



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Alleviation of salinity stress effects on agro-physiological traits of wheat by auxin, glycine betaine, and soil additives

Rania A. Khedr^a, Sobhy Gh. R. Sorour^b, Saad H. Aboukhadrah^b, Neveen M. El Shafey^b, Hassan E. Abd Elsalam^c, Mohamed E. El-Sharnouby^d, Amira M. El-Tahan^{e,*}^a Crops Physiology Research Department, Field Crops Research Institute, ARC, Egypt^b Agronomy Department, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt^c Department of Food Science and Nutrition, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia^d Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia^e Plant Production Department, Arid Lands Cultivation Research Institute, The City of Scientific Research and Technological Applications, SRTA-City, Borg El Arab, Alexandria, Egypt

ARTICLE INFO

Article history:

Received 14 July 2021

Revised 6 September 2021

Accepted 9 September 2021

Available online 20 September 2021

Keywords:

Salinity

Wheat

Molasses

Humic acid

Naphthalene acetic acid

Glycine betaine

ABSTRACT

Soil salinity is a major constraint to wheat production; it causes a severe reduction in wheat growth and yield. Alleviation of salinity effects on physiological, biochemical, and yield of wheat cultivars; Sids 14 and Misr 3 using some soil additions (control, Molasses and Humic acid), compatible solutes, and growth regulators (water as control, Naphthalene acetic acid, and Glycine betaine) were investigated in salt-affected soils. Results indicated that Misr 3 was superior to Sids 14 in all studied characteristics except flag leaf area, relative water content, plant height and recorded lower and desirable value of leaf temperature. The addition of Molasses (24 L ha⁻¹) or Humic acid (12 L ha⁻¹) significantly increased physiological and biochemical characteristics. At the same time, flag leaf temperature, proline, and malondialdehyde (MDA) content were decreased, yield and its attributes also increased except No. kernel spike-1. Foliar spray of Naphthalene acetic acid (NAA) at 30 mg L⁻¹ or glycine betaine (GB 100 mM) also positively affected the studied characteristics, where Glycine betaine recorded the highest Relative water content and Fv/Fm. In contrast, NAA recorded the most increased Catalase (CAT) activity, and the Number of spikes m⁻² and insignificant differences were observed between them in grain yield. It could be recommended the cultivation of Misr 3 with Molasses and GB under saline soils.

© 2021 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Salinity is a significant problem worldwide, and Egypt is one of the countries that is particularly affected Al-Naggar et al. (2015). Wheat (*Triticum aestivum* L.) is a major cereal crop with a global yield of more than 8.8 million tons FAO (2020). Egypt's wheat yield is insufficient for human use. To reduce the gap between consumption and production, wheat growing was expanded to newly reclaimed fields. However, salinity stress (which is expected in the Egyptian North) significantly impacts wheat growth and yield,

and yields might drop dramatically, making crop farming unprofitable. (Mujeeb-Kazi et al., 2019; Zeeshan et al., 2020). Plants change morphologically, physiologically, biochemically, and molecularly due to salinity Abd El-Hamid et al. (2020). Different tactics for maximizing plant development could help to mitigate these effects. Molasses or humic acid additives to the soil are one of these ways. Sugar beets are used in this recipe. Molasses enhances nitrogen absorption efficiency and soil biological activity in agriculture. Humic, fulvic, and amino acids are present in various concentrations Samavat and Samavat (2014).

Molasses increased wheat chlorophyll a and b, according to Kaushik et al. (1994). Under varied salt levels, green bean plants sprayed with molasses had better vegetative development, chlorophyll content, relative water content, enzymatic activity, proline concentrations, plant height, and leaf area Abdelmotib et al. (2019). Betaine was first discovered in sugar beet juice at a rate of 100 mmol kg⁻¹ plant tissue Abdelmotib et al. (2019). It was the most abundant nitrogenous compound found in molasses with

* Corresponding author.

E-mail address: aeltahan@srtacity.sci.eg (A.M. El-Tahan).

Peer review under responsibility of King Saud University.



an average of 3–4% El-Geddawy et al. (2012) for protection against osmotic stresses, drought, high salinity, and high temperature.

Numerous researchers have identified multiple benefits of humic acid for plants in salt-affected soils. Tahir et al. (2012) discovered that humic acid treatments improved wheat development under salinity. Humic acid may have induced an increase in chlorophyll synthesis or delayed chlorophyll degradation, according to El-Bassiouny et al. (2014). Phytohormone concentrations in plant tissue are inhibited by salt stress Egamberdieva et al. (2017). Exogenous phytohormone treatment is an essential method for increasing plant tolerance (Javid et al., 2011; Iqbal et al., 2012). The use of Naphthalene acetic acid (NAA) resulted in an increase in chlorophyll content, plant height, and flag leaf area Abed Jeber and Khaeim (2019), and spike length, number of grains spike⁻¹ and 1000 kernel weight Jahan et al. (2019). Exogenous spraying with indole-3-acetic acid (IAA) increased leaf area and pigment content while decreasing proline content (Akbari et al., 2007; Zoubida and Gherroucha, 2017). Spraying wheat plants with IAA grew water content but had no influence on proline content, according to Abd El-Samad (2013).

Glycine betaine is also used as an osmoregulator chemical for optimal plant growth under salinity stress. It regulates osmotic pressure within the plant, allowing it to absorb water, and it also has essential roles in a variety of plant activities (Dawood, 2016; Roychoudhury and Banerjee, 2016). As a result, glycine betaine (GB) reduced proline and lipid peroxidation (MDA) in plants, alleviating salt stress (Salama et al., 2015; Dawood, 2016). It also increased leaf water potential, increased the enzyme antioxidant catalase (CAT) activity, and improved growth and production Raza et al. (2014).

This study aimed to minimize the harmful effects of salt stress on wheat by using limited, widely available fertilizers and foliar spraying treatments to improve wheat growth and yield under salinity stress.

2. Materials and methods

2.1. Experimental locations

Two-year field research was carried at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt, during the winter seasons 2018/2019 and 2019/2020. The site is at 31°0–07' N latitude, 30°0–57' E longitude, and around 6 m above mean sea level. The response of two wheat cultivars to three soil addition treatments and three foliar spray treatments under saline soil conditions was studied in this area, representing the circumstances in Egypt's North-Middle Nile Delta region. Rice (*Oryza Sativa*, L) was the previous crop in both seasons.

2.2. Environmental data

The soil analysis was performed at the Laboratory of Water Requirements and Field Irrigation Research Institute, Sakha Agricultural Research Station, according to Jackson (1973). The soil type was clay, and EC mean values were ranged from 9.40 to 7.90 dsm⁻¹, while pH values were ranged from 7.96 to 7.65 at 0–30 and 30–60 cm depth in the two seasons, respectively. According to the Sakha meteorological station, average minimum temperatures in the studied area in the North Middle Nile Delta region at Kafr El-Sheikh Governorate during the two growing winter seasons 2018/2019 and 2019/2020 were 15.6 and 15.0 °C, while average maximum temperatures were 28.7 and 27.8 °C, respectively, during the two growing winter seasons 2018/2019 and 2019/2020. In addition, rainfall totaled 71.34 and 200.10 mm in the first and

second seasons, respectively, with relative humidity averages of 57.73 and 53.68 percent.

2.3. Wheat cultivars

Two wheat cultivars; Sids14 and Misr 3 were kindly provided by Wheat Research Depart., Field Crops Research Institute, Agricultural Research Center, Egypt. The name and pedigree of these cultivars are presented in Table 1.

2.4. The experiment design

The experiments were laid out in a split-split plot design with four replications. The main plots were devoted to the two wheat cultivars; Sids 14 and Misr 3, the subplots were assigned to soil addition treatments (Control, sugar beet Molasses at the rate of 24 L ha⁻¹ and Humic Acid at the rate of 12 L ha⁻¹) and the sub-subplots were allocated to different foliar spray treatments (water as control, Naphthalene acetic acid as auxin (NAA) at the concentration of 30 mg L⁻¹ and glycine betaine (GB) at the concentration of 100 mM). The sub-sub plot area was 4.2 m², including six rows 3.5 m long and 20 cm apart (1.2 × 3.5 m). Sowing was carried out on 27th and 30th November in 2018/2019 and 2019/2020, respectively. Molasses was provided by Delta Sugar Factory, Kafr El-sheikh governorate, Egypt. Soil addition treatments (Molasses and Humic acid) were distributed to the soil between the rows of wheat plants using a sprinkler before the second irrigation. The analysis of Molasses and Humic acid is presented in Tables 2 and 3. The foliar application treatments (water as control, Naphthalene acetic acid, and glycine betaine) were sprayed two times (30 and 45 days after sowing). All cultural practices were recommended for wheat crops in the region to obtain healthy plants and better yield.

2.5. Yield and growth components

At heading, flag leaves of ten plants were randomly taken from each sub-sub-plot to estimate physiological and biochemical characteristics as follows:

2.5.1. Physiological characteristics

Flag leaf area in cm² was measured using Area Meter (L1-Cor, Model L1 3000A).

Specific leaf weight (SLW) in g cm⁻² was calculated according to Pearce et al. (1968) using the formula:

$$SLW = \frac{\text{leaf dry weight(g)}}{\text{leaf area(cm}^2\text{)}}$$

Relative water content (RWC %) was calculated based on Gonzalez and Gonzalez (2001) using the formula:

$$RWC = \frac{Fw - Dw}{Tw - Dw}$$

where: FW = the sample fresh weight, TW = the turgid weight & DW = the dry weight.

Leaf chlorophyll fluorescence was measured using chlorophyll fluorometer (OS-30, opti sciences, inc. The USA) to calculate the

Table 1
Names and pedigrees of the studied wheat cultivars.

Cultivar	Pedigree
Sids 14	Bow's's'/Vee's's'//Bow's'/Tsi/3/BAN
Misr 3	ATTILA*2/PBW65*2/KACHU

Table 2
Chemical analysis of Molasses and Humic acid.

Compound	Moisture %	Crud protein %	Ash %	Fat %	Total Carbohydrates %
Molasses	22.10	6.6	10.52	0	60.76
Humic acid	18.48	4.1	8.1	0	69.3

Table 3
Molasses and humic acid concentrations of amino acids (g ml⁻¹).

Compound	Aspartic	Glutamic	Serine	Histidine	Glycine	Threonine	Arginine	Alanine	Valine	Tyrosine	Tryptophan	Phenylalanine	IsoLeucine	Leucine	Lysine	Proline
Molasses	159.76	38.82	3.20	2.75	0.78	113.83	2.38	20.40	5.58	122.12	4.14	5.29	12.73	1.32	38.84	2.70
Humic Acid	150.17	22.34	30.83	0.31	1.62	40.81	2.28	13.50	2.04	30.66	1.97	1.50	6.67	0.96	17.74	0.90

Analyzed at National Research Center – Central Laboratories, Network- Chromatographic Lab.

maximum quantum yield of photo-system II (PSII) by the formula of Maxwell and Johnson (2000) as follow:

$$\frac{Fv}{Fm} = \frac{Fm - Fo}{Fm}$$

where: Fv/Fm = the maximal quantum efficiency of PSII (MQE), Fm = the maximal chlorophyll fluorescence, and Fo = minimum chlorophyll fluorescence (in the dark).

Leaf temperature (°C): During the mid-day time, and in the absence of cloud cover, a portable steady state the promoter (LI-COR model LI- 1600) was used to measure leaf temperature on a central portion of fully extended flag leaves from three randomly selected plants in each plot.

2.5.2. Biochemical characteristics

Chlorophyll content (Chll a and b) in µg ml⁻¹ was determined by using the Spectro-Photometer apparatus as described by Moran (1982) according to the equation:

$$Chlla = 12.64A664 + 2.99A647$$

$$Chllb = -5.6A664 + 23.26A647$$

Proline content of leaves (mg g⁻¹FW) was determined according to Bates et al. (1973).

MDA (µmol g⁻¹ FW.), the level of lipid peroxidation in the plant as malondialdehyde, was estimated according to Heath and Packer (1968) and expressed as MDA formed using an extinction coefficient of 155 mM cm⁻¹.

Catalase activity (CAT) in µmol min⁻¹ g protein⁻¹ was estimated according to Lum et al. (2014).

2.5.3. Yield And it's attributes

At harvest, two square meters were randomly selected from each sub-sub plot to determine Plant height (cm), No. Kernel spike-1, No. Spikes m-2, 1000-Kernel weight (g) (1000-k wt.), and Grain yield (t ha⁻¹).

2.6. Statistical analysis

According to Gomez and Gomez (1984), the gathered data were subjected to analysis of variance and combined analysis throughout the two seasons. Duncan's Multiple Range Test was used to compare treatment means Duncan (1955). All statistical analysis was carried out with the help of the "MSTATC" computer software program, which used the analysis of variance technique.

3. Results

3.1. Physiological and biochemical characteristics

Data in Table 4 show that Sids 14 had higher flag leaf area and RWC % (45.34 cm² and 76.44%) than Misr 3 (40.89 cm² and 73.84 %) for flag leaf area and RWC, respectively. Also, it had a lower and desirable flag leaf temperature (27.14 °c), while Misr 3 had higher values of Fv/Fm and SLW (0.728 and 0.025 g cm⁻²) than Sids 14 (0.709 and 0.21 g cm⁻²).

Concerning soil addition treatments, data in Table 4 clearly showed that both Molasses and Humic acid significantly increased physiological characteristics compared to the control treatment with insignificant differences between the two treatments in RWC, flag leaf area, SLW, and flag leaf temperature. In contrast, the addition of Molasses recorded the highest efficiency of photosynthesis (Fv/Fm) (0.730) and ranked first.

Regarding foliar spray treatments, spraying wheat plants with NAA or GB caused significant increases in RWC %, flag leaf area, SLW, Fv/Fm, and significantly decreased flag leaf temperature compared to untreated plants (control treatment). There were insignificant differences between the two treatments in flag leaf area, SLW, and flag leaf temperature. Still, GB recorded the highest RWC (77.02 %) and Fv/Fm (0.723), followed by NAA (75.38% and 0.721) for RWC and Fv/Fm respectively.

Concerning biochemical characteristics, Table 5 showed that the cultivar Misr 3 gave higher values of chll a (9.90 µg ml⁻¹), chll b (2.96 µg ml⁻¹), proline content (0.390 mg g⁻¹FW), and CAT activity (2.49 µmol min⁻¹g protein⁻¹) than Sids 14. The lowest value of lipid peroxidation product as MDA content (248.0 µmols g⁻¹ FW) was obtained from Misr3.

Soil addition treatments significantly increased chlorophyll content (chll a and b) and activity of antioxidant enzyme CAT, where Molasses recorded the highest concentration of both chll b (3.008 µg ml⁻¹) and CAT (2.39 µmol min⁻¹g protein), followed by HA, which gave 2.819 µg ml⁻¹ for chll b and 2.19 µmol min⁻¹ g protein for CAT. On the contrary, soil additions caused a significant decrease in Proline content and MDA compared with control treatments. There were insignificant differences between Molasses and Humic acid in chll a and proline content.

Also, using NAA or GB as a foliar spray led to a significant increase in chll a and chll b, with insignificant differences. While the inverse was true in flag leaves, the content of Proline and MDA where the significant decrease was observed in the two traits with using NAA or GB compared with control treatment.

Table 4

Flag leaf area (cm²), SLW (g cm⁻²), RWC (%), Fv/Fm and flag leaf temperature (°C) of the two wheat cultivars as affected by soil additions and spraying treatments in combined analysis for 2018/2019 and 2019/2020 seasons.

Factors	Flag leaf area (cm ²)	SLW (g cm ⁻²)	RWC (%)	Fv/Fm	Flag leaf temperature (°C)
Cultivars (C)	**	*	*	**	*
Sids14	45.34	0.021	76.44	0.709	27.14
Misir 3	40.89	0.025	73.84	0.728	27.6
Soil addition (A)	**	**	*	**	*
Control	37.99b	0.019b	71.97b	0.706c	27.73 a
Molasses	46.69 a	0.025 a	77.61 a	0.730 a	27.11b
Humic acid	44.67 a	0.024 a	75.84 a	0.718b	27.26b
Foliar spray treatments (F)	**	**	**	**	**
Control	39.46b	0.022b	73.01c	0.710c	27.55 a
NAA	45.72 a	0.024 a	75.38b	0.721b	27.25b
GB	44.16 a	0.023 a	77.02 a	0.723 a	27.30b

Where SLW: specific leaf weight and RWC: relative water content. *, ** and NS indicate P < 0.05, P < 0.01, and not significant, respectively. Means within the same column for each factor designated by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

Table 5

Overall mean values of some biochemical characteristics of the two wheat cultivars as affected by soil additions and foliar spraying in combined analysis for 2018/2019 and 2019/2020 seasons.

Factors	chl a (μg ml ⁻¹)	chl b (μg ml ⁻¹)	Proline (mg g ⁻¹ FW)	MDA (μmols g ⁻¹ FW)	CAT (μmolmin ⁻¹ g protein ⁻¹)
Cultivars (C)	*	*	**	*	**
Sids14	9.16 b	2.69 b	0.299 b	312.1 a	1.74 b
Misir 3	9.90 a	2.96 a	0.390 a	248.0b	2.49 a
Soil addition (A)	**	**	*	**	**
Control	8.70 b	2.651 c	0.399 a	334.1 a	1.77 c
Molasses	10.37 a	3.008 a	0.297b	243.6 b	2.39 a
Humic acid	9.51 ab	2.819 b	0.338 ab	262.5 b	2.19 b
Foliar spray treatments (F)	*	*	**	*	**
Control	8.90 b	2.64 b	0.382 a	288.2 a	1.94 c
NAA	9.90 a	2.89 a	0.341 b	275.9 b	2.26 a
GB	9.78 a	2.94 a	0.310 c	276.0 b	2.15 b

Chl a: chlorophyll a, chl b: chlorophyll b, MDA: malondialdehyde, CAT: catalase. *, ** and NS indicate P < 0.05, P < 0.01, and not significant, respectively. Means within the same column for each factor designated by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

3.2. Plant height and yield and its attributes

Plant height (cm), No. kernel spike⁻¹, No. spikes m⁻², 1000 k. wt (g). and grain yield (t ha⁻¹) as affected by the two wheat cultivars, three soil additions, and three spray treatments are presented in Table 6.

Data in Table 6 indicated that Misr 3 was significantly superior Sids 14 in No. spikes m⁻², 1000 k. wt. (g) and grain yield (t ha⁻¹), while Sids 14 was significantly taller (98.12 cm) than Misr 3 (89.36 cm). On the other side, there were insignificant differences between them in No. kernel spike⁻¹.

Concerning soil addition treatments, results indicated a significant increase in plant height, No. spikes m⁻², 1000 k wt., and grain yield, while an insignificant increase in No. kernel spike⁻¹ was

observed. In general addition of Molasses caused highly significant increases in all traits and consequently in grain yield followed by Humic acid compared to control treatment.

Regarding spray treatment, data indicated that spraying with NAA or GB significantly increased plant height (cm), No. spikes m², 1000kwt. (g) and grain yield (t ha⁻¹), but there was an insignificant increase in No. kernel spike⁻¹. Also, minor differences were observed between NAA and GB in plant height, 1000 k wt (g), and grain yield (t ha⁻¹).

3.3. Interaction effect

Data in Fig. 1 indicated that Misr 3 with Molasses as a soil addition had the highest values of efficiency of photosynthesis

Table 6

Overall mean values of plant height and grain yield and the characteristics of its components of two wheat cultivars as affected by soil additions treatments and foliar spray treatments in combined analysis for 2018/2019 and 2019/2020 seasons.

Factors	Plant height (cm)	No. spike m ⁻²	No. kernel spike ⁻¹	1000 - k. wt. (g)	Grain yield t ha ⁻¹
Cultivars (C)	*	**	NS	**	**
Sids14	98.12 a	389.3 b	55.85	41.04 b	4.31 b
Misir 3	89.36 b	428.9 a	58.02	43.06 a	5.45 a
Soil addition (A)	*	*	NS	**	**
Control	89.01 b	359.9 c	55.47	40.57 b	4.24 c
Molasses	97.03 a	449.5 a	58.04	43.07 a	5.50 a
Humic acid	95.18 a	418.0 b	57.3	42.51 a	4.88 b
Foliar spray treatments (F)	*	**	NS	**	**
Control	92.05 b	382.0 c	55.26	41.18 b	4.42 b
NAA	94.87 a	428.6 a	57.66	42.45 a	5.07 a
GB	94.31 a	416.8 b	57.9	42.52 a	5.13 a

*** and NS indicate P < 0.05, P < 0.01, and not significant, respectively. Means within the same column for each factor designated by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

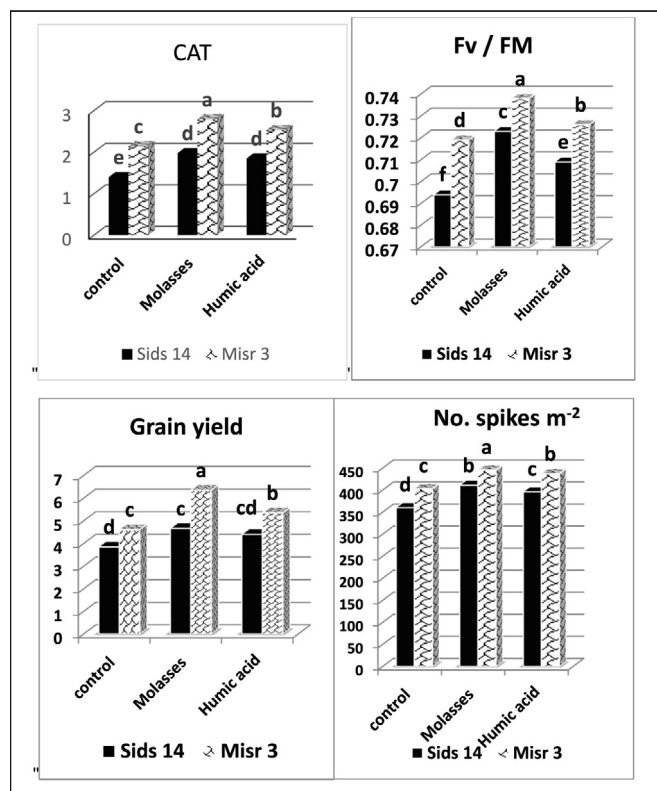


Fig. 1. Interaction effect between cultivars and soil addition treatments on Fv/Fm, CAT activity, No. spikes m^{-2} and grain yield.

(Fv/Fm) (0.738), CAT activity ($2.79 \mu\text{molmin}^{-1}\text{g protein}^{-1}$), No. spikes m^{-2} (445.7), and grain yield (6.35 t ha^{-1}) while the lowest were obtained from Sids 14 without soil addition (control treatment).

Presented data in Fig. 2 revealed that the highest values of flag leaf area (48.28 cm^2) and RWC % (79.83 %), chl a ($11.08 \mu\text{g ml}^{-1}$), No. spikes m^{-2} (463.8) and grain yield (5.97 t ha^{-1}), were obtained from Molasses as a soil addition and GB as a foliar application treatment.

4. Discussion

Saline soils limit plant growth due to osmotic stress, ionic toxicity, and reduced ability to take up essential minerals Zeeshan et al. (2020), affecting different physiological and biochemical processes related to plant growth and development. These effects could be reduced by soil additions of Molasses or Humic acid. Molasses (Sugar beet extract) is a by-product of the sugar manufacturing from sugar beets; it is a cheap and available material; sugar beet extract contains a considerable amount of GB and a range of other essential nutrients Abdelmotlb et al. (2019). Humic acid is widely used as a soil conditioner and growth regulator Kenawy and Mona (2017).

NAA is a vital growth regulator that decreased under salinity stress, causing severe plant growth and yield defect. The exogenous application of NAA is a crucial way to increase plant tolerance Iqbal et al. (2012).

GB stands for quaternary ammonium compounds, which are organic compatible solutes that assist plants in coping with osmotic stress induced by drought, excessive salinity, or high warmth. Exogenous application of GB has been proposed to promote crop growth and yield in salt-stressed environments.

Flag Leaf is one of the essential parts of the plant; it significantly contributes to the grain filling; flag leaf area is inhibited by salinity

Chamekh et al. (2014). In comparison to control treatments, adding molasses or humic acid-enhanced flag leaf area and SLW significantly. These results are very similar to those reported by Abdelmotlb et al. (2019).

The optimum criterion for representing plant water status was presented as relative water content (RWC). By reducing the water potential within the cell, high salt concentrations create osmotic stress. RWC is a dehydration tolerance to salinity stress index Abd El-Hamid et al. (2020). RWC % decreased as a result of salinity stress (Farhat et al., 2020). Molasses treatment increased significantly RWC, which means that it helps plant tissues to improve their water content. These results are in good agreement with those reported by (Kaushik et al., 1994; Abdelmotlb et al., 2019).

PSII's photochemical efficiency is reflected in the Fv/Fm ratio. Because of the disordering of chloroplast integrity, NaCl stress can disrupt the photosynthesis process, decreasing the effectiveness of the two photosystems Ibrahim et al. (2015). The efficiency of photosynthesis was increased either with the addition of Molasses or Humic compared with the control treatment. These treatments help keep leaf water contents, which represent one of the main elements of the photosynthesis process.

Leaf temperature could be used as an indicator for plant water status and transpiration. The decrease in leaf potential and relative water content is associated with a rise in leaf temperature Zada et al. (2020) due to less leaf transpiration.

It's clear that both soil addition Molasses and Humic acid treatments significantly increased flag leaf area, SLW, RWC %, and Fv/Fm and caused a desirable decrease in flag leaf temperature compared to control treatments. These positive effects of Molasses and Humic acid may be due to their chemical composition and the contents of amino acids Tables 2 and 3. Where amino acids have a huge role in stimulating the physiological and biochemical processes, they have critical roles in many metabolic processes, including nitrogen assimilation pathways Marschner (2011) which plays a vital role in protein synthesis and chlorophyll formation, thereby increases leaf area production and photosynthesis efficiency; also, Molasses stimulates nutrient elements uptake efficiency and activity of soil biology, and that gave good growth for wheat plants.

The chlorophyll content of leaves is an indicator of the photosynthetic potency of plant tissues. Chlorophyll content (a and b) was decreased under salt stress (Abd El-Hamid et al., 2020; Farhat et al., 2020). Soil addition treatments caused a significant increase in chlorophyll content (a and b). These results are corroborated with the findings of those obtained by Abedmotlb et al. (2019), who reported that Molasses addition increased chlorophyll content, Humic acid, according to El-Bassiouny et al. (2014), may produce an increase in chlorophyll synthesis or delay chlorophyll degradation. Higher chlorophyll levels caused by soil addition treatments could also be due to the stimulating effects of amino acids on chlorophyll biosynthesis and a reduction in chlorophyll degradation, where it can likely act against peroxidation and degradation of cell components, particularly chlorophylls, and thus extend cell life. These results are consistent with Noroozlo et al. (2019).

Proline is an osmotic adjustment component, and it is a typical response in plants exposed to salt stress Abd El-Hamid et al. (2020).

Salinity also causes reactive oxygen species (ROS) production in plant cells, which is toxic to plants and causes oxidative damage that leads to cell death Caverzan et al. (2016). Lipid peroxidation is induced by these ROS, resulting in enhanced membrane fluidity and permeability. Malondialdehyde (MDA), a natural result of oxidation of polyunsaturated fatty acids in the membrane produced by the accumulation of oxidants, was measured and expressed as lipid peroxidation (ROS). MDA levels were higher in the (control

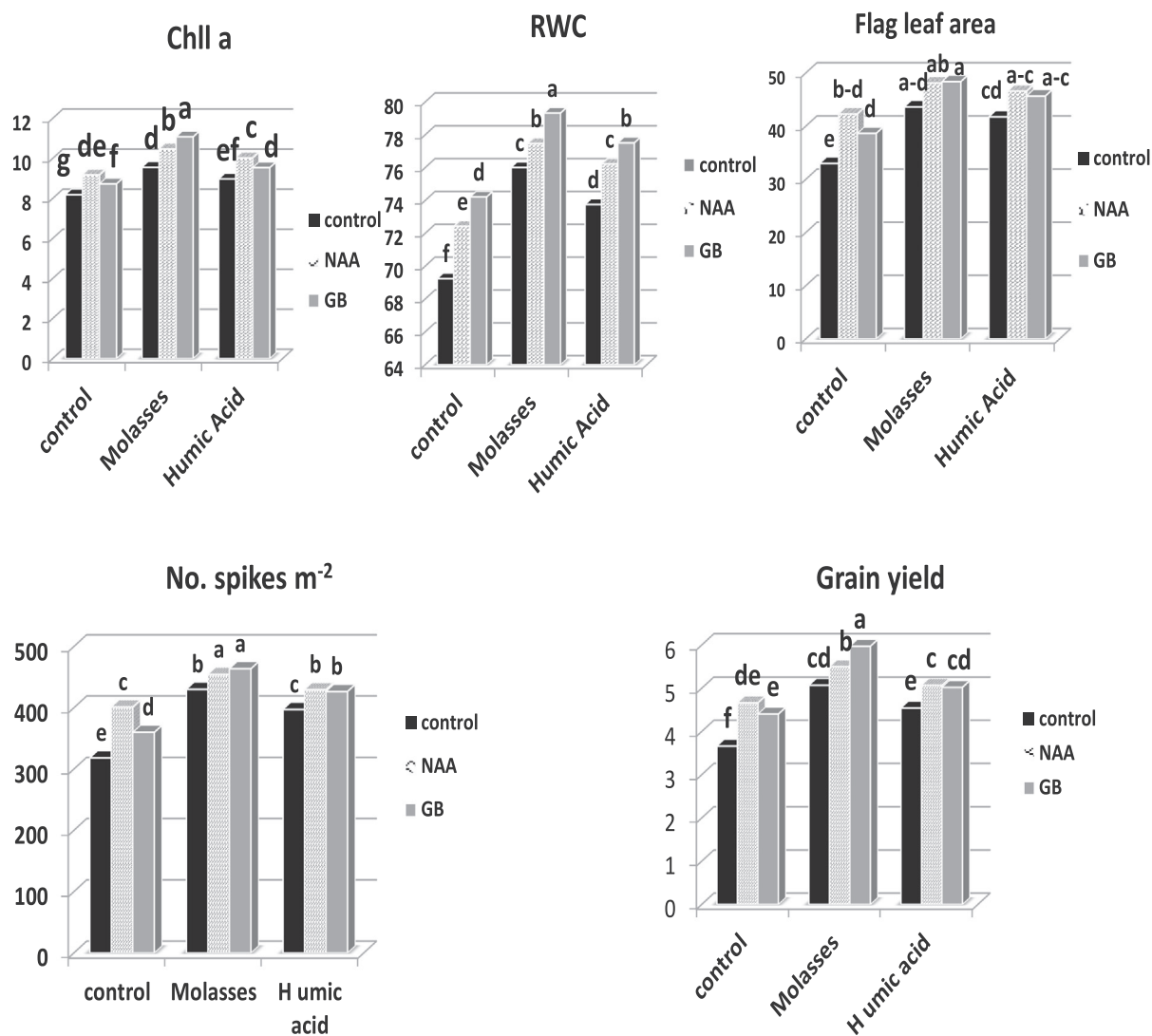


Fig. 2. Effect of the interaction between soil addition and foliar spray treatments on flag leaf area, RWC, chl a, No. spikes m⁻² and grain yield.

treatment), whereas Molasses and Humic significantly reduced them. Molasses treatment resulted in a significant reduction in proline and MDA content compared to control treatments; these reductions may be attributed to the involvement of soil addition treatments in minimizing the harmful effects of soil salinity on wheat plants compared to untreated plants. These results are consistent with those of (Kaushik et al., 1994; Abdelmotlb et al., 2019). Humic acid also reduces proline and MDA levels, which helps plants cope with salt stress. (Salama et al., 2015; Dawood, 2016; Roychoudhury and Banerjee, 2016). These data are compatible with those of Kenawy and Mona (2017), who found that adding Humic acid to saline soils decreased the electrical conductivity of the soil (EC).

Catalase is a major antioxidant enzyme that protects cells against oxidative stress by scavenging ROS intracellular Zeeshan et al. (2020). CAT activity was increased significantly with soil addition treatments compared with the control treatment. This increase caused good plant growth and decreased ROS's adverse effects on cells under salinity stress by converting harmful free radicals into harmless compounds. The CAT enzyme converts H₂O₂ into H₂O and O₂.

Foliar spraying treatments of GB decreased proline and MDA content compared to control treatment. These results are in har-

mony with those obtained by (Salama et al., 2015; Dawood, 2016), where GB plays a vital role in the stabilization and protection of cell membranes and proteins during stress Khan et al. (2015).

Humic acid treatments improve wheat development by unavailable chelating nutrients, buffering soil pH, and enhancing macronutrient uptake Tahir et al. (2012). Humic substances also help plants cope with the detrimental effects of salt by limiting the uptake of toxic components Kenawy and Mona (2017). Humic, Fulvic, and amino acids are also available in different levels in molasses (Samavat and Samavat, 2014). Protein hydrolysates and amino acids (in Molasses and Humic acid) improve plant salt tolerance and nitrogen uptake, resulting in higher yields (Calvo et al., 2014; Colla et al., 2014; Kenawy and Mona, 2017). GB reduces the accumulation of Na and increases the accumulation of K and Ca₂ in wheat plants which improves leaf water potential, increases the activities of enzymatic antioxidant CAT Raza et al. (2014) as well as enhancing growth and yield; the obtained results are in agreement with those obtained by (Abd El-Samad, 2013, and Zoubida and Gherroucha, 2017).

Phytohormones, such as auxin, are known to regulate plant responses to salt stress and mitigate the stress's adverse effects (Javid et al., 2011). NAA increased chlorophyll, plant height, area

of the flag leaf, and 1000 grain weight; these results in a good agreement (Abed Jeber and Khaeim, 2019; Jahan et al., 2019).

Concerning the interaction effect, it's clear that using both Molasses and GB gave the highest values of photosynthetic pigments, yield and its attributes, lower MDA and proline content, and that may be due to the effect of betaine in sugar beet juice Abdelmotlb et al. (2019), which is considered as an organic osmolyte and addition of betaine as soil addition and foliar spray had a better effect on plants under saline conditions. GB enhances the plant's growth and increases yield (Abd El-Samad, 2013; Raza et al., 2014; Zoubida and Gherroucha, 2017).

5. Conclusion

Based on the results of the findings in the present study, it could be recommended to cultivate Misr 3 with Molasses as a soil addition and Glycine betaine as a foliar application to obtain high productivity of wheat from the unit area under saline soil.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was carried out using the facilities and materials in Taif University Researches Supporting Project number (TURSP-2020/139), Taif University, Taif, Saudi Arabia.

References

- Abd El-Hamid, E.A.M., El-Hawary, M.N.A., Khedr, Rania. A., Shahein, Alaa M.E.A., 2020. Evaluation of some bread wheat genotypes under soil salinity conditions. *J. Plant Product., Mansoura Univ.* 11 (2), 167–177.
- Abed Jeber, B., Khaeim, H.M., ed Jeber and Khaeim 2019. Effect of foliar application of amino acids, organic acids, and naphthalene acetic acid on growth and yield traits of wheat. *Plant Archives*, 824–826.
- Abdelmotlb, N.A., Abdel-Ail, F.S., Abd EL-Hady, S.A., EL-Miniawy, S.M., Ghoname, A. A., 2019. Glycine betaine and sugar beet extract ameliorated salt stress adverse effect on green bean irrigated with saline water. *Middle East J. Appl. Sci.* 09, 142–154.
- Abd El-Samad, M. Hamdia, 2013. The physiological response of wheat plants to exogenous application of gibberellic acid (GA3) or indole-3- acetic acid (IAA) with endogenous ethylene under salt stress conditions. *Int. J. Plant Physiol. Biochem.* 5 (4), 58–64.
- Akbari, G., Sanavy, S.A., Yousefzadeh, S., 2007. Effect of auxin and salt stress (NaCl) on seed germination of wheat cultivars (*Triticum aestivum* L.). *Pakistan J. Biol. Sci.* 10, 2557–3256.
- Al-Naggar, A.M.M., Sabry, S.R.S., Atta, M.M.M., El-Aleem, O.M.A., 2015. Effects of salinity on performance, heritability, selection gain, and correlations in Wheat (*Triticum aestivum* L.) doubled haploids. *Scientia* 10 (2), 70–83.
- Bates, L.S., Walden, R.P., Teare, I.D., 1973. Rapid determination of free proline for water studies. *Plant Soil* 39:, 205–208.
- Calvo, P., Nelson, L., Klopper, J.W., 2014. Agricultural uses of plant biostimulants. *Plant Soil* 383, 3–41.
- Caverzan, A., Casassola, A., Sandra Brammer, P., 2016. Antioxidant responses of wheat plants under stress. *Genetics Mol. Biol.* 39 (1), 1–6.
- Chamekh, Z., Ayed, S., Sahl, A., Ayad, S., Hammem, Z., Jalloul, S., Trifa, Y., Amara, H., 2014. Effect of salt stress on the flag leaf area and yield components in twenty-five durum wheat genotypes (*Triticum turgidum* ssp. durum). *J. New Sci.* 6 (3), 23.
- Colla, G., Rouphael, Y., Canaguier, R., Svecova, E., Cardarelli, M., 2014. Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. *Front Plant Sci.* 5 (448), 1–6.
- Duncan, D.B., 1955. Multiple Range and Multiple f Test. *Biometrics* 11, 1–24.
- Dawood, M.G., 2016. Influence of osmoregulators on plant tolerance to water stress. *Sci. Agric.* 13 (1), 42–58.
- El-Bassiouny, Hala S.M., Bakry, B.A., Attia, Amany A., Maha, M., Allah, Abd. 2014. Physiological role of humic acid and nicotinamide on improving plant growth, yield, and mineral nutrient of wheat (*Triticum durum*) grown under newly reclaimed sandy soil. *Agric. Sci.* 5, 687–700.
- Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd-Allah, E.F., Hashem, A., 2017. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. *Front. Microbiol.* 8, 2104.
- El-Geddawy, Mennat-Allah, M.A., Omar, M.B., Seleim, Magda M., Elsyiad, S.I., 2012. Composition and properties of Egyptian beet molasses. *J. Food Dairy Sci., Mansoura Univ.* 3 (12), 669–679.
- FAO, 2020. Food and Agriculture Organization. Faostat, FAO Statistics Division, March, 2020.
- Farhat, W.Z.E., Shehab-Eldeen, M.T., Khedr, Rania A., 2020. Agronomic and physiological studies on some exotic and local bread wheat genotypes under saline soil conditions in North Delta region. *Egypt. J. Plant Breed.* 24 (2), 465–491.
- Gomez, K.A., Gomez, A.A., 1984. *Statistical Procedures for Agricultural Research*. John Wiley and Sons, Inc., New York, USA.
- Gonzalez, L., Gonzalez-Vilar, M., 2001. Determination of relative water content. In: Reigosa, M.J. (Ed.), *Handbook of Plant Ecophysiology Techniques*. Kluwer Academic Publishers, Dordrecht, pp. 207–212.
- Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 189–198.
- Ibrahim, W., Ahmed, I.M., Chen, X., Cao, F., Zhu, S., Wu, F., 2015. Genotypic differences in photosynthetic performance, antioxidant capacity, ultrastructure and nutrients in response to combined stress of salinity and Cd in cotton. *Bio. Metals* 28, 1063–1078.
- Iqbal, N., Masood, A., Khan, N.A., 2012. Phytohormones in salinity tolerance: ethylene and gibberellins cross talk. In: Khan, N.A., Nazar, R., Iqbal, N., Anjum, N. A. (Eds.), *Phytohormones and Abiotic Stress Tolerance in Plants*. Springer, Berlin, pp. 77–98.
- Jackson, M., 1973. *Soil Chemical Analysis*. Prentice Hall of India Private, LTD, New Delhi, India.
- Javid, M.G., Sorooshzadeh, A., Moradi, F., Sanavy, S.A.M.M., Allahdadi, I., 2011. The role of phytohormones in alleviating salt stress in crop plants. *AJCS* 5 (6), 726–734.
- Jahan, M.A.H.S., Hossain, A., Teixeira DA Silva, J.A., Elsabagh, A., Rashid, M.H., Barutcular, C., 2019. Effect of naphthalene acetic acid on root and plant growth and yield of ten irrigated wheat genotypes. *Pak. J. Bot.* 51 (2), 451–459.
- Kaushik, A., Kadyan, B.R., Kaushik, C.P., 1994. Sugarmill effluent effects on growth, photosynthetic pigments and nutrient up take in wheat seedlings in aqueous vs. soil medium. *Water Air, Soil Pollut.* 87, 39–46.
- Kenawy, H., Mona, M., 2017. Impact of amino acids and humic substances application on some soil chemical properties along with nutritional status and productivity of wheat grown under saline conditions. *J. Soil Sci. Agric. Eng., Mansoura Univ.* 8 (6), 231–240.
- Khan, M.S., Ahmad, D., Khan, M.A., 2015. Utilization of genes encoding osmoprotectants in transgenic plants for enhanced abiotic stress tolerance. *Electron. J. Biotechnol.* 18, 257–266.
- Lum, M.S., Hanafi, M.M., Rafii, Y.M., Akmar, A.S.N., 2014. Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *J. Animal Plant Sci.* 24 (5), 1487–1493.
- Marschner, P., 2011. *Marschner's Mineral Nutrition of Higher Plants*. Elsevier, London, p. 672.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence. A-a practical guide. *J. Exper. Bot.* 51 (345), 659–668.
- Moran, R., 1982. Formulae for determination of chlorophyll pigments with N-N-dimethyl formamid. *Plant Physiol.* 69, 1376–1381.
- Mujeeb-Kazi, A., Munns, R., Rasheed, A., Ogbonnaya, F.C., Ali, N., Hollington, P., Dundas, I., Saeed, N., Wang, R., Rengasamy, P., Saddiq, M.S., De León, J.L.D., Ashraf, M., Rajaram, S., 2019. Breeding strategies for structuring salinity tolerance in wheat. *Adv. Agronomy.* 155, 121–187.
- Noroozlo, Y.A., Sour, M.K., Delshad, M., 2019. Stimulation effects of foliar applied glycine and glutamine amino acids on lettuce growth. *Open Agric.* 4, 164–172.
- Pearce, R.B., Brown, R.H., Blaster, R.E., 1968. Photosynthesis of alfalfa leaves as influenced by age and environment. *Crop. Sci.* 6, 677–680.
- Raza, M.A., Saleem, M.F., Shah, G., Khan, I.H., Raza, A., 2014. Exogenous application of glycinebetaine and potassium for improving water relations and grain yield of wheat under drought. *J. Soil Sci. Plant Nutr.* 14, 348–364.
- Roychoudhury, A., Banerjee, A., 2016. Endogenous glycine betaine accumulation mediates abiotic stress tolerance in plants. *Tropical Plant Res.* 3 (1), 105–111.
- Salama, K.H.A., Mansour, M.M.F., Almalawi, H.A., 2015. Glycine betaine priming improves salt tolerance of wheat. *Biologia* 70 (10), 1334–1339.
- Samavat, S., Samavat, S., 2014. The effects of fulvic acid and sugar cane molasses on yield and qualities of tomato. *Int. Res. J. Appl. Basic Sci.* 8 (3), 266–268.
- Tahir, M.M., Khurshid, M., Khan, M.Z., Abbasi, M.K., Kazmi, M.H., 2012. Lignite-derived humic acid effect on growth of wheat plants in different soils. *Pedosphere* 21, 124–131.
- Zada, A., Ali, A., Shah, A., Gill, S., Hussain, I., Ullah, Z., Sher, H., 2020. Physiological and molecular characterization of bread wheat (*Triticum aestivum* L.) for drought resistance. *Authorea*. September 17.
- Zeeshan, M., Lu, M., Sehar, Sh., Holford, P., Wu, F., 2020. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. *Agronomy* 10, 127.
- Zoubida, B., Gherroucha, H., 2017. Improvement of salt tolerance in durum wheat (*Triticum Durum* Desf.) by auxin and kinetin application. *Eur. Sci. J.* 13 (9), 96–110.