



Research article

Synergistic effect of salicylic acid and biochar on biochemical properties, yield and nutrient uptake of triticale under water stress

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ABSTRACT

In arid regions, one of the practical solutions to overcome the water shortage and increasing soil fertility is application of salicylic acid (SA) with biochar. A pot experiment was conducted to consider the combination of SA with biochar on biochemical and physiological parameters of triticale as a factorial experiment using a completely randomized design (RCD) with four replicates. Treatments consisted of irrigation regime (normal irrigation and irrigation according to 50 % field capacity), salicylic acid application [without SA (SA0) and 3 mM SA (SA3)] and fertilizer type including without fertilizer (control), application of 50 kg ha⁻¹ phosphorus (P), and application of wheat biochar (WB), cotton biochar (CB) and sesame biochar (SB) (2 % w/w). Under water stress, CB at SA0 and SA3 could improve the total chlorophyll by 119.4 and 70.6 %, compared to control, respectively. Also, carotenoid content in SA3 treatments increased in the range of 75.8 to 34.6 % compared to SA0. CB at SA3, increased catalase activity by 11.4 % compared to SB. At SA3, the highest RWC was observed in WB and CB by 26.7 and 18.1 % increases compared to SA0, respectively. At SA3, CB could enhance grain yield by 24.8 % under water stress. Under water stress, at SA3, remobilization efficiency from 63.2 % in control was enhanced to 69.2, 74.3 and 68.1 % in WB, CB and SB, respectively. CB and WB had better chemical properties in terms of EC, N, P, K and micronutrients compared to SB. These properties of BC and WB enhanced their ability to increase the nutrient availability, biochemical properties and consequently the grain yield enhancement, especially when applied with SA3.

1. Introduction

Water stress is one of the most important abiotic stresses in reducing crop yield. The agricultural fields are exposed to a continuous decrease in water resources, especially in arid and semi-arid areas [1,2]. Crops are subjected to water deficit daily due to a rise in canopy temperature, and evaporation and unsuitable distribution of precipitation [3]. In these conditions, crop yield may be reduced by more than 50 % [4]. Indeed, water stress can limit stomatal conductance, relative water content (RWC), and photosynthetic pigment contents, which cause decrease carbon fixation and dry matter accumulation [5].

Triticale (*X Triticosecale* Wittmack) is a hybrid of rye (*Secale* spp.) as male parent and wheat (*Triticum* spp.) as female parent. It has

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been produced due to a combination of good characteristics of rye, including tolerance to biotic and abiotic stresses and the high yield potential and grain quality of wheat [6]. In Iran, triticale grows better than wheat in unfavorable biotic and abiotic conditions. In the world, under water stress, triticale culture is a promising crop to overcome the detrimental effects of water stress on crop productivity [7,8]. Triticale is grown as a cover crop, fodder and for the production of flour for bread [7]. Practically, in arid and semi-arid regions, triticale is adapted to a wide range of soil and climatic conditions and can create higher grain yields in comparison to wheat [7–9].

Many crops such as wheat, triticale, rapeseed, sunflower and corn can limit the negative effects of water stress by leaf rolling, expanding root systems, and decreasing the water potential of cells by producing osmolytes [1,3–6,10]. Some crops, when exposed to water stress, by accumulating plant growth regulators (PGRs) such as salicylic acid (SA) can maintain the cell turgor in mesophyll cells [1,11]. Salicylic acid (SA) is a phenolic PGR which is able to regulate some vital plant processes, mainly water and nutrient uptake and carbon fixation, especially under water deficit [2]. Recently, Akhtar et al. [10] reported that foliar application of SA can enhance the photosynthetic pigments, antioxidative enzyme activity and consequently, dry matter production of *Lolium perenne* when plants are subjected to water stress. Under water stress, SA as an effective therapeutic agent can inhibit the deleterious effects of reaction oxygen species (ROS) by regulation of antioxidant enzyme activity including catalase (CAT) and peroxidase (POX) [1,12].

On the other hand, to mitigate the negative effects of water stress in crop production, application of nutrients such as biochar is another promising approach which can be extended in arid areas. Biochar, with its porous structure, is known to increase the water retention capacity of the soil and can decrease POX activity when plants are exposed to water stress [13,14]. Biochar is a carbon-rich material provided by the pyrolysis process which has various benefits including increasing the available water in the rhizosphere, improving the chemical and physical characteristics of the soil, facilitate the gradual release of macro and micronutrients, and promote the accessibility and absorption of nutrients by the crop roots [15]. Application of organic fertilizers such as biochar has been recommended compared to chemical fertilizers due to their higher safety, low cost and improvement in soil properties [16,17]. Improvement in the water holding capacity of the soil by biochar application can be a main reason for dry matter and yield improvement of the crops in arid areas [13,18].

Abd El-Mageed et al. [19] suggested that biochar application under water deficit could be a suitable approach among the different water management practices for enhancing water use efficiency. Biochar has a favorable potential to increase crop yield by 11 % and resistance to biotic and abiotic stresses because biochar is associated with the enhancement of soil microorganisms and regulation of biochemical and physiological properties of crops [14]. In a study, Wu et al. [20] reported that biochar amended to the soil can enhance water uptake and nutrient availability, and limits ROS creation by regulation of antioxidant enzyme activities under water and salt stresses. In fact, improving soil characteristics by biochar can alleviate the detrimental effects of water stress through ameliorating the photosynthetic rate, chlorophyll content, enzyme activity and RWC [13,21]. Overall, many studies demonstrate that the efficiency of SA applied alone or combined with biochar is depends on different factors, including the crop type, application rate of SA and biochar, type of biochar, crop growth stages and environmental conditions [12,19,20,22].

Water stress, especially at the reproductive stages of crops in arid and semi-arid areas, is one of the main problems which limits crop productivity. In sustainable agriculture, use of PGRs combined with biochar is necessary to yield improvement via increasing nutrient uptake and ameliorate the biochemical properties of plants when exposed to water deficit [22]. To the best of our knowledge, there is little information about the synergistic effects of SA with biochar on the biochemical and physiological characteristics of triticale under water stress. In the current study, it was hypothesized that application of SA combined with different sources of biochar (wheat, cotton and sesame) would alleviate the adverse effects of water deficit through changes in the biochemical and physiological characteristics of triticale. In fact, application of SA with biochar can overcome water deficit via improving the pigment content, relative water content, enzyme activity, dry matter remobilization and nutrient uptake, synergistically. In the synergistic effect of SA with biochar, the cumulative and positive effects of two agents can be observed in yield improvement of triticale, under water stress. The aim of this study, is to consider the effects of SA with different sources of biochar derived from cotton, wheat and sesame on the biochemical and physiological characteristics, nutrient uptake and yield of triticale when it is exposed to water stress.

Table 1
Physical and chemical properties of the soil.

Soil property	amount
Sand (%)	58.28
Silt (%)	33.28
Clay (%)	8.44
pH	8.37
EC (dS m ⁻¹)	0.34
Organic carbon (%)	0.32
Total N (%)	0.03
Available P (mg kg ⁻¹)	7.11
Available K (mg kg ⁻¹)	152.33
Available Fe (mg kg ⁻¹)	0.68
Available Mn (mg kg ⁻¹)	2.73
Available Zn (mg kg ⁻¹)	1.01
Available Cu (mg kg ⁻¹)	3.05

2. Materials and methods

2.1. Treatments and plant material

A pot experiment was conducted to investigate the performance of SA with different sources of biochar on biochemical and physiological parameters of triticale at the greenhouse of the Agroecology Department of Shiraz University, Fars Province, Iran. The soil used in the experiment was classified as Coarse-loamy, carbonatic, hyperthermic Typic Ustifluvents [23]. Some physical and chemical properties of the soil are given in Table 1. Experimental treatments consisted of irrigation regime at 2 levels (normal irrigation and irrigation according to 50 % field capacity), salicylic acid application (SA) at 2 levels [without SA (SA0) and 3 mM SA (SA3)] and fertilizer type at 5 levels including without fertilizer (control), application of 50 kg ha⁻¹ phosphorus from super phosphate triple source (P), and application of wheat biochar (WB), cotton biochar (CB) and sesame biochar (SB) (2 % w/w) alone. The pot study was set up as a factorial experiment 2 × 2 × 5 using a completely randomized design (RCD) with four replicates.

Hashemi is a new and early mature cultivar (125–141 days) of triticale with a medium plant height of 67–85 cm, and an average grain yield of 6814 kg ha⁻¹ which is adapted to moderate and semi-arid areas [24]. The seeds of the Hashemi cultivar were obtained from the Agriculture and Natural Resources Research Center of Darab, Fars Province, Iran. First, triticale seeds were sterilized by sodium hypochlorite solution (5 %) for 5 min and then washed several times by distilled water. Before seed planting, each fertilizer treatment (P or biochar) was incorporated to the soil. Then, six uniform seeds of triticale were cultured in depth of 3 cm in 5 kg pots filled with the soil. The seedlings were thinned to four seedlings at the three-leaf stage (ZGS13) [25]. The temperature in the greenhouse was 25 ± 5 °C, with 65 ± 10 % relative humidity, and light intensity was in the range from 700 to 1250 μmol. m⁻² s⁻¹. In normal irrigation treatment, each pot was weighted daily and irrigated up to 100 % field capacity (17 wt %) throughout the growing season. In water stress treatment, the soil water content of each pot was kept at 50 % field capacity after stem elongation stages (ZGS31). Also, foliar application of 3 mM SA was applied at booting stage (ZGS41) in pots which were treated with SA.

2.2. Biochar preparation and analysis

The biochar of wheat, cotton and sesame was prepared from abundant plant residues in the Darab region (28° 45.0'N, 54° 26.8'E), Fars province, Iran. The crop residues were dried and ground to pass through a 2-mm sieve. The dried and ground plant residues (at 72 °C for 48h) were pyrolyzed in a muffle furnace (Shimifan, F47) under limited oxygen conditions at a temperature of 400 °C for 4 h. The temperature was increased at a rate of 5 °C min⁻¹. Biochar was ground and sieved (<0.5 mm) before being applied to the soil [17]. Some characteristics of wheat, cotton and sesame biochars including pH, EC and macro and micronutrients are presented in Table 3. To determine the photosynthetic pigments, antioxidant enzyme activity, and RWC, the flag leaf of seedlings in each pot was sampled at the end of the flowering stage (ZGS69).

2.3. Chlorophyll and carotenoid content determination

The chlorophyll content of the flag leaf was extracted using 10 ml of 80 % acetone that was added to 200 mg of leaf tissue gradually, and ground with a mortar and pestle. After creating a homogenized solution which was described by Arnon [26], the absorbance was determined by a double-beam UV-VIS spectrophotometer (UV-1900, Shimadzu, Japan) at λ = 645, 663, and 470 nm. Then, the chlorophyll *a*, *b* and total [26] and carotenoid [27] were used for pigment assay.

2.4. Antioxidant enzymes assay

The catalase enzyme activity (CAT) was evaluated with a spectrophotometer (UV-160A) based on the method described by Aebi [28]. CAT activity was expressed as units (μmol H₂O₂ consumed per minute) per milligram of protein. Also, the peroxidase enzyme activity (POD), was determined by the method of Chance and Maehly [29]. POD was expressed as units (μmol guaiacol oxidized per minute) per milligram of protein.

Table 2

Some characteristics of the wheat, cotton and sesame biochars.

Biochar properties	Wheat biochar	Cotton biochar	Sesame biochar
pH	10.58 ± 0.07 ^a	10.10 ± 0.05 ^b	9.81 ± 0.02 ^b
EC (dS m ⁻¹)	7.64 ± 0.11 ^a	3.52 ± 0.23 ^b	6.84 ± 0.22 ^a
Total N (%)	1.1 ± 0.2 ^b	3.0 ± 0.3 ^a	1.3 ± 0.1 ^b
Total P (%)	0.07 ± 0.01 ^b	0.18 ± 0.02 ^a	0.05 ± 0.01 ^b
Total K (%)	0.63 ± 0.05 ^a	0.74 ± 0.03 ^a	0.38 ± 0.04 ^b
Total Fe (mg kg ⁻¹)	183.4 ± 21.3 ^b	361.5 ± 32.2 ^a	122.8 ± 11.3 ^c
Total Cu (mg kg ⁻¹)	5.1 ± 0.9 ^b	10.2 ± 0.7 ^a	7.1 ± 0.3 ^b
Total Zn (mg kg ⁻¹)	18.2 ± 0.2 ^{3b}	27.3 ± 1.9 ^a	11.6 ± 1.4 ^c
Total Mn (mg kg ⁻¹)	50.1 ± 7.2 ^b	241.9 ± 5.1 ^a	48.3 ± 6.2 ^b

Means in each row with the same letter do not differ significantly by LSD test at 5 % probability level. Data presented with ±SE.

Table 3

Interaction effect of irrigation regime, salicylic acid and fertilizer type on pigment content of triticale.

Irrigation regime	Salicylic acid (mM)	Fertilizer type	Chlorophyll a content (mg g ⁻¹ FW)	Chlorophyll b content (mg g ⁻¹ FW)	Total chlorophyll (mg g ⁻¹ FW)	Carotenoid content (mg g ⁻¹ FW)	
Normal irrigation	SA0	C	3.11 ± 0.03 ^{hi}	1.68 ± 0.01 ^{sh}	4.79 ± 0.02 ⁱ	0.35 ± 0.01 ^d	
	SA0	P	3.81 ± 0.02 ^{ef}	2.83 ± 0.01 ^d	6.64 ± 0.05 ^{ef}	0.34 ± 0.01 ^d	
	SA0	WB	4.29 ± 0.01 ^{cd}	3.53 ± 0.02 ^{ab}	7.82 ± 0.02 ^{bc}	0.35 ± 0.02 ^d	
	SA0	CB	4.86 ± 0.02 ^b	3.68 ± 0.03 ^a	8.54 ± 0.03 ^a	0.37 ± 0.02 ^d	
	SA0	SB	4.13 ± 0.01 ^{de}	3.23 ± 0.02 ^{bc}	7.36 ± 0.04 ^{cd}	0.33 ± 0.01 ^d	
	SA3	C	3.82 ± 0.01 ^{ef}	3.29 ± 0.01 ^{bc}	7.11 ± 0.03 ^{de}	0.52 ± 0.01 ^{cd}	
	SA3	P	4.31 ± 0.02 ^{cd}	3.36 ± 0.01 ^b	7.67 ± 0.02 ^b	0.57 ± 0.01 ^{cd}	
	SA3	WB	4.92 ± 0.03 ^{ab}	3.72 ± 0.03 ^a	8.64 ± 0.01 ^a	0.55 ± 0.02 ^{cd}	
	SA3	CB	5.14 ± 0.02 ^a	3.61 ± 0.02 ^{ab}	8.75 ± 0.01 ^a	0.54 ± 0.02 ^{cd}	
	SA3	SB	4.62 ± 0.01 ^{bc}	3.25 ± 0.02 ^{bc}	7.87 ± 0.03 ^{bc}	0.53 ± 0.01 ^c	
	Water stress	SA0	C	2.13 ± 0.03 ^j	0.54 ± 0.01 ^j	2.67 ± 0.01 ^k	0.98 ± 0.03 ^b
		SA0	P	2.93 ± 0.03 ^h	1.17 ± 0.03 ⁱ	4.10 ± 0.02 ^j	0.74 ± 0.03 ^c
SA0		WB	3.32 ± 0.02 ^{gh}	2.01 ± 0.03 ^{fg}	5.33 ± 0.01 ^h	0.71 ± 0.03 ^c	
SA0		CB	3.14 ± 0.01 ^h	2.72 ± 0.02 ^f	5.86 ± 0.03 ^{gh}	0.68 ± 0.01 ^c	
SA0		SB	3.06 ± 0.02 ^h	2.23 ± 0.01 ^f	5.29 ± 0.01 ^{hi}	0.62 ± 0.01 ^c	
SA3		C	2.72 ± 0.02 ⁱ	1.33 ± 0.01 ^h	4.05 ± 0.02 ^j	1.32 ± 0.02 ^a	
SA3		P	3.11 ± 0.02 ^h	1.72 ± 0.01 ^g	4.83 ± 0.01 ⁱ	1.21 ± 0.02 ^{ab}	
SA3		WB	3.64 ± 0.04 ^{fg}	2.69 ± 0.02 ^{de}	6.33 ± 0.01 ^{fg}	1.11 ± 0.01 ^{ab}	
SA3		CB	3.93 ± 0.04 ^{def}	2.98 ± 0.03 ^{cd}	6.91 ± 0.02 ^{de}	1.03 ± 0.01 ^b	
SA3		SB	3.22 ± 0.01 ^{gh}	2.36 ± 0.03 ^{ef}	5.58 ± 0.01 ^h	1.09 ± 0.02 ^b	

SA0: without salicylic acid; SA3: application of 3 mM SA, C: without fertilizer, P: application of 50 kg ha⁻¹ phosphorus, WB: application of wheat biochar (2% w/w), CB: application of cotton biochar (2% w/w), SB: application of sesame biochar (2% w/w). Means in each column followed by the same letters are not significantly different at 5% probability level using the least significant differences (LSD) test. Bars represent mean ± SE.

2.5. Leaf relative water content (RWC) measurement

The leaf RWC was measured by the method of Machado and Paulsen [30] at the end of the flowering stage (ZGS 69). Eight leaf discs (10 mm in diameter) were sampled from a fully expanded flag leaf in each pot, and were weighed for determination of fresh weight (FW).

Then, the leaf discs were kept in distilled water for 6 h, and dried on filter paper and weighed for total weight (TW). The samples were oven dried for a dry weight measurement (DW). Finally, the RWC was calculated as:

$$\text{RWC} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100$$

2.6. Dry matter remobilization and remobilization efficiency

To calculate the dry matter remobilization and remobilization efficiency of triticale, one plant in each pot was harvested at the flowering and maturity stages. These traits were calculated based on the formula of Ercoli et al. [31] and Dordas [32] as follows:

Dry matter remobilization (g plant⁻¹) = dry matter in flowering stage - dry matter of leaves, culms, and chaff, at maturity.

Remobilization efficiency (%) = (dry matter remobilization/dry matter of the whole plant at flowering) × 100.

2.7. Measurement of yield components and yield

In each pot, at the end of the maturity stage (ZGS 99), the plants were manually harvested, and plant height measured in the laboratory. Then, the plants were oven dried at 72 °C for 48 h and grain yield, harvest index and yield components consisted of grain number spike⁻¹, and 100-grain weight were determined.

2.8. Grain nutrient analysis

After harvesting, the macro and micronutrients of triticale grain were measured by the following methods. First, the dry grain of each pot was powdered using an electric mill, then ashed at 550 °C and digested with 2 M HCl. The total N content of the grain was measured using the Kjeldhal method [33]. Also, the total P concentration was measured colorimetrically and total K using flame photometer (Corning 510, UK). Finally, the total concentration of micronutrients such as Fe, Cu, Zn and Mn in the acid extract was determined using atomic absorption spectroscopy (PG 990, PG Instruments Ltd. UK) [34].

2.9. Statistical analysis

Data were analyzed using SAS software 2012 (version 9.4), and the data means were compared by the least significant differences

(LSD) test at a 0.05 probability level ($p \leq 0.05$). To check the normal distribution of data, Kolmogorov-Smirnov and Shapiro-Wilk tests were used and the skewness and kurtosis indices of data confirmed that the distribution of data was normal. To reveal the relationships between all traits, Pearson’s correlation coefficients were used.

3. Results

3.1. Physical and chemical characteristics of biochars

The physical and chemical characteristics of wheat, cotton and sesame biochars are given in Table 2. Among the different sources of biochar, the highest amounts of the pH were observed in wheat biochar (WB) (10.58 ± 0.05), while the EC of WB and sesame biochar (SB) was more than cotton biochar (WB), significantly ($p \leq 0.05$). The source of biochar had a noticeable effect on macronutrient contents. The highest contents of N, P and K were obtained in CB by 130.7, 260.0 and 94.7 % increases compared to SB, respectively. Also, the highest content of micronutrients, including the Fe, Cu, Zn and Mn, was significantly recorded by CB in comparison to WB and SB.

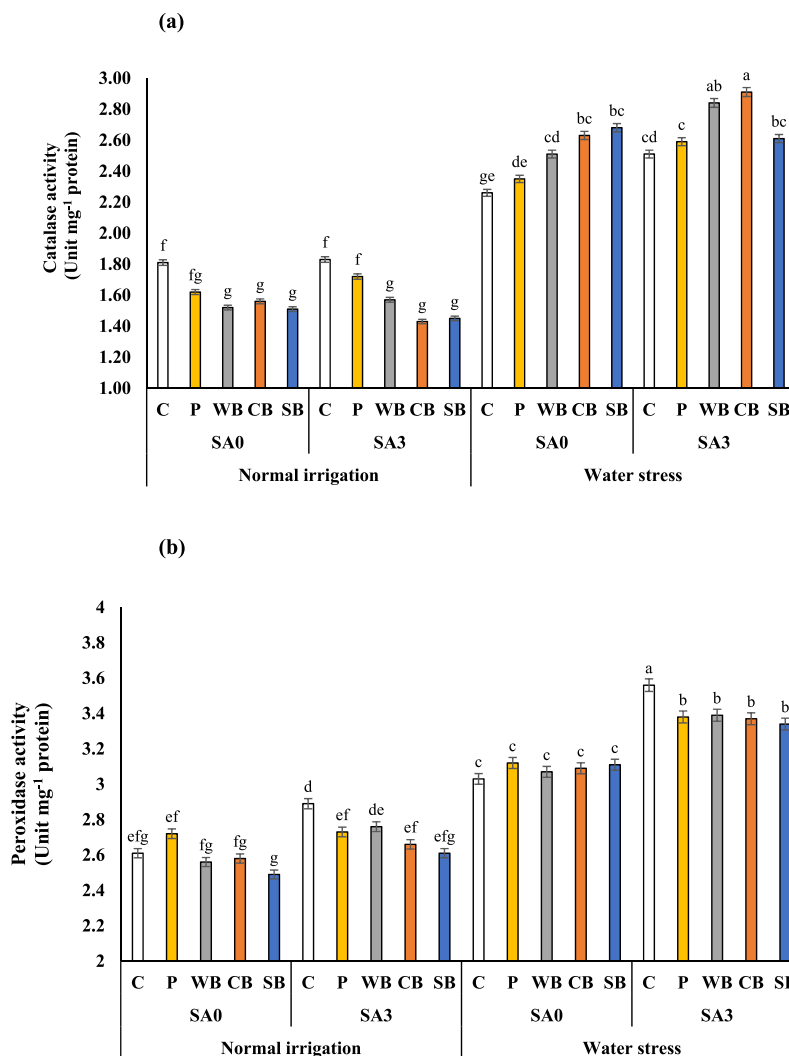


Fig. 1. Interaction effect of irrigation regime, salicylic acid and fertilizer type on catalase (a) and peroxidase activity (b) of triticale. SA0: without salicylic acid; SA3: application of 3 mM SA, C: without fertilizer, P: application of 50 kg ha⁻¹ phosphorus, WB: application of wheat biochar (2 % w/w), CB: application of cotton biochar (2 % w/w); SB: application of sesame biochar (2 % w/w). Means followed by the same letters are not significantly different at 5 % probability level using LSD test. Vertical bars represent ±SE.

3.2. Pigments content

Findings from this study showed that water stress and no application of salicylic acid and biochar (control treatment) affected chlorophyll *a*, *b* and total chlorophyll, negatively (Table 3). The highest amount of chlorophyll *a* content was observed in the normal irrigation regime when 3 mM of salicylic acid (SA3) was applied with CB or WB. At SA3 level, WB and CB could enhance the chlorophyll *a* content by 33.8 and 44.4 % compared to control respectively. Overall, phosphorous application (P treatment) could not alleviate the detrimental effects of water stress compared to CB and WB (Table 3). The interaction effect of the irrigation regime, SA level and fertilizer type had a significant effect on chlorophyll *b* content. Applying SA3 with CB and WB could mitigate the negative effects of water stress on chlorophyll *b* content in comparison to P treatment. When triticale is subjected to water stress, total chlorophyll in all SA and fertilizer treatments increased significantly ($p \leq 0.05$). However, under water stress, CB at SA0 and SA3 levels could improve the total chlorophyll more than the other fertilizer treatments by 119.4 and 70.6 % compared to control respectively (Table 3). Interestingly, in all fertilizer treatments, SA3 application was more effective in increasing the carotenoid content compared to SA0 when triticale is subjected to water stress. Under normal irrigation, in each SA level, the type of fertilizer had no significant effect on carotenoid content.

3.3. Catalase and peroxidase activity

In all fertilizer treatments, antioxidant enzyme activity including catalase (CAT) and peroxidase (POX) was increased by water stress compared to normal irrigation (Fig. 1). Under normal irrigation, the type of biochar had no noticeable effect on CAT activity (Fig. 1a). Controversy, under water stress, at SA3, CB created the highest CAT activity (11.4 % increase) compared to SB, with no significant difference with WB. At both SA levels, P treatment could not ameliorate CAT activity when triticale exposed to water stress. Overall, in all fertilizer treatments, water stress increased the POX activity compared to normal irrigation (Fig. 1b). Additionally, in all fertilizer treatments, SA3 could excite the POX activity more than SA0 and the highest POX activity (3.56 Unit mg^{-1} protein) was observed in control (SA3 without fertilizer) by significant difference with fertilizer treatments.

3.4. Relative water content

Relative water content (RWC) was influenced by irrigation regime and fertilizer type (Fig. 2). Under normal irrigation, CB created the highest RWC by 16.4 and 17.6 % increases compared to control at SA0 and SA3, respectively. Water stress depressed RWC in all fertilizer treatments, but the SA3 application was able to improve the RWC of triticale significantly ($p \leq 0.05$). Overall, P application at all SA levels, could not mitigate the detrimental effect of water deficit on RWC. Also, under water stress at SA3 level, the WB and CB had the better performance in RWC improvement, while there was no significant difference between biochar treatments at SA0.

3.5. Plant height, yield and yield components

Water stress hampered the plant height of triticale statistically ($p \leq 0.05$). Under normal irrigation, the highest plant height was observed in biochar treatments (WB, CB and SB) in the range of 52.26–52.64 cm at SA3 (Table 4). In fact, no application of SA (SA0) could not improve plant height in all fertilizer treatments. Under water stress, CB and SB at SA3 could ameliorate the plant height by

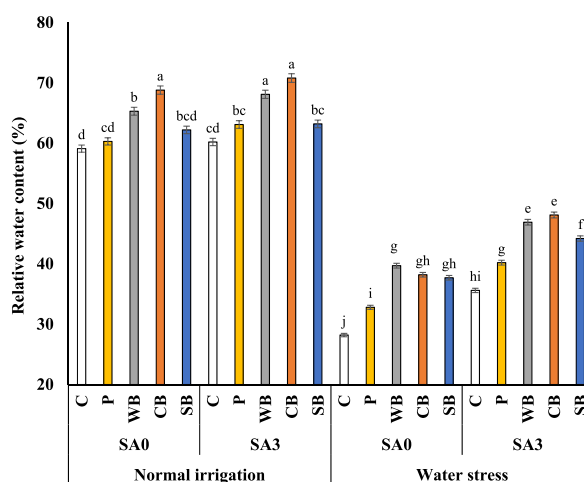


Fig. 2. Interaction effect of irrigation regime, salicylic acid and fertilizer type on relative water content of triticale. SA0: without salicylic acid; SA3: application of 3 mM SA, C: without fertilizer, P: application of 50 kg ha^{-1} phosphorus, WB: application of wheat biochar (2 % w/w), CB: application of cotton biochar (2 % w/w); SB: application of sesame biochar (2 % w/w). Means followed by the same letters are not significantly different at 5 % probability level using LSD test. Vertical bars represent \pm SE.

Table 4

Interaction effect of irrigation regime, salicylic acid and fertilizer type on plant height, yield components, dry matter remobilization and remobilization efficiency of triticale.

Irrigation regime	Salicylic acid (mM)	Fertilizer regime	Plant height (cm)	Grain no. spike ⁻¹	100-grain weight (g)	Harvest index (%)	Dry matter remobilization (g m ⁻²)	Remobilization efficiency (%)
Normal irrigation	SA0	C	43.95 ± 0.9 ^{bc}	51.00 ± 0.3 ^d	3.47 ± 0.06 ^{def}	43.95 ± 0.4 ^{cd}	1.64 ± 0.06 ^e	40.77 ± 0.3 ^f
	SA0	P	44.15 ± 0.8 ^{bc}	51.67 ± 0.4 ^d	3.55 ± 0.03 ^{cde}	44.15 ± 0.4 ^{bcd}	1.35 ± 0.04 ^g	32.52 ± 0.1 ^g
	SA0	WB	45.31 ± 0.5 ^{bc}	52.33 ± 0.2 ^d	3.71 ± 0.02 ^{bcd}	45.11 ± 0.3 ^{bc}	1.41 ± 0.06 ^{fg}	32.80 ± 0.2 ^g
	SA0	CB	46.11 ± 0.4 ^b	53.33 ± 0.6 ^d	3.75 ± 0.03 ^{bcd}	46.11 ± 0.3 ^b	1.36 ± 0.04 ^g	33.54 ± 0.3 ^g
	SA0	SB	44.85 ± 0.6 ^{bc}	52.33 ± 0.4 ^d	3.67 ± 0.02 ^{cd}	44.85 ± 0.3 ^{bcd}	1.35 ± 0.03 ^g	31.55 ± 0.4 ^g
	SA3	C	45.19 ± 0.4 ^{bc}	54.00 ± 0.3 ^d	3.86 ± 0.04 ^{bc}	45.19 ± 0.2 ^{bc}	1.81 ± 0.04 ^{cde}	39.21 ± 0.3 ^f
	SA3	P	39.14 ± 0.3 ^{de}	59.33 ± 0.2 ^c	4.00 ± 0.02 ^b	39.14 ± 0.3 ^{fgh}	1.92 ± 0.06 ^{cde}	31.70 ± 0.2 ^g
	SA3	WB	52.64 ± 0.2 ^a	71.67 ± 0.4 ^a	5.20 ± 0.04 ^a	52.64 ± 0.4 ^a	1.76 ± 0.03 ^{def}	24.84 ± 0.1 ^h
	SA3	CB	54.41 ± 0.4 ^a	73.67 ± 0.5 ^a	5.31 ± 0.02 ^a	54.41 ± 0.6 ^a	1.34 ± 0.04 ^g	26.48 ± 0.2 ^h
	SA3	SB	52.26 ± 0.4 ^a	66.00 ± 0.2 ^b	5.11 ± 0.02 ^a	52.26 ± 0.2 ^a	1.44 ± 0.03 ^{fg}	26.42 ± 0.3 ^h
Water stress	SA0	C	29.38 ± 0.5 ^h	29.67 ± 0.4 ^f	1.59 ± 0.04 ^k	29.38 ± 0.3 ^j	2.14 ± 0.01 ^{bc}	56.20 ± 0.4 ^e
	SA0	P	33.68 ± 0.3 ^g	31.00 ± 0.3 ^f	1.83 ± 0.02 ^{jk}	33.68 ± 0.4 ⁱ	2.23 ± 0.03 ^b	41.30 ± 0.2 ^f
	SA0	WB	36.35 ± 0.2 ^{fg}	33.33 ± 0.4 ^f	2.32 ± 0.01 ^{hi}	38.45 ± 0.4 ^{gh}	2.24 ± 0.04 ^b	55.10 ± 0.6 ^e
	SA0	CB	38.45 ± 0.1 ^{ef}	42.33 ± 0.3 ^e	2.58 ± 0.04 ^h	41.40 ± 0.3 ^{def}	2.38 ± 0.06 ^{ab}	69.60 ± 0.3 ^{ab}
	SA0	SB	37.68 ± 0.3 ^{ef}	33.00 ± 0.2 ^f	2.10 ± 0.04 ^{ij}	37.68 ± 0.1 ^h	2.03 ± 0.04 ^{bcd}	56.90 ± 0.2 ^e
	SA3	C	39.32 ± 0.2 ^{de}	42.67 ± 0.4 ^e	2.93 ± 0.03 ^g	39.32 ± 0.2 ^{fgh}	2.31 ± 0.07 ^{ab}	63.20 ± 0.1 ^{cd}
	SA3	P	37.10 ± 0.4 ^e	44.33 ± 0.3 ^e	3.02 ± 0.04 ^{fg}	40.70 ± 0.3 ^{efg}	2.27 ± 0.01 ^b	62.10 ± 0.3 ^d
	SA3	WB	41.20 ± 0.2 ^d	44.31 ± 0.4 ^e	3.24 ± 0.02 ^{efg}	43.24 ± 0.2 ^{cd}	2.46 ± 0.03 ^{ab}	69.20 ± 0.4 ^{ab}
	SA3	CB	43.64 ± 0.1 ^{bc}	46.35 ± 0.3 ^e	3.37 ± 0.01 ^{ef}	45.91 ± 0.1 ^{bc}	2.64 ± 0.04 ^a	74.30 ± 0.4 ^a
	SA3	SB	42.31 ± 0.2 ^{cd}	45.46 ± 0.2 ^e	3.14 ± 0.02 ^{fg}	42.21 ± 0.1 ^{de}	2.31 ± 0.03 ^{ab}	68.10 ± 0.2 ^{bc}

SA0: without salicylic acid; SA3: application of 3 mM SA, C: without fertilizer, P: application of 50 kg ha⁻¹ phosphorus, WB: application of wheat biochar (2 % w/w), CB: application of cotton biochar (2 % w/w), SB: application of sesame biochar (2 % w/w). Means in each column followed by the same letters are not significantly different at 5 % probability level using the least significant differences (LSD) test. Bars represent mean ± SE.

17.6 and 14.1 % compared to P. The grain number spike⁻¹ is one of the important yield components of triticale yield which was affected by water stress, negatively (Table 4). Under normal irrigation, the highest grain number spike⁻¹ was observed in SA3 with WB and CB, by 32.7 and 36.4 % increase compared to control, respectively. In all fertilizer treatments, application of SA3 compared to SA0 improved the grain number spike⁻¹ significantly ($p \leq 0.05$) except the CB. Overall, in both of the irrigation regimes, regardless of fertilizer type, SA3 application enhanced the grain number spike⁻¹ in comparison to SA0. The 100-grain weight was affected by the interaction effect of the irrigation regime, SA level and fertilizer type (Table 4). In each irrigation regime and SA level, there was no significant difference between the P treatment and control in terms of 100-grain weight. In addition, when SA3 was combined with biochar treatments under water stress the changes in 100-seed weight were less than normal irrigation conditions. SA3 application with CB and WB improved the grain yield by 87.1 and 78.1 %, respectively under normal irrigation (Fig. 3). Also, under normal irrigation, SA3 with P treatment could not improve the grain yield of triticale. CB can alleviate the disturbance effect of water stress on grain yield, so that at SA0 and SA3, grain yield was enhanced by 131.9 and 24.8 %, respectively. Overall, in all fertilizer types, grain yield in SA3 improved compared to SA0, significantly ($p \leq 0.05$). In each irrigation regime, CB and WB at SA3 could improve the HI of triticale by significant differences with P or control treatments (Table 4). Also, in each fertilizer type, SA3 application was able to enhance HI compared to SA0, significantly ($p \leq 0.05$).

3.6. Dry matter remobilization and remobilization efficiency

Dry matter remobilization had a main role in grain yield improvement mainly under water stress conditions. The highest dry matter remobilization (2.64 g m⁻¹) was observed in SA3 and CB by 16.2 % increase compared to P treatment when plant was exposed to water stress (Table 4). Similarly, remobilization efficiency was improved by water stress and biochar application compared to P treatment (Table 4). Under water stress, at SA3, remobilization efficiency from 63.2 % in control was enhanced to 69.2, 74.3 and 68.1 % in WB, CB and SB, respectively. Under normal irrigation, biochar application could not enhance the remobilization efficiency and the highest amount was obtained in control treatment at both SA levels.

3.7. Nutrients contents

Water stress could decrease the N content of grain triticale in all fertilizer types, but biochar treatments could mitigate the negative effect of water deficit compared to P and control treatments, significantly (Table 5). Under normal irrigation and water stress conditions, SA3 application in P treatment increased the grain P content of triticale by no significant difference with CB (Table 5). In contrast, the grain P content in P treatment was more than all biochar treatments at SA0 levels. Grain K content was another macronutrient which was affected by irrigation regime, SA level and fertilizer type. At each SA level, water stress depressed the grain K content of fertilizer treatments significantly ($p \leq 0.05$) except for P treatment (Table 5). The highest Fe content was created in WB, CB and SB in the range of 106.5–111.5 mg kg⁻¹ DW by significant difference ($p \leq 0.05$) with other treatments. Also, water stress decreased the grain Fe content in control at both SA levels (Table 5). Under normal irrigation, SA3 created the highest Cu content in WB and CB by significant difference with P and control treatments (Table 5). Biochar treatments improved the grain Cu content under stress, so that the Cu content in CB from 4.35 mg kg⁻¹ DW in SA0 reached to 6.11 mg kg⁻¹ DW (40.4 % increase) in SA3 and in SB from 4.25 mg kg⁻¹ DW reached to 6.17 mg kg⁻¹ DW (45.1 % increase). When triticale was exposed to water stress, all biochar treatments enhanced the grain Zn content significantly compared to P and control treatments, at both SA levels (Table 5). Under normal irrigation, the grain Mn content in WB and CB was significantly ($p \leq 0.05$) higher than P and control treatments, which demonstrated the positive effects of

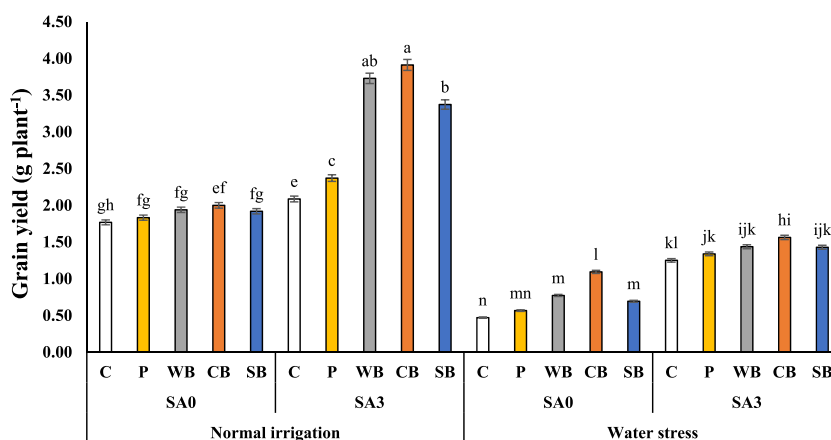


Fig. 3. Effect of irrigation regime, salicylic acid and fertilizer type on grain yield of triticale. SA0: without salicylic acid; SA3: application of 3 mM SA, C: without fertilizer, P: application of 50 kg ha⁻¹ phosphorus, WB: application of wheat biochar (2 % w/w), CB: application of cotton biochar (2 % w/w); SB: application of sesame biochar (2 % w/w). Means followed by the same letters are not significantly different at 5 % probability level using LSD test. Vertical bars represent \pm SE.

Table 5

Interaction effect of irrigation regime, salicylic acid and fertilizer type on macro and micronutrient contents of triticale grain.

Irrigation regime	Salicylic acid (mM)	Fertilizer regime	Grain N content (%)	Grain P content (%)	Grain K content (%)	Grain Fe content (mg kg ⁻¹ DW)	Grain Cu content (mg kg ⁻¹ DW)	Grain Zn content (mg kg ⁻¹ DW)	Grain Mn content (mg kg ⁻¹ DW)	
Normal irrigation	SA0	C	0.39 ± 0.02 ⁱ	0.22 ± 0.01 ^{def}	1.23 ± 0.04 ^{fg}	79.40 ± 1.2 ^{ef}	4.31 ± 0.12 ^g	10.41 ± 0.11 ^{fg}	17.41 ± 0.16 ^{de}	
	SA0	P	0.45 ± 0.01 ^g	0.27 ± 0.02 ^b	1.58 ± 0.06 ^{de}	81.20 ± 1.8 ^{ef}	7.41 ± 0.13 ^d	15.36 ± 0.14 ^{de}	19.86 ± 0.12 ^{cd}	
	SA0	WB	0.56 ± 0.03 ^d	0.23 ± 0.02 ^{cde}	1.85 ± 0.03 ^c	85.30 ± 1.6 ^{de}	7.43 ± 0.09 ^d	20.14 ± 0.10 ^b	22.14 ± 0.11 ^b	
	SA0	CB	0.63 ± 0.01 ^c	0.23 ± 0.01 ^{cde}	1.94 ± 0.04 ^{bc}	88.30 ± 2.0 ^{cd}	7.89 ± 0.08 ^{cd}	22.31 ± 0.14 ^b	22.41 ± 0.08 ^b	
	SA0	SB	0.57 ± 0.03 ^d	0.21 ± 0.01 ^{ef}	1.81 ± 0.05 ^{cd}	79.50 ± 2.0 ^{ef}	7.46 ± 0.07 ^d	20.95 ± 0.21 ^c	20.13 ± 0.06 ^c	
	SA3	C	0.44 ± 0.02 ^h	0.26 ± 0.01 ^{bcd}	1.36 ± 0.02 ^{ef}	92.10 ± 0.9 ^{bc}	5.98 ± 0.08 ^f	13.38 ± 0.04 ^{ef}	19.85 ± 0.07 ^{cd}	
	SA3	P	0.53 ± 0.02 ^{de}	0.32 ± 0.01 ^a	1.61 ± 0.02 ^d	97.80 ± 0.8 ^b	8.41 ± 0.07 ^{bc}	18.91 ± 0.09 ^{cd}	22.31 ± 0.14 ^b	
	SA3	WB	0.76 ± 0.01 ^a	0.28 ± 0.02 ^{abc}	2.11 ± 0.03 ^a	111.50 ± 2.3 ^a	9.86 ± 0.06 ^a	36.63 ± 0.09 ^a	25.36 ± 0.12 ^a	
	SA3	CB	0.77 ± 0.03 ^a	0.27 ± 0.01 ^{abc}	2.06 ± 0.01 ^a	108.30 ± 1.5 ^a	9.32 ± 0.07 ^a	25.14 ± 0.05 ^b	25.16 ± 0.10 ^a	
	SA3	SB	0.71 ± 0.02 ^b	0.26 ± 0.01 ^{bcd}	2.09 ± 0.01 ^{ab}	106.50 ± 1.6 ^a	9.11 ± 1.02 ^{ab}	22.39 ± 1.01 ^b	24.76 ± 0.11 ^{ab}	
	Water stress	SA0	C	0.24 ± 0.02 ^k	0.19 ± 0.01 ^{ef}	1.09 ± 0.04 ^g	66.30 ± 1.4 ^h	3.36 ± 0.03 ^h	9.43 ± 0.09 ^g	16.41 ± 0.09 ^e
		SA0	P	0.38 ± 0.01 ⁱ	0.21 ± 0.01 ^{ef}	1.36 ± 0.03 ^{ef}	72.30 ± 1.3 ^g	3.92 ± 0.05 ^h	11.21 ± 0.08 ^{fg}	17.23 ± 0.06 ^{de}
		SA0	WB	0.45 ± 0.03 ^g	0.13 ± 0.01 ^h	1.42 ± 0.04 ^e	79.20 ± 1.4 ^{ef}	4.23 ± 0.07 ^g	19.56 ± 0.09 ^{bc}	19.12 ± 0.02 ^{cd}
		SA0	CB	0.47 ± 0.04 ^{fg}	0.14 ± 0.01 ^{gh}	1.48 ± 0.01 ^{de}	80.40 ± 0.9 ^{ef}	4.35 ± 0.09 ^g	20.15 ± 0.05 ^{bc}	19.44 ± 0.03 ^{cd}
SA0		SB	0.46 ± 0.02 ^{fg}	0.11 ± 0.01 ^h	1.36 ± 0.03 ^{ef}	80.30 ± 0.8 ^{ef}	4.25 ± 0.05 ^g	18.41 ± 0.04 ^{cd}	19.21 ± 0.01 ^{cd}	
SA3		C	0.32 ± 0.02 ^j	0.22 ± 0.03 ^{def}	1.13 ± 0.04 ^{fg}	69.30 ± 0.7 ^{gh}	4.11 ± 0.08 ^g	10.97 ± 0.06 ^{fg}	17.23 ± 0.06 ^{de}	
SA3		P	0.41 ± 0.01 ^{hi}	0.26 ± 0.02 ^{bcd}	1.47 ± 0.04 ^{de}	75.20 ± 0.5 ^{fg}	5.68 ± 0.06 ^f	11.39 ± 0.04 ^{fg}	19.86 ± 0.02 ^{cd}	
SA3		WB	0.44 ± 0.03 ^{sh}	0.19 ± 0.01 ^{efg}	1.61 ± 0.02 ^d	80.20 ± 1.5 ^{ef}	6.06 ± 0.04 ^{ef}	21.16 ± 0.07 ^b	20.16 ± 0.03 ^c	
SA3		CB	0.49 ± 0.02 ^{ef}	0.22 ± 0.02 ^d	1.62 ± 0.02 ^d	83.40 ± 1.6 ^{de}	6.11 ± 0.07 ^{ef}	21.59 ± 0.06 ^b	20.59 ± 0.04 ^c	
SA3		SB	0.45 ± 0.01 ^g	0.18 ± 0.01 ^{fg}	1.56 ± 0.01 ^{de}	76.20 ± 1.7 ^f	6.17 ± 0.04 ^{ef}	19.47 ± 0.08 ^b	20.56 ± 0.06 ^c	

SA0: without salicylic acid; SA3: application of 3 mM SA, C: without fertilizer, P: application of 50 kg ha⁻¹ phosphorus, WB: application of wheat biochar (2 % w/w), CB: application of cotton biochar (2 % w/w), SB: application of sesame biochar (2 % w/w). Means in each column followed by the same letters are not significantly different at 5 % probability level using the least significant differences (LSD) test. Bars represent mean ± SE.

Table 6
Correlation coefficients (Pearson's) between all measured traits of triticale.

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1.Chlorophyll a content	1																				
2.Chlorophyll b content	0.404*	1																			
3.Total chlorophyll content	0.551**	0.541*	1																		
4.Carotenoid content	0.298*	0.957**	0.321*	1																	
5.Relative water content	0.753**	0.780**	0.431*	-0.381**	1																
6.Catalase	-0.283*	0.806**	0.547**	0.813**	-0.521*	1															
7.Peroxidase	-0.481*	-0.864**	0.325*	0.761**	-0.428*	0.732**	1														
8.Plant height	0.461*	-0.825**	0.431**	-0.658*	0.481**	-0.513**	-0.431*	1													
9.Grain no. spike ⁻¹	0.532**	0.828**	0.613**	-0.456*	0.630**	0.231*	0.302*	0.351*	1												
10.100-grain weight	0.416**	0.863**	0.761**	-0.521*	0.578*	0.132 ^{ns}	0.856**	0.359*	-0.436**	1											
11. Harvest index	0.391*	0.841**	0.549**	-0.421**	0.611**	0.241*	-0.799**	0.391*	0.439**	0.569*	1										
12.Grain yield	0.631*	0.889**	0.753**	-0.653*	0.436*	-0.221*	-0.889**	0.289 ^{ns}	0.766**	0.611**	0.748**	1									
13.Dry matter remobilization	-0.156 ^{ns}	0.709**	0.349*	0.636**	0.113 ^{ns}	0.486*	-0.696**	0.306*	0.507*	0.473**	0.466**	0.446*	1								
14.Remobilization efficiency	-0.211 ^{ns}	0.667**	0.651**	0.543*	0.146 ^{ns}	0.318*	-0.674**	0.426*	0.413*	0.416*	0.691**	0.651**	0.718**	1							
15.Grain N content	0.303*	0.724**	0.771**	0.213 ^{ns}	0.413*	0.231*	-0.631**	0.131 ^{ns}	0.314*	0.377*	0.314*	0.418*	0.398*	0.307*	1						
16. Grain P content	0.286*	0.257*	0.411*	0.112 ^{ns}	0.174*	0.286*	-0.267*	0.203 ^{ns}	0.421*	0.213*	0.391*	0.315*	0.203 ^{ns}	0.202 ^{ns}	0.316*	1					
17. Grain K content	0.431**	0.831**	0.534*	0.091 ^{ns}	0.345**	0.313*	-0.893**	0.166 ^{ns}	0.513**	0.450*	0.414*	0.761**	0.319*	0.288*	0.403*	0.314*	1				
18. Grain Fe content	0.403**	0.756**	0.894**	0.103 ^{ns}	0.366*	0.102 ^{ns}	-0.771**	0.123 ^{ns}	0.341*	0.329*	0.315*	0.516*	0.410*	0.276*	0.476**	0.113 ^{ns}	0.718**	1			
19. Grain Cu content	0.531**	0.537**	0.711**	0.067 ^{ns}	0.582*	0.201 ^{ns}	-0.545**	-0.103 ^{ns}	0.115 ^{ns}	0.345*	0.298*	0.403*	0.113 ^{ns}	0.101 ^{ns}	0.303*	0.171 ^{ns}	0.598*	0.705**	1		
20. Grain Zn content	-0.491**	0.640**	0.457**	0.131 ^{ns}	0.453*	0.259 ^{ns}	-0.647**	0.096 ^{ns}	0.143 ^{ns}	0.459*	0.113 ^{ns}	0.299*	0.268*	0.107 ^{ns}	0.402*	0.086 ^{ns}	0.439**	0.403*	0.471*	1	
21. Grain Mn content	0.580**	0.178**	0.536**	0.119 ^{ns}	0.235*	0.131 ^{ns}	-0.516**	0.141 ^{ns}	0.115 ^{ns}	0.298*	0.274*	0.312*	0.114 ^{ns}	0.090 ^{ns}	0.376*	0.103 ^{ns}	0.504*	0.398*	0.368*	0.509*	1

ns, * and **: non-significant, and significant at 5 % and 1 % probability levels, respectively.

them on grain Mn improvement (Table 5).

3.8. Correlation between all measured traits of triticale

Results of Pearson's correlation between all traits are given in Table 6. Total chlorophyll demonstrated a positive and significant correlation with all traits at 0.01 % and/or 0.05 % probability levels. RWC as a main indicator of the water status of the leaves, had a significant and negative correlation with CAT ($r = -0.521^*$) and POX ($r = -0.428^*$) activity. A positive correlation was observed between carotenoid content with CAT and POX ($r = 0.732^{**}$) while it correlated with RWC, plant height, yield and yield components, negatively. Grain no. spike⁻¹ correlated with 100-grain weight ($r = -0.436^{**}$) negatively, but it correlated with harvest index ($r = 0.439^{**}$) and grain yield ($r = 0.766^{**}$) positively. A positive correlation was observed between grain yield with dry matter remobilization and remobilization efficacy at 0.05 and 0.01 probability levels, respectively. Macronutrients including N, P and K correlated to grain yield, yield component, chlorophyll *a* and *b*, total chlorophyll positively except the carotenoid content and CAT. On the other hand, there was a significant correlation between N and K with micronutrients including Fe, Cu, Zn and Mn. In contrast, P correlated with N ($r = 0.316^*$) and K ($r = 0.314^*$) with no significant relationship with micronutrients.

4. Discussion

This study considered the role of SA and biochar application alone or combined in improving the performance of triticale under normal irrigation and water stress conditions. The SA application level and type of biochar from different sources is more effective in promoting biochemical and physiological characteristics of triticale, which supports our hypothesis.

4.1. Changes of pigments content

Considering the pigment contents of leaf including leaf chlorophyll *a*, *b*, total chlorophyll and carotenoid is a good index for evaluating the detrimental effect of water stress on photosynthetic performance and dry matter production [2,5,10]. Under water deficit, the decline in the leaf water content is responsible for loss of cell turgor, which limits the pigment content, nutrient uptake and assimilate translocation from the leaf [10]. SA can maintain the cell integrity and photosynthetic pigments of the mesophyll cells through the regulation of metabolic functions and enzyme activities [35]. Similar to our results, Ahmad et al. [1] declared that SA application can increase the leaf chlorophyll contents when wheat cultivars were exposed to water stress, however, pigment contents under normal irrigation were higher than water stress conditions. Indeed, under water deficit, SA can affect the volume of chloroplast, grana expansion and cellular contents to overcome fragmentation and disturbance of chlorophyll [36,37]. The co-application of SA with biochar can diminish the negative effects of water stress in some plants. In wheat, under water stress SA with biochar application alone or in combination demonstrated a considerable improvement in chlorophyll *a* and *b* and carotenoid contents in the range of 74–168 % [2]. Recently, Lalarukh et al. [38] showed that in wheat biochar is able to raise photosynthetic pigments under water stress. However, this increment was not significant with control treatment (without biochar). In contrast, Sattar et al. [39] showed a considerable increase in chlorophyll content, RWC and dry matter production when corn was subjected to water stress. Mohammadi Alagoz et al. [40] suggested that carotenoid content responds to water and salt stress. The main role of carotenoids is protection of the chlorophyll pigments against oxidative disturbance to inhibit the production of ROS. In agreement to our results, Shanazari et al. [41] showed that carotenoid content improved significantly in comparison to control when wheat and triticale were subjected to water stress.

In sunflower, water stress decreased the chlorophyll and carotenoid contents up to 64 % while the use of SA alleviates the disturbance effects of water stress, significantly [35]. In the current study, water stress dampened the chlorophyll content of triticale but the combination of SA3 application with CB increased the total chlorophyll by 70.6 % compared to control. It seems the higher N content (3.0 ± 0.3) in CB creates a favorable condition in increasing the total chlorophyll compared to WB, SB and P treatments. Also, under water stress, carotenoid content in SA3 treatments increased in the range of 75.8 to 34.6 % compared to SA0. Under water stress, the higher carotenoid content in C and P treatments in both of the SA levels might be attributed to the lack of macro and micronutrient contents compared to biochar treatments which excited the carotenoid in protection of chlorophyll disturbance. Overall, the positive effects of SA and biochar in reducing the impact of stress conditions may vary with the application rate and timing of SA, source of biochar and environmental conditions [2].

4.2. Enzyme activity

Under water stress, dry matter production is dampened especially due to loss in cell turgor and increasing ROS. ROS production due to low water potential in the cells is a common phenomenon under water stress. One of the main mechanisms to overcome the negative effect on water stress is production of concentrations of antioxidant enzymes including CAT and POX, which SA application can excite this mechanism [1]. Islam et al. [12] showed that elevation activity of CAT and POX is related to decreasing ROS in barley. In fact, SA triggers the mechanism of antioxidant activity of plants and ameliorates the detrimental effects of water stress by improving photosynthesis rate and dry matter production [10,42]. In response to water stress, isochorismate synthase gene is overexpressed for SA biosynthesis and consequently enhances POX activity for inhibition of ROS production [43]. On the other hand, Alkharabsheh et al. [44] suggested that under water deficit, biochar causes to enhance enzyme activity, water and nutrient uptakes and creates favorable conditions for dry matter production. Also, Khan et al. [3] declared that biochar can promote CAT and POX activity through decreasing

the ROS under water stress. Biochar having oxygen functional groups and a porous structure can increase the soil water holding capacity, which improves cell turgor and regulate the enzyme activity of crops when exposed to water deficit [17]. Our results are in line with Zulfiqar et al. [45] who suggested that under water stress, wheat biochar application improved the activities of antioxidant enzymes of CAT and POX by 24.11 and 13.14 %, respectively. Molina et al. [46] showed that CAT and POX were enhanced under water stress and co-application of SA with ammonium sulphate caused a significant reduction in the activity of enzymes in comparison to normal irrigation. In the current study, under water WB and CB with SA3 improved the CAT activity by 10.6 and 13.1 % compared to SA0 respectively, while the CAT activity in SB, P and C treatments in SA3 was lower than SA0. Also, POX in water stress was higher than normal irrigation, regardless SA and biochar treatments. In SA3, the higher amount of POX in C treatment (without biochar) under water stress is due to lack of nutrients which excited crop growth. It appears that the interaction effect of biochar source and SA level created different responses in terms of CAT and POX activity, especially when triticale was exposed to water stress.

4.3. Variation in RWC

In the new study, Yadav et al. [11] demonstrated that wheat and millet decrease RWC in the range of 33–64 % under water stress, while SA combined with thiourea kept the cell turgor and consequently improved the RWC through osmolytes accumulation. Decreasing the RWC of leaf might be related to cell dehydration which destroys the cell wall through lipid peroxidation, as well. In reaction to water stress, RWC was dampened and consequently the photosynthetic capacity of mesophyll cells was reduced. RWC was correlated with CAT and POX, chlorophyll *a* and *b* contents but not with carotenoid [10]. In contrast to our results, González-Villagra et al. [42] reported that SA under moderate water stress plants (Irrigation according to 60 % FC) demonstrated a similar RWC in comparison to plants without SA (SA0). Also, under normal irrigation in each biochar treatment, no huge variation was observed between SA0 and SA3. Hafez et al. [47] asserted that water deficit causes a decrease in pigment content, RWC, stomatal conductance of wheat, which is related to water availability depression. Biochar can improve the RWC by increasing the water holding capacity of the soil, which enhances water availability and nutrient uptake [15]. In a similar study, Rasool et al. [48] reported biochar improved total chlorophyll content and RWC in water stress conditions. Elshayb et al. [18] declared that biochar with ZnO NPs treatment enhances chlorophyll content in the range of 14.5 to 13.6 %, and RWC from 12.7 to 14.9 % in comparison to control. Our study showed the significant impact of SA and biochar on RWC maintenance under water stress, so that at SA3, the highest RWC was observed in WB and CB by 26.7 and 18.1 % increases compared to SA0, respectively. Indeed, biochar can enhance the meso-pores holding crop-available water through improving macroaggregates. Under water stress, these pores are responsible for keeping water in the soil structure for root uptake under drought stress, which, in turn, enhances photosynthetic pigments and RWC [49].

4.4. Plant height changes

Mohammadi Alagoz et al. [40] reported that water deficit at spike emergence and flowering stages decreased the plant height of triticale by 10.8 and 13.2 % compared to normal irrigation, significantly. In wheat, application of SA at 0.3 and 0.5 mM improved the plant height and grain No. spike⁻¹ but SA at 0.5 mM had better performance [50]. In wheat, increasing the plant height by 58 % was also reported by SA combined with biofertilizer under water stress [2]. Khan et al. [5] reported that SA can increase plant height and grain yield of wheat through decreasing the wilting of leaves and lipid peroxidation of membranes in mesophyll cells. Our results showed that plant height was decreased by water stress in the range of 19.7–43.2 % compared to normal irrigation, but SA3 application with biochar improved it significantly. Also, the synergistic effects of SA3 with CB on plant height was more than application of CB, alone.

4.5. Yield components changes of triticale

Maghsoudi et al. [51] in a study on wheat declared that water stress decreased the 1000-grain weight by 38.77 % in Shiraz cultivar, while co-application of SA with silicon improved the 1000-grain weight and grain yield, significantly. In accordance to our results, Shemi et al. [37] reported that water deficit hampered the photosynthesis activity of wheat and consequently decreased the plant height, grain no. spike⁻¹, 1000-grain weight, harvest index and grain yield. Indeed, the ROS induced by water deficit can destroy the cell membrane and chlorophyll structure while foliar application of SA can overcome water deficit by improving chlorophyll *a*, *b* and RWC which results in the grain yield enhancement [36].

4.6. The relationship between plant height and yield components with grain yield

Zulfiqar et al. [45] showed that wheat biochar limited the detrimental impacts of water deficit in wheat and improved the grain yield through enhancing plant height, grain no. spike⁻¹ and 1000- grain weight by 15.7,13.8 and 10.4 % compared to control (without biochar). In a study with different sources of biochar (wheat straw, rice husk and oilseed rape straw), regardless of water stress conditions, biochar (2 % w/w) enhanced the grain no. pod⁻¹ of fenugreek in the range of 9.0–14.5 % [52]. Also, biochar of rice husk and oilseed rape straw improved seed yield by 16.7 and 21.6 %, respectively. In agreement with our results, Hafez et al. [47] suggested that decreasing the yield and yield components of wheat might be attributed to depression of RWC and chlorophyll content, which caused a reduction in photosynthesis rate. However, biochar application alone or combined with vermicompost enhanced 1000-grain weight, the grain no. spike⁻¹, harvest index and grain yield when plants were exposed to water stress. They concluded that co-application of biochar with vermicompost positively increased water availability and nutrient uptake by roots compared to

application alone. Raza et al. [53] showed the synergistic effect of nano-biochar with brassinosteroids that increased the plant height, 1000-grain weight, grain no. spike⁻¹, harvest index and grain yield, statistically under water stress. Useviciute et al. [6] showed that in triticale the highest grain yield was obtained under normal irrigation by direct drilling of triticale associated with co-application of chemical fertilization (NPK), and 15 t/ha pine wood biochar. Our results showed that SA3 application with different sources of biochar, in both irrigation regimes, was more effective in improving grain no. spike, 100-grain weight, harvest index and consequently triticale grain yield compared to SA0 (without SA). In addition, in both irrigation regimes, SA3 with biochar synergistically increased yield and yield components of triticale in spite of P and control treatments. Overall, the efficiency of SA in decreasing the detrimental effects of water stress on morpho-physiological process is dependent on type of crop, source of biochar, SA application level and severity of water stress [2,17,52]. Also, antioxidant activity and osmotic adjustment are the other mechanisms for improving yield and yield components by SA application under water stress [42].

4.7. Dry matter remobilization and remobilization efficiency

Ercoli et al. [31] stated that severe water stress affected dry matter remobilization and remobilization efficiency of durum wheat, negatively. Although the remobilization of pre-anthesis assimilates to grain is enhanced to compensate the current assimilation reduction due to water deficit during the grain filling period [54], some studies showed decrease in the assimilate remobilization of pre-anthesis to grain due to severe water stress [55]. Recently, Barati et al. [7] reported that irrigation cut-off after anthesis decreased assimilate remobilization, harvest index and grain yield of triticale when high chemical N fertilizer (150 kg N ha⁻¹) was applied. Bijanzadeh et al. [9] also declared that the decrease in the grain yield of Juanillo triticale cultivar is attributed to a reduction in remobilization efficiency. In our study, under water stress, application of SA3 with biochar improved the dry matter remobilization (6.5–14.5 %) and remobilization efficiency (9.4–175 %) of triticale due to the synergistic effect of them in increasing the nutrient uptake and water holding capacity [2]. Indeed, this combination creates a favorable condition for more contribution of pre-anthesis assimilate to grain especially under water stress.

4.8. Macro and micronutrient uptake

Depending on the type of raw materials, biochar can have different effects on plant nutrition in drought stress conditions, and these effects may be positive or negative. On the one hand, biochar has an effect on the physical characteristics of the soil and improves the water retention in the soil, and on the other hand, due to its nutritional elements, it causes better plant nutrition and helps in better root growth and absorption of water and nutrients in water stress conditions [48,49,52]. In this research, it was found that all the biochars used, especially cotton biochar, caused a significant increase in various nutrients, especially N, K and Zn in triticale seeds. On the other hand, the use of biochar in water stress conditions can reduce the P content of seed, which can be solved by using salicylic acid in these conditions. Of course, this problem does not cause a serious problem considering the optimal amount of P in the seed. However, it should be considered in P-depleted soils. Khan et al. [3] asserted that biochar by porous structure can enhance the water holding capacity of the rhizosphere, which causes nutrient uptake increment of N, P, K, and Mg under water deficit. Also, Rodríguez-Vila et al. [56] stated that biochar as a main nutrient source can increase the nutrient availability of N, P, K and Mg by keeping more water in the soil compared to control (without biochar). Interestingly, biochar absorbed nutrients and slowly released them in the rhizosphere, causing improvement in the nutrient use efficiency [16]. In another study, Agegnehu et al. [57] declared that the wood biochar enhanced the water holding capacity of the soil and consequently dampened the N and P leaching, and improved the nutrient availability and grain yield of peanut plants.

Ahmad et al. [1] suggested that SA under water stress significantly affected the P and K of wheat cultivars, significantly. Also, SA was more effective in enhancing the N content compared to control. Similar to our results, they stated that N content under normal irrigation with SA was more than water stress conditions. On the other hand, under water stress, when wheat was treated with SA, the higher N, P and K contents were taken up compared to control (without SA). In a study on faba bean, Abd El-Mageed et al. [19] reported that biochar depending on application amount can increase the N content in the range of 20.9–22.5 %, P content from 25.4 to 16.9 % and K content from 20.7 to 28.0 %. Hafez et al. [47] concluded that biochar can improve the nutrient availability and water holding capacity of the soil, which substantially enhances the RWC, chlorophyll content, nutrient uptake (N, P and K), CAT and POX activity. In the current study, under water stress the contents of macro and micronutrients in the grain of triticale was decreased due to decline the water availability in the soil, while SA3 with CB could enhance the N (53.1 %), K (62.0 %), Fe (20.3 %), Cu (48.6 %), Zn (10.62 %) and Mn (18.8 %). Actually, among the biochar treatments, CB and WB had better chemical properties in terms of EC, N, P, K and micronutrients compared to SB. Overall, these properties of BC and WB enhanced their ability to increase the nutrient availability in the rhizosphere, and consequently the grain yield enhancement compared to other treatments.

4.9. Correlation coefficient between traits

Considering the correlation coefficient between all traits is more important in grain yield improvement. Similar to our results, Azmat et al. [2] stated that in wheat RWC correlated with chlorophyll content positively, but with CAT and POX activity, negatively. Also, in *Zinnia elegans* a positive correlation was observed between chlorophyll and yield in spite of carotenoid [10]. Bijanzadeh et al. [58] showed a positive and significant correlation between K content of grain triticale and RWC of the third leaf under water stress. Disagree with our results, a positive and significant correlation was reported by Zamaninejad et al. [59] between corn grain yield and plant height. Recently, Raza et al. (2023) stated that plant height had significant and negative correlation with CAT and POX of wheat

[53]. Also, they showed N, P and K contents of grain had significant correlation with CAT and POX, negatively. Our results showed that increasing chlorophyll *b* content, total chlorophyll, RWC, dry matter remobilization, grain no. spike⁻¹, 100-grain weight, harvest index, and grain K content were highly significant with grain yield of triticale at 0.01 probability level. The negative correlation between grain yield with carotenoid content, CAT and POX activity is related to increasing the plant's maintenance respiration, which decreased the grain yield [13,60].

5. Conclusion

These results highlight the efficiency of SA combined with biochar as a suitable strategy for enhancing crop performance and productivity of triticale when exposed to water stress. Application of 3 mM salicylic acid with cotton biochar can improve the photosynthetic pigments, relative water content, plant height, 100-grain weight, remobilization efficiency and macro and micro-nutrient compared to control under water stress. The wheat biochar was the second-best treatment in terms of these traits, but phosphorous treatment alone could not improve the biochemical and physiological properties compared to biochar treatments. It seems the better physical and chemical properties of cotton and wheat biochar play a main role in water and nutrient uptake especially when combined with salicylic acid under water stress. Also, cotton and wheat biochar when combined with salicylic acid could mitigate the detrimental effects of water stress through enhancing the pigment content, relative water content, yield components and regulation of enzyme activity of triticale. Further experiments are required to investigate the effect of the other sources of biochar at different levels of salicylic acid on soil properties, biochemical and physiological properties and nutrient uptake of triticale under water deficit.

Ethics approval and consent to participate

The study was conducted following the highest ethical standards. The data presented in this manuscript are accurate and authentic.

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Data availability statement

The data are available in the public repository zenodo at the following link: <https://doi.org/10.5281/zenodo.11356035>.

CRedit authorship contribution statement

Hesameddin Khajepour Tadvani: Validation, Software, Investigation. **Ehsan Bijanzadeh:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Data curation, Conceptualization. **Mahdi Najafi-Ghiri:** Writing – review & editing, Resources, Investigation.

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.

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