cell wall components, including cellulose (Thalman & Santelia, 2017). These solutes can interact with cellular macromolecules such as antioxidant enzymes, thereby stabilizing their structure and function (EL Sabagh et al., 2021).

Overall, the findings showed that the physiological characteristics, total soluble sugars, and proteins in plants treated with 10 g slag/ kg soil treatment (D1) indicated enhanced development of durum wheat under salt-stress conditions. This is due to enhanced photosynthesis, less oxidative damage, an increase in the relative abundance of the beneficial microbial community in the soil, and the assimilation of mineral elements (Das et al., 2020). In the other hand, due to the growing interest in using slags for soil conditioning, heavy metal concentrations in these materials have become a subject of interest. The majority of slag materials contain of metal contaminants such as V, Cr, As, Pb, Cd, Co, Ba, Hg, Se, Sb, Ag, Zn, and Ni, which are influenced by the steel manufacturing process's quality (Chen et al., 2019). Studies conducted in Finland have indicated that the concentrations of certain elements, such as Cr and Zn, are low due to the high temperatures of the processes. Conversely, long-term experiments conducted in Germany have demonstrated that using steel slag as a liming material does not elevate the content of mobile chromium in the soil. Furthermore, there have been no significant increases in the Cr content of plants after using steelmaking slags

as fertilizer (Hiltunen 2004). Nevertheless, it is important to carry out further investigations focused on the heavy metals behavior on the soil and crops in order to better understand the effects of long-term use of steelmaking slags in agriculture.

Conclusion

Slag promoted the growth of *T. durum* plants at lower amendment levels, specifically at 10 g slag/kg soil. The total protein and total soluble sugar content increased at both slag amendment levels, 10 g slag/ kg soil and 20 g slag/ kg soil. In contrast, the fertilization with slag at a rate of 20 g slag/kg soil inhibited shoot extension and accumulation of biomass, thus resulting in a decline in the growth and physiological parameters of wheat. However, there was an increase in the quantum efficiency of photosystem II (Fv/Fm) and stomatal conductance (gs) of durum wheat amended with 10 g slag/ kg soil under salt stress (100 mM NaCl) and normal conditions (0 mM NaCl). These results support the hypothesis that slag-based fertilizers applied at a rate of 10 g slag/kg soil improve the salinity tolerance of *T. durum* plants. This in turn enhances plant processes through better efficiency of PSII, osmolytes accumulation, and mineral nutrition.

Slag-based fertilizers at a low rate will be tested in the field in order to evaluate the effect of such slag application on biomass and grain yield. Treatments with drought stress in combination with the slag fertilizers will be also evaluated.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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