Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XIII, 2024
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSE OF TWO DURUM WHEAT VARIETIES TO WATER STRESS COMBINED WITH A SLUDGE AMENDMENT

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Abstract

Sewage sludge, as biosolids, can improve the fertility of degraded soils in arid and semi-arid regions. The use of these biosolids reduces reliance on increasingly expensive chemical fertilizers. They also limit carbon emissions and promote soil microbial growth, while stimulating plant growth. The objective of this study is to investigate the physiological and biochemical response of two durum wheat varieties (Hedba and BiDi) to the application of residual sludge (D0 = control, D1 = 30 g and D2 = 60 g of sludge per pot) under water stress conditions (S0 = 100%, S1 = 40%, S2 = 25% FC). The analyses indicated a linear and significant accumulation in the biochemical variables (sugars, prolineandN) under the effect of stress and residual sludge. Contrary to the physiological variables with (WRC, Cell Integrity). However, chlorophyll a content accumulates with stress and residual sludge. Hedba variety is more resistant to stress compared to BD.

***Key words**: biochemistry, Durum wheat, physiology, residual sludge, water stress.*

INTRODUCTION

Agriculture plays a crucial role in economic and social development by providing food, raw materials, and generating employment (Lallaouna et al., 2023). This role is even more important given that global food demand is expected to increase by 70% by 2050, driven by rapid population growth, bringing the world population to over 9 billion people, thus threatening human food security (Hunter et al., 2020). Faced with this imminent challenge, ensuring future food security requires a multidimensional approach that includes assessing and improving soil quality, optimizing agricultural productivity, and strategic food planning (El Behairy et al., 2024). Environmental stresses, particularly droughts, cause significant decreases in cereal crop yields, affecting durum wheat particularly, an essential crop in North Africa and the main staple food in Algeria (Boudjabi et al., 2023). Indeed, in arid and semi-arid regions, durum wheat cultivation faces the constant challenge of recurring water stress (Boudjabi et al., 2023). This alarming situation threatens the food security of North African populations, making the search for sustainable solutions to optimize durum wheat production in these challenging environments all the more urgent. The use of residual sludge as an amendment emerges as a promising alternative to mineral fertilizers (Singh et al., 2011; Ababsa et al., 2023; Boudjabi & Chenchouni, 2023). Indeed, these biosolids, rich in organic matter and nutrients (Sharma et al., 2017; Vera-García et al., 2023; Boudjabi et al., 2019), contribute to soil quality improvement and increased yields (Boudjabi & Chenchouni, 2023). They offer an economic, ecological, and more efficient solution to restore soil fertility and sustainably boost agricultural yields. Moreover, the use of residual sludge as an amendment aligns with the principles of the circular economy by encouraging waste reuse and reducing dependence on non-renewable resources. In this study, we simulated water stress conditions frequently encountered in semi-arid regions of Algeria. Our objective was to assess the effect of soil amendment with residual sludge on the response of two durum wheat genotypes (Hedba and BD) to variable water stress. To achieve this, we analyzed various physiological and biochemical parameters of Hedba and BD plants, such as cellular integrity, relative water content, proline and sugar content, leaf nitrogen content, and chlorophyll a, under different doses of residual sludge (D0 = 0, D1 = 30, and D2 = 60 g/pot) and three levels of water stress (S0 = 100%, S1 = 40%, and S2 = 25% FC).Our hypothesis is that water stress will negatively affect physiological parameters and induce an increase in biochemical variables. However, our hypothesis regarding the amendment of residual sludge is that the effect of stress will be paradoxically corrected and mitigated by the application of sludge, and this correction will be linear with the doses of sludge applied.

MATERIALS AND METHODS

Experimental Site

To study the physiological and biochemical response of two durum wheat varieties to increasing doses of residual sludge under water deficit conditions, an experiment was conducted in a plastic greenhouse at the Biology Department of the Cheikh Larbi Tebessi, Tébessa University during the academic year 2021-2022.

Experimental Design

The experiment was carried out in identical plastic pots with a capacity of 3 kg, considering three factors. The first factor is the variety effect, with two varieties selected Hedba and BIDI(BD). The varieties used were obtained from the OAIC of Tébessa (Algeria). The application of residual sludge and water stress were applied at three levels: S0 = 100% FC, 40% FC, and 25% FC for water stress, and D0 = control (without sludge), D1 = 30 g of residual sludge, D2 = 60 g for residual sludge. The residual sludge used was urban sludge, brought from the wastewater treatment plant located in Ain Beida (a region in eastern Algeria). It underwent air drying, grinding, and sieving through a 2 mm sieve. Before its application in the pots, a fraction of this residual sludge was taken for chemical analysis, and a fraction of the soil used was also analyzed at the same time. Their physicochemical characteristics are shown in Table 1.

Table 1. Physicochemical characteristics of soil and sewage sludge used in experiments

Physico chemical parameters	Soil	Sewage sludge
Organic carbon [%]	1.60	30.47
Total nitrogen [%]	0.18	5.88
Assimilable phosphorus [μg/g]	2.67	19.34
Total $CaCO_3$ [%]	14.68	–
Active $CaCO_3$ [%]	2.32	–
Electrical conductivity [dS/cm]	0.323	1.58
pH	7.45	7.68
Nitrate [mg/kg]	14.3	52.28
Iron [ppm]	4.6	7.6
Zinc [ppm]	1.86	22.19
Copper [ppm]	7.24	12.69
Soil texture	Silt-clay	–

Description of the experiment

Six seeds of each durum wheat variety (Hedba and BD) were sown in pots filled with soil from Tébessa. The pots were then amended with sludge according to the defined doses and placed in a greenhouse. Water stress was applied to the plants at the jointing stage. The experiment was repeated three times for each treatment combination (sludge and water stress), for a total of 54 pots (27 per variety).

Plant analyses

Determination of Biochemical parameters

The method of Dubois et al. (1956) was used to quantify sugars in durum wheat leaves. 100 mg of fresh leaves are macerated in 80% ethanol for 48 hours, then the ethanol was evaporated. The volume is brought up to 20 ml with distilled water. 1 ml of the extract is reacted with phenol and sulfuric acid. The optical density (OD) is measured at 496 nm, and soluble sugars are calculated according to the equation Y = 0.0029 OD - 0.323. The method of Troll and Lindsley in Pérez-Alfocea (1995) is used to measure proline content in durum wheat leaves. 100 mg of leaves are macerated in 40% methanol heated to 85°C for one hour.

1 ml of the extract is then reacted with acetic acid, ninhydrin, and a mixture of distilled water and orthophosphoric acid. The solution is boiled for 30 minutes, cooled, and toluene is added. The upper organic phase, containing proline, is recovered and its optical density (OD) is measured at 528 nm. Proline content is calculated using the equation $y = 0.913\ OD + 0.004$. The Kjeldahl method (Sáez-Plaza et al., 2013) was used to determine the nitrogen (N) content of the samples. 1 g of dry plant material was mineralized with concentrated sulfuric acid and a catalyst ($CuSO_4$ + K_2SO_4) to convert organic nitrogen into ammonium sulfate [$(NH_4)2SO_4$]. Ammonia was then released from the ammonium sulfate with sodium hydroxide and titrated with a standard solution of sodium hydroxide to quantify the nitrogen content.

Determination of physiological parameters

The leaf cell turgor was measured according to the method of Barrs (1968). Leaf segments of 1 cm are weighed fresh (FW), then placed in water for 24 hours to obtain the turgid weight (TW). They are then dried at 80°C for 48 hours and weighed dry (DW). The relative water content (RWC) is calculated using the formula RWC (%) = (FW-DW)/(TW-DW) x 100. Membrane integrity was assessed by measuring electrolyte leakage using the method of Matos et al. (2002). Leaf discs were immersed in water for 4 hours to obtain the free conductivity (EC), and then autoclaved to obtain the total conductivity (TC). The percentage of membrane integrity (CI) was calculated using the formula CI (%) = (1 - EC) / TC × 100.Chlorophyll (a) was extracted using the methods of Luo (2023), employing acetone and anhydrous ethanol. Fresh leaf material (0.1 g) was cut and placed in a graduated test tube. Then, 10 mL of a 95% ethanol and 80% acetone mixture (1:1 v/v) was added, and the mixture was incubated in the dark for 48 hours until the green leaves became colorless. A blank control was prepared using the same solvent mixture. Absorbance values were measured at 645 nm and 663 nm using a UV spectrophotometer. Chlorophyll (a) concentration was calculated using the following equations: **Chlorophyll a = (12.72 × A663 - 2.59 × A645) × V × N / W.** where V represents the extract volume, N represents the dilution factor, and W represents the fresh weight of the sample (g).

Statistical Analysis

The values obtained for the physiological and biochemical traits of the plants observed between the combinations of residual sludge doses and water stress levels were subjected to ANOVA testing. For all variables that presented significant differences, the analysis was complemented with Tukey's post-hoc tests (HSD) to perform multiple comparisons of means between residual sludge doses, water stress levels, and their combinations by groups. The statistical significance threshold was set at $p < 0.05$. All statistical operations were done using Statistica 13.0 software.

RESULTS AND DISCUSSIONS

Analysis of variance for the results related to sugar content in the plants indicated a significant genotype effect (p = 0.001) (Table 2), showing a higher accumulation for the Hedba variety with a content of 0.56±0.030 μg/g DW, compared to BD with a content of 0.51±0.039 μg/g DW (Figure 1). The application of water stress also induced a highly significant increase in sugars ($p < 0.001$) (Table 2) in the plants used. The Tukey HSD test for mean comparison indicated two groups: S2, S1 > S0. The sugar content at the S0 moisture level was 0.48±0.054 μg/g DW, while the highest content of 0.57±0.035 μg/g DW was obtained with the S2 stress level (Figure 1).

Amending the soil with residual sludge showed the same trend, with a highly significant increase in sugars ($p < 0.001$). The highest sugar content of 0.72±0.018 μg/g DW was observed with the highest sludge dose, D2, followed by D1 with a content of 0.54±0.023 μg/g DW, and finally, the control plants, D0, with a content of 0.35± 0.030 μg/g DW (Figure 1).

Regarding proline content, the statistical analysis showed a highly significant accumulation ($p < 0.001$) in the Hedba variety with a value of 0.10±0.018 μg/g DW compared to the BD variety, which had a content of 0.35±0.048 μg/g DW (Figure 1). The effect of water stress was also significant (p = 0.02) (Table 2). The proline content at the S0 stress level was

0.15±0.051 µg/g DW, while at the S2 level; it was 0.28±0.057 µg/g DW (Figure 1). The Tukey HSD test indicated two groups: S0, S1 < S1, S2.

According to the results obtained in this study, amending the soil with residual sludge significantly favored the formation and accumulation of proline ($p < 0.001$), indicating the following two groups: D2 > D1, D0. With the dose D2, a proline content of 0.34±0.052 µg/g DW was obtained, while the lowest content of 0.16±0.062 µg/g DW (Figure 1) was observed with D0. Nitrogen content in the plants showed no significant difference ($p = 0.09$) (Table 2) between the two varieties used. The Hedba variety had a slightly lower content of 0.53±0.045 µg/g DW compared to BD, which had a content of 0.56±0.059 µg/g DW (Figure 1).

Table 2. Tow-way Anova testing the effects of Sewage Sludge doses (D0 = 0 g of Sewage sludge /kg of soil, D1 = 30 g of Sewage sludge/kg of soil, D2 = 60 g de Sewage sludge /Kg of soil), and drought stress levels (S0 = 100%, S1 = 40%, et S2 = 25% of field capacity) on the variation of physiological plants parameters

Factors	df	SS	*p*-value	Sig	SS	*p*-value	Sig	SS	*p*-value	Sig
		Sugar			Proline			Nitrogen		
G	1	0.032	=0.001	**	0.792	<0.001	***	0.012	0.093	ns
S	2	0.091	<0.001	***	0.213	<0.01	**	1.054	<0.001	***
D	2	1.254	<0.001	***	0.436	<0.001	***	2.442	<0.001	***
GxS	2	0.118	<0.001	***	0.035	0.320	ns	0.002	=0.790	ns
GxD	2	0.029	<0.05	*	0.155	0.010	**	0.118	<0.001	***
SxD	4	0.148	<0.001	***	0.153	0.055	ns	0.067	<0.01	**
GxSxD	4	0.032	<0.05	*	0.262	<0.01	**	0.067	<0.01	**

df: degrees of freedom, SS: Sum of Squares, Sig: statistical significance, ***: p < 0.001, **: p < 0.01, *: p ≤ 0.05, ns: p >0.05)

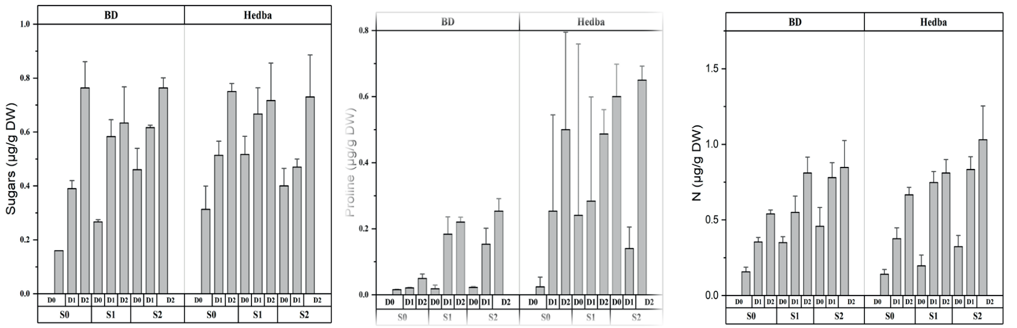

Figure 1. Bares with SD representing the variation of wheat plant biochemical parameters for different sludge amendments D0 = 0 g of Sewage sludge /kg of soil, D1 = 30 g of Sewage sludge /kg of soil, D2 = 60 g de Sewage sludge/kg of soil and drought stress treatments (100%, 40%, and 25%FC).

Contrary to the genotype effect, the application of water stress and the amendment of residual sludge induced a highly significant increase in nitrogen content ($p < 0.001$) for both factors. With water stress, the nitrogen content at the S0 level was 0.37±0.046 µg/g DW, and it increased to a higher value of 0.71±0.061 µg/g DW for S2. However, the S1 level noted a content of 0.57±0.058 µg/g DW (Figure 1).

Nitrogen content in the plants increased linearly with the increasing doses of residual sludge applied. Indeed, the highest nitrogen content of 0.78±0.040 µg/g DW (Figure 1) was observed with the highest dose, D2, while the lowest content of 0.27±0.029 µg/g DW (Figure 1) was obtained with the lowest dose, D0. The Tukey HSD test indicated three groups in ascending order: D0<D1<D2.The difference in

the accumulation of sugars and proline in the leaves of the two varieties used indicates that the Hedba variety is more resistant compared to the BD variety. This is confirmed by the strong capacity for the synthesis of both osmoticums analyzed (sugars and proline). Several authors (Chaib et al., 2015) report that the accumulation of osmoticums in the leaves of durum wheat varieties is a criterion that allows the selection of the most resistant varieties or genotypes. In our case study, the evolution of sugar and proline content follows the same trend with increasing stress levels. Our results concur with those of Chaib et al. (2015), who, by using several durum wheat genotypes subjected to multiple stress levels, obtained a similar trend in the evolution of sugars and prolines for 75% FC, 35% FC, and 25% FC. With 75% FC as an example, the highest proline content was observed with the Hedba variety, reaching 3.75 µmol/mg DW.

The more a plant accumulates sugars or proline, the better it can resist and maintain life for as long as possible under stress conditions (Akcay et al., 2023). Soluble sugars and proline are believed to play a major role in osmotic adjustment by contributing to the change in the plant's osmotic potential, enabling it to cope with adverse environmental stress conditions.

It is clear that the accumulation of sugars and proline increases with the severity of water stress, and this observation is consistent with the literature. Indeed, several authors (Bekka, 2021) report in their research a linear accumulation of sugars and proline as water stress becomes more severe. In the same vein, our results corroborate the findings of Chahbar & Belkhodja (2016), who state that the effect of water deficit translates into a marked increase in the concentration of certain constituents, which can be nitrogenous compounds, carbohydrates, or organic acids.

The accumulated proline plays a role in regulating cytoplasmic pH or serves as a nitrogen reserve for the plant. Additionally, sugars are considered an energy source that promotes various metabolic reactions within the cell (Bajji et al., 2000).

The enhanced synthesis of sugars and prolines tested in the leaves of plants under the influence of residual sludge can be attributed to the fertilizing role of this bio-solid, which acts as a source of several mineral elements, primarily nitrogen and carbon (Hao et al., 2024). Indeed, the analysis of this bio-solid in our laboratory reveals its richness in nitrogen and carbon, aligning with the findings of several authors (Souza Filho et al., 2023), who report that residual sludge is a source of nitrogen. Amending the soil with this fertilizer allows for the release of nitrogen into the soil and its subsequent incorporation into the existing nitrogenous compounds in the plant. Additionally, the applying of this fertilizer in the soil facilitates the utilization of mineral elements (nitrogen and carbon) and their integration into the formation of sugars and proline within the plant. This is supported by the observations in this study, which show that the increase in leaf nitrogen content under stress conditions is positively correlated with the increase in proline content.

Under stress conditions, plants absorb nitrogen to concentrate on proline synthesis (Mahdid & Simonneau, 2023). The decrease in RWC is significant with the increase in water stress. It appears that BD retains water in its leaves better than the Hedba variety, and this difference in behavior can be explained by the variation in leaf surface area. The larger the leaf surface, the greater the effect of water evaporation through the pores (Semida et al., 2020). Indeed, in this study, the leaf surface area of the BD variety is smaller than that of Hedba, which explains its superior water retention capacity compared to the latter (El Yamani et al., 2020).

Analysis of variance for the values obtained for leaf water content revealed a highly significant genotype, stress, and sludge amendment effect ($p<0.001$) (Table 2). The Hedba variety retained more water in its leaves 60.29±3.410% compared to BD 51.48±3.809% (Figure2). Overall, according to the results, leaf water content decreased with increasing water stress. At the control level, S0, the water reserve in the leaves was 68.68±3.716%, and it decreased to the lowest content of 45.81±3.146% with S2 (Figure 2). It appears from the obtained values that the application of residual sludge negatevely affects the water content of the plants. Indeed, it is noticeable that WRC decreases as the doses increase, ranging from 71.82±2.719% for D0 to 38.77±2.810% (Figure 2).

The statistical analysis of cell integrity in the plants revealed highly significant effects ($p < 0.001$) for genotype, water stress, and residual sludge amendment. It appears that the membrane of the BIDI variety is more resistant compared to Hedba. The integrity observed for BD 21.18±0.811%, is higher than that of Hedba, 17.66±0.691% (Figure 2).

According to the obtained averages, it is clear that the increase in water stress leads to a loss and a decrease in cell integrity. The Tukey HSD test indicated two groups: S0 with a value of (21.22±0.930%) followed by a second group, S2 (18.66±0.709%), and S1 (18.38±1.223%). The amendment of residual sludge showed the same trend as water stress, with a decrease in integrity as the amendment doses increased. The value obtained for D0 22.83±0.833%, decreased to 15.61±0.583% for the highest sludge dose, while D1 had a value of 19,83±0.755%. The following groups were obtained: D0>D1>D2.

The evolution of chlorophyll a content was significant ($p = 0.0003$) (Table 3) between the two varieties used in this study. Hedba revealed chlorophyll a content of 0.86±0.066 mg/g DW, while BIDI noted a lower content of 0.69±0.565 mg/g DW (Figure2). Water stress induced a significant increase in the pigment ($p < 0.0001$) (Table 3). The comparison of means showed two groups: S1, S2 > S0. At the S0 level, the content was 0.64±0.059 mg/g DW, while the highest content of 0.88±0.700 mg/g DW (Figure 2) was observed at the S2 level. The variance analysis of the results obtained for the amendment of residual sludge indicated a highly significant effect ($p < 0.001$), showing an improvement in chlorophyll a content from 0.48±0.037 mg/g DW for D0 to 1.00±0.089 mg/g DW for S2 (Figure 2).

The decrease in RWC of plants under water stress conditions was correlated with the increase in water stress levels. This observation is entirely logical; the less irrigation the soil receives, the less water will be available to the plants (Ahmad et al., 2023). Several authors who report similar observations further support these results. Regarding the effect of residual sludge, it is evident that it negatively influences plant water restoration. This effect can be attributed to the role of this amendment as an adsorbent matrix that retains water in its interstices, limiting the water passage to the plants (Głąb et al., 2020), leading to a decrease in leaf water content. Contrary to our findings, several studies (Benalioua, 2023) indicate that residual sludge improves plant water reserves. These authors report that plants amended with residual sludge exhibit a significantly higher relative water content compared to control plants because this organic fertilizer establishes sufficient moisture in the soil and enhances its water-holding capacity (Delibacak et al., 2020). Boudjabi & Chenchouni (2021) also reported an improvement in soil water retention after amending the soil with residual sludge.

The accumulation and synthesis of proline and sugars in the plant require the utilization and integration of water molecules, leading to an increase in their concentration and a consequent elevation in osmotic potential. In contrast, there is a decrease in leaf water content. The Hedba variety exhibits higher cell membrane integrity compared to the BD variety. This observation confirms that Hedba is more resistant than BD. Furthermore, according to the observed results, it is evident that the synthesis of proline and sugars is more pronounced in Hedba, and these elements, one based on nitrogen (proline) and the other on carbon (sugars), play a role in the formation of the cell membrane, contributing to its resistance to water stress (Khan et al., 2020). In the same vein, it is worth mentioning that the accumulation of osmolytes, particularly proline, glycine betaine, and soluble sugars, can protect membranes from desiccation (Abasi et al., 2024). Sugars contribute to maintaining phosphorylation reactions and energy production (Loretti et al., 2001). They protect enzyme synthesis processes, which could enhance plant tolerance to drought (Ahmad et al., 2021). However, it is observed that cell membrane integrity decreases with increasing water stress. This finding corroborates the existing literature and the findings of several authors (Harsha et al., 2024), who mention a loss of cell membrane integrity under severe water stress. As previously noted for WRC, the amendment of residual sludge limits water passage to the plants, and this effect negatively influences the synthesis of essential components for all the biochemical reactions necessary for cell membrane synthesis

(Quilambo, 2004). It is worth mentioning that this decrease becomes more pronounced with increasing doses of residual sludge applied to the soil. Indeed, as the doses increase, water retention intensifies, and plants are less able to restore essential elements for biochemical reactions. Valentovič et al. (2006) reported that the application of water stress on two maize varieties for 24 hours, induced by 0.3 M sorbitol (-1.4 MPa), resulted in cell membrane damage, which they attributed to the effect of lipid peroxidation observed in their experiment. Stressed plants responded by increasing the amount of chlorophyll (a).

Table 3. Tow-way Anova testing the effects of Sewage Sludge doses (D0 = 0 g of Sewage sludge/kg of soil, D1 = 30 g of Sewage sludge/kg of soil, D2 = 60 g of Sewage sludge/kg of soil), and drought stress levels (S0 = 100%, S1 = 40%, S2 = 25% of field capacity) on the variation of biochemical plants parameters

Factors	Df	SS	*p*-value	Sig	SS	*p*-value	Sig	SS	*p*-value	Sig
		WRC			CI			Chlo (a)		
G	2	1045.9	<0.001	***	167.13	<0.001	***	0.391	<0.001	***
S	2	4707.7	<0.001	***	87.81	<0.001	***	0.565	<0.001	***
D	2	9866.5	<0.001	***	473.93	<0.001	***	2.520	<0.001	***
G×S	4	468.7	<0.01	**	25.15	<0.05	*	0.319	<0.01	**
G×D	4	228.5	0.071	ns	13.48	0.180	Ns	0.086	0.192	Ns
S×D	4	383.4	0.069	ns	31.63	0.100	ns	0.615	<0.001	***
G×S×D	8	1053.2	<0.001	***	3074	0.108	ns	0.729	<0.001	***

df: degrees of freedom, SS: Sum of Squares, Sig: statistical significance, ***: $p < 0.001$, **: $p < 0.01$, *: $p \leq 0.05$, ^{ns}: $p > 0.05$)

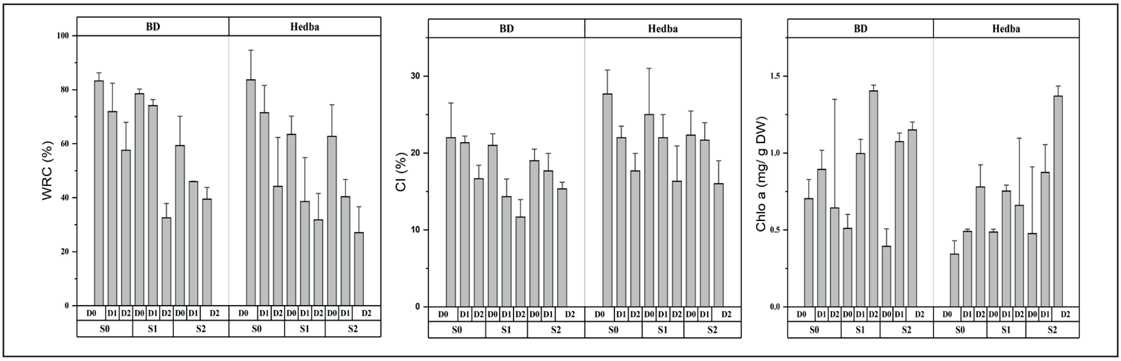

Figure 2. Bares with SD representing the variation of wheat plant physiological parameters for different sludge amendments (D0 = 0 g of Sewage sludge/kg of soil, D1 = 30 g of Sewage sludge/kg of soil, D2 = 60 g de Sewage sludge/kg of soiland drought stress treatments (100%, 40%, and 25% FC).

This increase is linked to a rise in nitrogen content in the leaves, as this mineral is an essential element for the formation of this pigment.

According to the results, it appears that nitrogen content increases linearly with the level of stress. As previously noted, amending the soil with sludge is an effective source of nitrogen for the plant. This bio-solid releases all forms of nitrogen into the soil, and once this mineral is taken up by the plant, it is incorporated into the synthesis of chlorophyll a (Kaya et al., 2024). Contrary to our findings, Gai et al. (2024) obtained a decrease in chlorophyll content in water-stressed sugarcane plants.

CONCLUSIONS

In conclusion, amending the soil with low doses of residual sludge in combination with water stress enables the plant to counter the effects of stress through a good accumulation of sugars, proline, and nitrogen. This observation is confirmed by the green color of the plant leaves, indicating a high content of chlorophyll (a), an essential pigment in energy reactions required by the plant. However, further experimentation with additional precautions is warranted. It is crucial to diagnose the heavy metal content in residual sludge to thoroughly understand and control the effects of this bio-solid amendment on both the soil and the plant.

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