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Effects of Slag-Based Fertilizer to Mitigate Salinity Stress on Greenhouse Durum Wheat (Triticum Durum Desf.) Cultivars

Farid Errouh
Abdelhamid Aouabe
Centre d'Agrobiotechnologie et de Bioingénierie, Unité de Recherche labellisée CNRST, Equipe "Physiologie des Stress Abiotiques", Université Cadi Ayyad, Marrakech, Morocco
Laboratoire d'Agroalimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad (UCA), Marrakech, Morocco
Ayoub Sadouki
Laboratoire d'Agroalimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad (UCA), Marrakech, Morocco
Hassan Chagiri
Hicham Khalisse
Concamine company Berrechid, Morocco
Brahim Oudra
Laboratoire Eau, Biodiversité et Changement Climatique (EauBiodiCC), Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad (UCA), Marrakech, Morocco
Julio Cesar Rodriguez
Centre d'Agrobiotechnologie et de Bioingénierie, Unité de Recherche labellisée CNRST, Equipe "Physiologie des Stress Abiotiques", Université Cadi Ayyad, Marrakech, Morocco
Laboratoire d'Agroalimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad (UCA), Marrakech, Morocco
Departamento de Agricultura y Ganadería, Universidad de Sonora, Luis Encinas SN, Hermosillo, México
Salah Er-Raki
Centre d'Agrobiotechnologie et de Bioingénierie, Unité de Recherche labellisée CNRST, Equipe "Physiologie des Stress Abiotiques", Université Cadi Ayyad, Marrakech, Morocco
ProcEDE /AgroBiotech center, Faculty of Sciences and Techniques,

Cadi Ayyad University, Marrakech, Morocco
Mohammed VI Polytechnic University (UM6P),
Center for Remote Sensing Applications (CRSA), Morocco
Abdelilah Meddich
Centre d'Agrobiotechnologie et de Bioingénierie, Unité de Recherche labellisée CNRST, Equipe "Physiologie des Stress Abiotiques", Université Cadi Ayyad, Marrakech, Morocco
Laboratoire d'Agroalimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad (UCA), Marrakech, Morocco

Doi: 10.19044/esipreprint.2.2023.p300

Approved: 16 February 2023
Posted: 18 February 2023

Cite As:
Errouh F., Aouabe A., Sadouki A., Chagiri H., Khalisse H., Oudra B., Rodriguez J.C., Er-Raki S. & Meddick A. (2023). *Effects of Slag-Based Fertilizer to Mitigate Salinity Stress on Greenhouse Durum Wheat (Triticum Durum Desf.) Cultivars*. ESI Preprints. https://doi.org/10.19044/esipreprint.2.2023.p300

Abstract

For a modern agricultural, the search for sustainable practices to increase productivity is fundamental. Steel slag have been studied for their potential use in agriculture. These substances present a great possibility of agricultural applications since they are rich in nutrients, which enhance plant uptake. In this regard, the effect of steel slag based-fertilizer was investigated in the greenhouse durum wheat cultivation in pots under salt-stress conditions. Two slag doses: 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. Wheat exposure to salinity decreased its biomass, stomatal conductance, efficiency of photosystem II, protein content and increased total soluble sugars, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents. Amended plants with 10 g slag/ kg soil (D1), led to a significant improve 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 salinity conditions as compared to the control treatment 0 g slag/ kg soil (C), indicating a positive influence on durum wheat plants. However, soil

enrichment with 20 g slag/ kg soil (D2) decreased plant growth parameters and presented the highest levels of H_2O_2 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 improve productivity of durum wheat and mitigate salinity stress.

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

Introduction

With the world's population that is estimated to grow to around 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 the responsible consumption and sustainable food production. As an important source of carbohydrate, cereal yield plays a great 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 takes globally the fifth place after soft wheat, rice, corn, and barley as it contributes to 20% of the total dietary calories and proteins globally (Maccaferri et al., 2003; Shiferaw et al., 2013). One of the most popular cereal crops worldwide is the durum wheat (*Triticum turgidum* L. var. durum) which is mostly grown in South European, North African, and West Asian nations. Although durum wheat production areas typically overlap those of bread wheat, durum is planted less frequently than bread wheat. Additionally, durum wheat is more suited than bread wheat to the dry Mediterranean landscape (Xynias et al., 2020). This explains why durum wheat cultivation and production are concentrated in the Mediterranean region (Turki et al., 2023). Due to climate change, these regions, where durum wheat is cultivated, are experiencing 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). 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. 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). Hence, the need to develop rational fertilization

approaches to minimize 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 a waste as a way for the steel industry to contribute to a circular economy due to growing awareness of environmental protection and economic benefits (Branca et al., 2020). As its rich 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 & Cai. (2006) and Das et al. (2019) have assessed the agronomic utility of steel slag as a fertilizer or as a liming material. Different types of steel slag had a good effect 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 the 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. The main objective of this work was to study the effectiveness of slag as a fertilizer to increase the tolerance of *Triticum durum* to salt-stress and to evaluate the effect of slag on growth, physiological and biochemical parameters to examine its contribution to the tolerance of wheat crop to salinization.

Materials and methods

Site description and biostimulant material

The experiment was conducted under greenhouse conditions at Cadi Ayyad University Marrakesh, Morocco. The environmental conditions inside for growing are as follows: average temperature of 25.5 °C, relative humidity average of 68.5% and 410 $\mu m^{-2} s^{-1}$ photon flux density average. The soil sample used in this experiment was characterized by a pH value of 8.10, electrical conductivity (EC): 0.73 mS/cm, organic matter: 0.86 %, available phosphorus: 7.96 mg kg^{-1}, K_2O: mg/kg: 168, Clay: 16 %, Fine silt: 3 %, Gross silt: 9 %, Fine sand: 38,3 % and Gross sand: 33,7 %.

The slag, which is a by-product obtained from the "Concamine" company based in Berrechid, Morocco, was composed of a variety of

chemical elements and a combination of oxides, with CaO making up the majority of them, as well as ortho-phosphates (PO_4^{3-}). The elemental analysis of the sample by energy dispersive X-ray (EDX) analysis (Figure 1) showed the presence of different chemical elements (main elements, secondary elements and trace elements) namely: Carbon, Oxygen, iron, sodium, magnesium, aluminum, silicon, phosphorus, sulfur, calcium and manganese. The slag products were used as a fine powder and its composition is presented in Table 1.

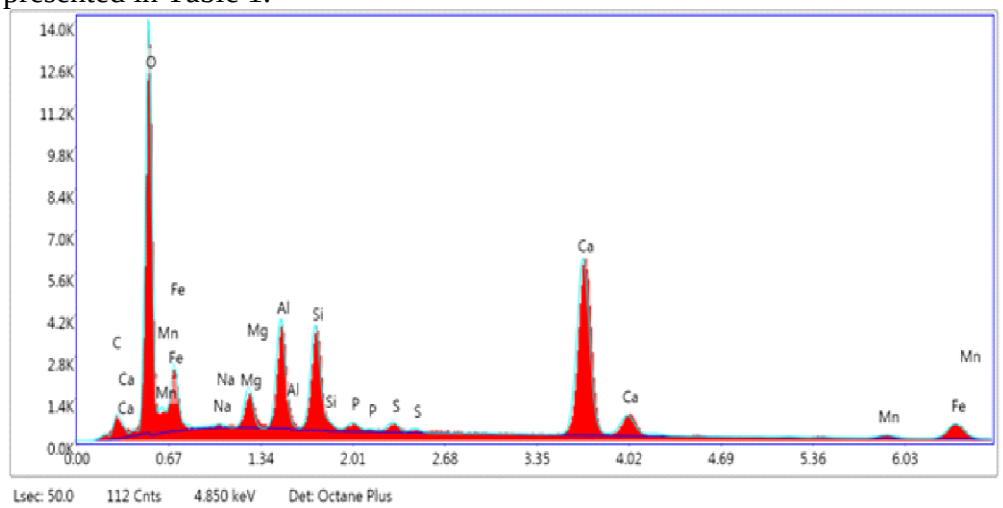

Fig. 1. The elemental analysis of the slag sample by energy dispersive X-ray (EDX) analysis

Table 1. Physico-chemical properties of the used slag

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	0.74	1.57	35.07	13.22	0.0035	1.1869	0.4014	1.0000
O K	35.02	55.63	1706.68	8.90	0.1302	1.1247	0.3306	1.0000
FeL	8.28	3.77	174.88	8.39	0.0339	0.8387	0.4878	1.0000
NaK	0.06	0.07	4.60	99.99	0.0004	1.0103	0.5818	1.0012
MgK	1.88	1.96	207.92	6.82	0.0139	1.0244	0.7197	1.0021
AlK	5.39	5.08	602.22	4.87	0.0432	0.9837	0.8113	1.0033
SiK	5.64	5.10	603.10	4.50	0.0489	1.0026	0.8617	1.0045
P K	0.41	0.34	35.19	24.34	0.0036	0.9606	0.8942	1.0078
S K	0.67	0.53	55.49	9.99	0.0062	0.9770	0.9318	1.0122
CaK	38.22	24.24	1402.04	3.51	0.3562	0.9316	0.9929	1.0072
MnK	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 moistened 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 arrangement with three treatments and six replications per each one under two level of salinity stress (0mM NaCl and 100mM NaCl). The following treatments were tested: (1) C: Control treatment 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 at 75% of field capacity using the technique described by Meddich et al. (2015).

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

Salinity stress level	**0 mM NaCl**			**100 mM NaCl**		
Treatments	**C**	**D1**	**D2**	**C**	**D1**	**D2**
Repelications	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 seeds 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. Measurements concerned the following parameters: 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 (Fm): 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 (Fm - F0) / Fm = Fv/Fm, where Fv is the variable fluorescence (Hosseinzadeh et al. 2015). The stomatal conductance (gs) is an indicator of

the rate of leaf transpiration, it is a parameter related to the water state of the plant 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 at a sunny day before harvest. Stomatal conductance measurements were taken in the second youngest leaf from six different plants from each treatment. This parameter is expressed in mmol of $H_2O.m^{-2}. s^{-1}$.

Measurement of Total Soluble Sugar (TSS) and Protein Contents

The total soluble sugar (TSS) content in the leaves were measured in extracts 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 4 ml 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 (H_2O_2)

According to the method given by Madhava Rao and Sresty (2000), malondialdehyde (MDA) was measured. 10 mL of 0.1% trichloroacetic acid (TCA) were used to extract lipid peroxides from the frozen leaf. 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 (A532 A600) 0.56A450.

Hydrogen peroxide (H_2O_2) contents in leaves were determined according to Velikova et al. (2000) method. Fresh material was 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 iodic 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 ± 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), ± slag) and their interactions. Comparisons between means 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

The effects of application of steel-slag on durum wheat growth were evaluated. 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 elongation (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) 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 the (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 0 g slag/ kg soil (C). The largest decrease in this parameter was recorded in plants amended with D2 treatment (20 g slag/ kg soil) (Table 3).

Table 3. Effect of salinity stress (**0 mM NaCl and 100 mM NaCl)** on growth performance of *Triticum durum* in absence and in presence of slag (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 resulted in a significant decrease in the shoot and root dry weights of wheat (Figure 2 and 3). However, the application of slag at the D1 treatment as a fertilizer has significantly improved the shoot dry weight of durum wheat plants in the absence (0 mM NaCl) and presence of salt stress (100 mM NaCl) (Figure 2). In addition, the 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 salinity conditions (100 mM NaCl) compared to the control (0 g slag/ kg soil) (Figure 3).

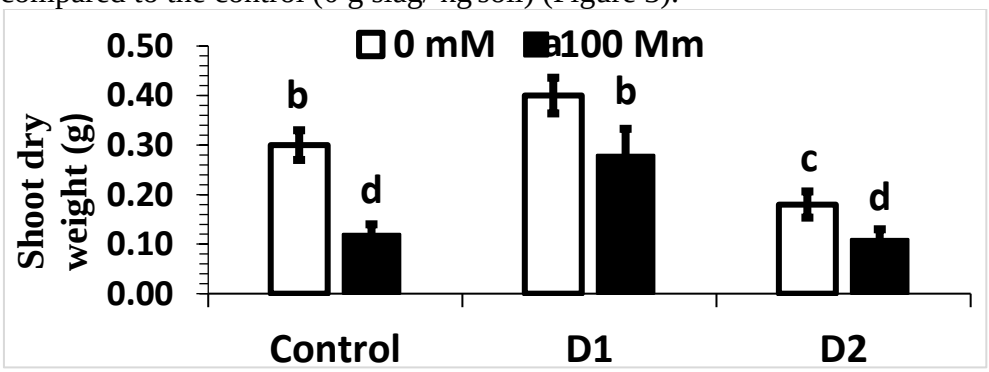

Fig. 2. Effect of salinity stress (**0 mM NaCl and 100 mM NaCl)** on shoot dry weight of *Triticum durum* in absence and in presence of slag (C: 0 g slag/kg soil, D1: 10 g slag/kg soil and D2: 20 g slag/kg soil) after 3 months of cultivation.

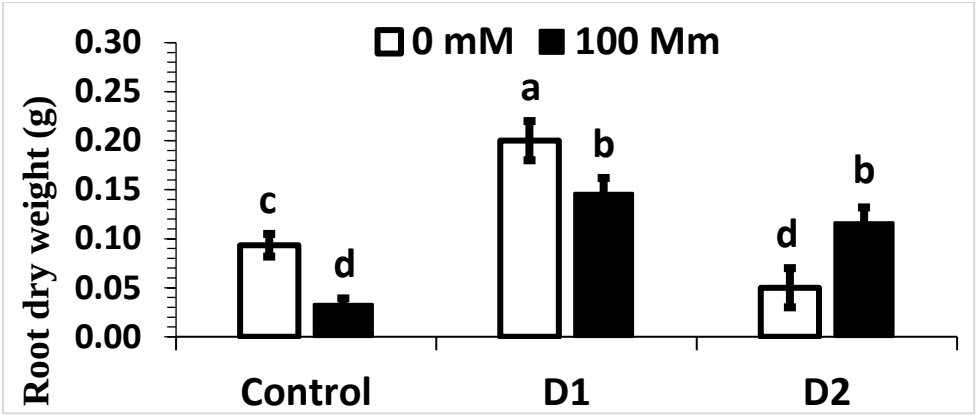

Fig 3. Effect of salinity stress (**0 mM NaCl and 100 mM NaCl)** on root dry weight of *Triticum durum* in absence and in presence of slag (C: 0 g slag/kg soil, D1: 10 g slag/kg soil and D2: 20 g slag/kg soil) after 3 months of cultivation

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 efficiency (Fv/Fm) were decreased with the application of salinity stress (100 mM NaCl) compared to normal conditions (0 mM NaCl) (Figure 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 3 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 salt level applied to soil, treatment D2 decreased both gs and Fv/Fm.

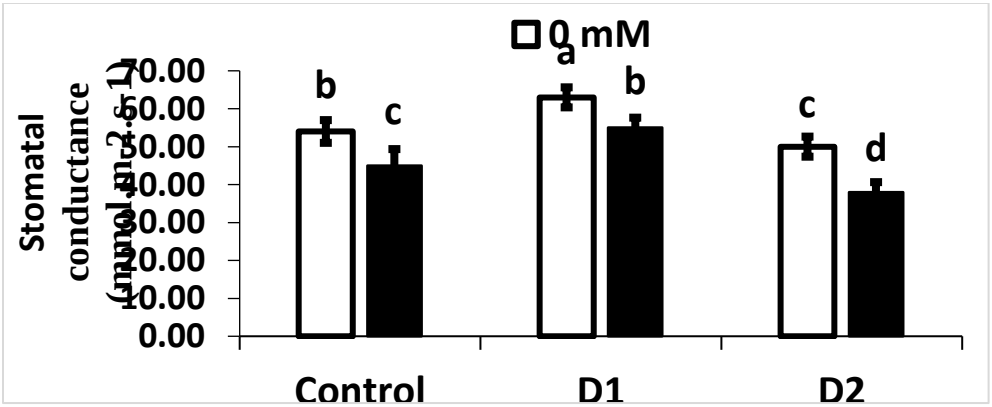

Fig 4. Effect of salinity stress (**0 mM NaCl and 100 mM NaCl)** on stomatal conductance of *Triticum durum* in absence and in presence of slag (C: 0 g slag/kg soil, D1: 10 g slag/kg soil and D2: 20 g slag/kg soil) after 3 months of cultivation.

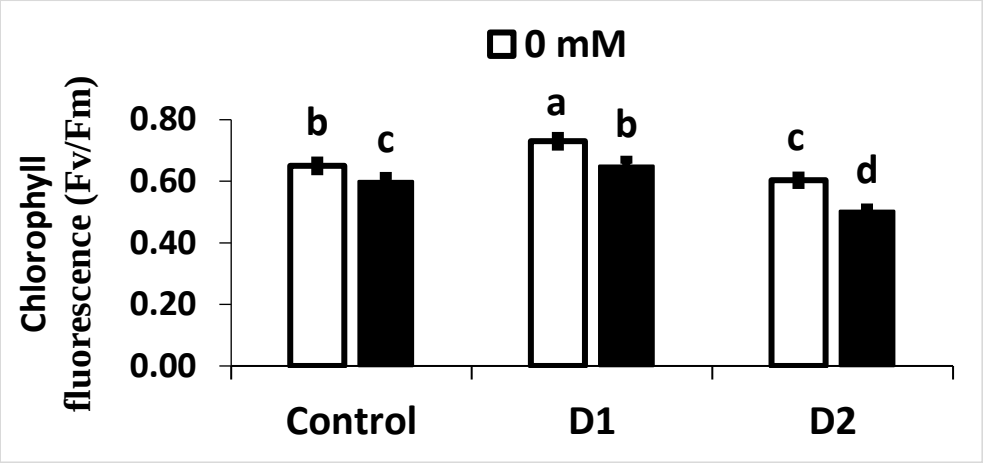

Fig 5. Effect of salinity stress (**0 mM NaCl and 100 mM NaCl)** on chlorophyll fluorescence (Fv/Fm) of *Triticum durum* in absence and in presence of slag (C: 0 g slag/kg soil, D1: 10 g slag/kg soil and D2: 20 g slag/kg soil) after 3 months of cultivation

Effect of slag on MDA, H_2O_2, 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 (H_2O_2) and malondialdehyde (MDA) in durum wheat are presented in Table 4. TSS, H_2O_2, 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 has 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 as compared to the control plants (0 g slag/ kg soil). Plants amended with 20 g slag/ kg soil treatment (D2) showed the highest levels of H_2O_2 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 (0 Mm NaCl and 100 Mm NaCl) on protein, total soluble sugars (TSS), hydrogen peroxide (H2O2) and malondialdehyde (MDA) contents of *Triticum durum* grown in the absence and presence of slag (Control: 0 g slag/kg, D1: 10 g slag/kg and D2: 20 g slag/kg soil) after 3 months of cultivation

Salinity stress level	Treatments	Protein (mg g^{-1} FM)	TSS (mg g^{-1} FM)	H_2O_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 serious limiting factors for crop growth and production (Parihar et al., 2015). Furthermore, 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 growing crop methods. However, the use of biostimulants can be advantageous to mitigate salinity stress on agricultural systems. In this context, the effect of applying slag at two concentrations, D1 (10 g slag/ kg soil) and D2 (20 g slag/ kg soil) on growth, physiological, and biochemical parameters of durum wheat (*Triticum durum*) plants under salt stress conditions was evaluated. The interaction between biostimulant rich in mineral elements and salt stress conditions may be important for understanding, analyzing, and improving defense strategies of durum wheat plant. Our results indicated that the salinity stress negatively affected growth of durum wheat plants. Hossain et al. (2021) reported also that salinity impairs the seedling establishment, stunted plant growth, poor reproductive development, and ultimately declines the crop 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 work, Wang and Cai (2006) have reported that treatment of the soil with steel slag (10 g slag/ kg soil) resulted in a 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 work, the application of 20 g slag/ kg soil, treatment (D2) showed a significant decrease in growth parameters compared to the control (C) 0 g slag/ kg soil under normal and salinity stress conditions. This could be probably due to the excess of mineral elements

present in high amount in the D2 concentration of slag (20 g slag/ kg soil) that 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), 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 be 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). The same study suggested that as CaO is the major component of slag, and Ca 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). The slag also increases the bioavailability of P, Si and Ca present in the soil to the plant (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, and improves yield also enhances nutrient concentrations in the plant (Ghisman et al. 2022; Moraes et al. 2017).

Measuring physiological characteristics that contribute to a 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). Indeed, the osmotic stress induced by salinity, which can lead to a reduction of CO_2 assimilation by the plant and consequently an inhibition of photosynthesis (Ezquer et al. 2020; Yang et al., 2020). In addition, salinity is a constraint that disrupt photochemical reactions of photosynthesis, especially at the PSII level (Baraldi et al. 2019; Zahra et al., 2022). This study also showed that the decrease in stomatal conductance was related to a reduction in chlorophyll fluorescence (Fv/Fm) in plants subjected to salt stress. This effect may be associated to destruction of chloroplasts due to the direct effect of salt stress and to 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. Our results 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 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)) than the tissues of the aerial part, because the root organs are the first to be affected by excess salt in the soil solution (Batool et al., 2020; Ait-El-Mokhtar et al., 2022). ROS cause oxidative damage to the different organic and inorganic molecules present in cells, including DNA, proteins, lipids, amino acids and sugars, resulting in lipid peroxidation and protein oxidation (Begum et al., 2020; Khan et al., 2021). The determination of 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). Our 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 for maintaining osmotic adjustment of wheat even under severe abiotic stress conditions such as salinity. It has been reported in one study that slag contains a variety of trace elements on their surface such as Fe, Mn and Ca, as well as metallic trace elements such as cadmium (Cd), lead (Pb), Al and Zn which are toxic at high levels but safe when in appropriate amounts (Chen et al., 2019).

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). Soluble sugars and proteins are among the metabolites commonly used to assess the degree of response to abiotic stresses (Evelin et al., 2019). In the present study, after 90-day 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 may be a defense response to improve the tolerance of durum wheat plants 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 (Thalmann and Santelia, 2017). These solutes can interact with cellular macromolecules such as antioxidant enzymes, there by stabilizing their structure and function (EL Sabagh et al., 2021).

Overall, our findings showed that the physiological characteristics, total soluble sugars, and proteins in plants treated with 10 g slag/ kg soil treatment (D1) enhanced the development of durum wheat and significantly protected the membrane stability of the photosynthetic machinery under salt stress conditions. This may result from enhanced photosynthesis, less oxidative damage, an increase in the relative abundance of the beneficial microbial community in the soil, assimilation and mineral elements (Das et al. 2020).

Conclusion

Slag based-fertilizers promote the growth of *T. durum* plant at lower amendment levels (10 g slag/ kg soil). The total protein and total soluble sugars content increased because of the application of slag with two doses 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 and caused a decline in growth and physiological parameters of wheat. However, there was an increase in the amount of 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). Interestingly, these results strongly support the hypothesis that slag-based fertilizers with a rate of 10 g slag/ kg soil develop salt-adaptive strategies through the influence of plant mechanisms, such as better efficiency of PSII, osmolyte accumulation and mineral nutrition, which are important mechanisms in tolerance of durum wheat seedlings to salinity.

As perspective of this work, Slag based-fertilizers with a level of 10 g slag/ kg soil will be tested in the field in order to evaluate the effect of this bio-stimulant on the biomass and grain yield, which is the most important for the farmers. Some treatments with hydric stress in combination with the slag fertlilizers will be also evaluated.

Acknowledgments

The present work was carried out in the framework of collaboration between the Faculty of Sciences Semlalia, Cadi Ayyad University Marrakech, Morocco and the “Concamine” company Berrechid, Morocco established on May 28, 2022 to valorize the slag on a large scale.

Conflict of interest: No potential conflict of interest was reported by the authors.

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