

## Impact of seed soak by ascorbic acid and foliar spray with boron in some morphological and physiological traits of wheat

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### Abstract

The effect of wheat seeds (Abu Ghraib variety) priming at different concentrations of ascorbic acid and boron foliar nutrition on certain morphological and physiological characteristics of wheat plants was investigated. A field experiment was executed in the city of Ramadi, Al-Jazeera region, from November 15, 2022, to April 19, 2023. The experiment included two factors: the first factor was seed priming by ascorbic acid (vitamin C) at different concentrations (0, 10, 20, and 40 mg.L<sup>-1</sup>), represented by (P<sub>0</sub>, P<sub>1</sub>, P<sub>2</sub>, and P<sub>3</sub>). The second factor was three concentrations of boron foliar spray at (0, 3, and 5) mg.L<sup>-1</sup> represented by (B<sub>0</sub>, B<sub>1</sub>, and B<sub>2</sub>) was added to plants as Boric acid (H<sub>3</sub>BO<sub>3</sub>). The experiment was carried out according to the randomized complete block design (RCBD) with three replicates. The results showed that all ascorbic acid concentrations, boron foliar fertilization, and their interactions increased significantly with concentrations increasing in all studied characteristics. The results showed significant differences at the concentration of 40 mg.L<sup>-1</sup> of ascorbic acid and 5 mg.L<sup>-1</sup> of boron for plant height (102.667cm), number of branches (14.000 branches. Plant<sup>-1</sup>), leaf area (68.55 cm<sup>2</sup>) and total chlorophyll (5.227 mg.gm<sup>-1</sup>).

**Keywords:** seed priming, ascorbic acid, boron, wheat plant, foliar spray, Total chlorophyll.

### Introduction

Wheat, which is known as the king of cereal crops, is one of the important food crops. Products made from wheat grain make up a significant portion of one of the primary meals eaten anywhere in the world [1]. It follows rice and maize in terms of output, and because it is highly adaptable to a wide range of climates and types of soil, it can be grown in every region of the world. As a result, the need for it continues to rise to meet the increasing requirements of a growing population [2]. Seed priming is one of the most important effective techniques used to improve germination and emergence quickly and increase the seed's ability to resist the difficult environmental conditions, as it showed a positive effect on germination and the emergence of seedlings [3]. In addition, the good and early germination of the crop increases its competition with bushes and its ability to withstand drought, it is environmentally safe [4], increases

yields, and reduces cost and time when re-sowing [5]. Also, the priming enhanced the seedlings growth in a variety of agricultural climate conditions [6] and reduced their sensitivity to external agents [7].

Ascorbic acid (known as vitamin C) is a water-soluble vitamin [8]. it is one of the most abundant antioxidants found in plants [9]. and is a non-enzymatic metabolic defense mechanism in plant cells [3] that helps to reduce the damage that is caused by the effect of oxidative stress by eliminating the toxicity and the harmful effects of effective oxygen in the chloroplast and cytosol, which reduces the intensity of stress [10]. It is found that ascorbic acid has a major role in organizing the complex series of biochemical defense plant responses to induce the production of protein and various defensive chemical compounds [11]. Ascorbic acid plays many roles in plant growth as well as the division and expansion of plant cell walls and other developmental processes [12]. Additionally, there was evidence that ascorbic acid had a beneficial influence on the growth of wheat [13]. Asghar et al. [14] demonstrated an acceleration in the rate of Maize seed germination, as well as an increase in the wet and dry weight of the shoot and the root system of the seeds treated with ascorbic acid at 20 and 40 milligrams per liter ( $\text{mg.L}^{-1}$ ) for 24 hours, The application of ascorbic acid by seed priming, foliar fertilization, and root system assisted wheat seedlings by maintaining growth, relative water content, cell membrane stability, and modifying osmosis [2]. This was accomplished through the accumulation of proline and the activation of antioxidant enzymes.

Boron is one of the essential micronutrients that play an important role in the formation of the cell wall, the conversion of sugars, the fixation of nitrogen, the carbohydrates transport, the increase in cell division, and the percentage of oil in seeds, it plays a role in increasing consumption efficiency of water and its role in balancing water relations, preserving flowers, forming pollen grains[15]. Foliar sprays of micronutrients are very useful when the roots are unable to provide or absorb nutrients [14]. A foliar spray of micronutrients is also very useful when the roots are unable to process or absorb nutrients [16]. The application of micronutrients through foliar spray to wheat crops has significant positive impacts on plant growth and yield indicators [13].

Rawashdeh and Sala [17] studied the effect of foliar spray applications of varying concentrations of boron (0, 250 and 500 ) ppm on the growth characteristics of wheat seedlings in vitro and the results showed a significant increase in seedlings height, roots length, stems and roots fresh weights and leaves content of chlorophyll as compared with controls. therefore, the purpose of this study was to investigate the effect of wheat seeds (Abu Ghraib variety) priming at different concentrations of ascorbic acid and boron foliar nutrition on certain morphological and physiological characteristics of wheat plants.

## **Materials and Methods**

An experiment was carried out in a field located in the city of Ramadi, Jazeera region, from November 15, 2022, to April 19, 2023. The experiment included two factors: The

first factor was seeds priming by ascorbic acid (vitamin C), at four concentrations (0, 10, 20 and 40)  $\text{mg.L}^{-1}$ , represented by ( $P_0$ ,  $P_1$ ,  $P_2$  and  $P_3$ ), and the second factor was boron by three concentrations foliar spray were (0, 3 and 5)  $\text{mg.L}^{-1}$  B was denoted by ( $B_0$ ,  $B_1$  and  $B_2$ ) Addition in the form of boric acid ( $\text{H}_3\text{BO}_3$ ) (17.4% B) to the plant. with three replicates the number of experimental units was 36. The experiment was carried out according to the randomized complete block design (RCBD). The soil analyses included taking soil samples before planting at a depth of 0-30 cm, then they were air dried, ground, and sifted with a sieve with a diameter of 2 mm. The soil was mixed well and samples were taken to conduct some analyses of the physical and chemical possessions of the soil visible in Table 1. Estimate the size distribution of soil particles using the pipette method as stated by the Day method presented in [4]. The EC, pH, Soluble potassium, calcium carbonate, and organic matter were estimated as stated by [18] and the amount of available nitrogen and phosphorous was determined according to [8]. Analysis was carried out in the Anbar Agriculture Directorate.

### Field practices

The soil of the field was prepared by conducting the plowing process twice perpendicularly by the inverter plow and smoothed by the masher. The field was divided into slabs, consisting of (36) slabs, the dimensions of which were 2 m x 2 m, and the area is 4  $\text{m}^2$ . The distance between slabs was seventy-five cm, and an area of fifty cm of dirt shall be left to prevent the transfer of water and fertilizers from one treatment to another. The earliest batch of nitrogen added in the form of urea (46% N) was added at a rate of 240  $\text{kg/ha}^{-1}$  in two batches, the first at planting. The second batch was added after 45 days of cultivation. Triple superphosphate fertilizer 46% ( $\text{P}_2\text{O}_5$ ) was added at a rate of 100  $\text{kg/ha}^{-1}$ , and  $\text{K}_2\text{O}$  (50% K) was added at a rate of 80  $\text{kg/ha}^{-1}$  at one time during planting for both seasons [13]. From the University of Anbar College of Agriculture, on lines with a deepness of five centimeters and a space of twenty centimeters between one hole and another, at a rate of three seeds per hole. It was reduced to one plant after 10 days of germination. The germination rate was 98%. Aphids struggled at the start of their looks on the leaves and stems of wheat, utilizing the pesticide DDVB 50 at a rate of 25 ml / 20 per liter of water. Spray the insecticide on the plants with a second spray 15 days after the first spray. The bushes were removed by hand hoeing.

### Seed priming

Four concentrations of 0, 10, 20, and 40  $\text{mg.L}^{-1}$  of an ascorbic acid were prepared. For 24 hours, the seeds were soaked at a rate of 300 seeds per concentration in nylon mesh bags and closed them tightly to avoid evaporation and changes in their concentrations. then the seeds were washed well with distilled water several times and left to dry in the shade until they returned to their original moisture. By recording their weight before and after soaking, the seeds were kept after activating them in thick, sealed plastic containers in the refrigerator at 4  $^{\circ}\text{C}$  until they were planted [19].

### Nutrition times

Three foliar sprays were conducted during the planting season. The first was forty-five days after sowing (tillering stage), the second was Sixty days after sowing (stem

elongation), and the third spray was seventy-five days after sowing (booting to heading), until the vegetation was completely wet. Twenty liters manual sprayer and 0.15 cm<sup>3</sup>.l of detergent solution as a dispersant material to reduce the water's surface tension and verify that the leaves were completely humidified achieved the spraying process to increase the efficiency of the spraying solution [7], including the control treatment.

**Table (1):** Some soil's chemical and physical characteristics before planting

Adjective	Result	Unit
EC	2.90	ds. M <sup>-1</sup>
pH	7.80	pH
Available nitrogen	0.017	%
Available Phosphorus	6.60	mg.kg <sup>-1</sup> soil
Available Potassium	0.18	cmol/kg soil
Organic matter	4.30	g/kg soil
Sand	11.20	%
Silt	54.70	%
Clay	34.10	%
Soil texture	Silty clay loam	--
Soluble boron	0.34	mg.kg <sup>-1</sup> soil
Lime	15	%
Gypsum	0	%

The morphological and physiological characteristics of ten intermediate plants from each experimental unit were calculated. morphological characteristics included plant height and leaf area according to the method used by [3] mentioned in [11] then calculation the number of branches. As for the physiological characteristics, the total chlorophyll content was estimated according to the method [3].

### Statistical analysis

Genstat program was used to analyze data. Differences between treatment means were compared using LSD at the 0.05 probability level.

## Results and Discussion

### Height of plant (cm)

Table 2 shows an increases in the plant height with increasing the concentration of ascorbic acid, as it reached the lowest value at a concentration of 0 mg.L<sup>-1</sup> at a rate of 95.444 cm, then it increased at a concentration of 10 mg.L<sup>-1</sup> and 20 mg.L<sup>-1</sup> to reach 96.778 and 97.778 cm for the two concentrations, respectively. It reached its maximum rate 98.889 cm at the concentration of 40 mg.L<sup>-1</sup>, with significant differences among the four concentrations. On the other hand, the plant height increased with the increase of boron concentrations, the plant height at the concentration 5 mg.L<sup>-1</sup> exceeded to

reach 100.167 cm, with a significant difference as compared with the plant heights measured at concentrations of 0 and 3 mg.L<sup>-1</sup>, as 97.583 and 93.917 cm respectively. It is clear from the results of the interaction between the ascorbic acid concentrations and the boron concentrations that the highest significant difference in plant height was at a concentration of 40 mg.L<sup>-1</sup> of ascorbic acid and a concentration of 5 mg.L<sup>-1</sup>, which was 102.667 cm, compared to the control treatment (without the addition of ascorbic acid and boron) which the plant height was 91.333 cm.

Ascorbic acid was found to increase the length of the root and stem or the root and shoot system in seed treatment [3]. The increase in the length of the root and shoot systems by ascorbic acid may be due to cell division and differentiation of meristematic cells [2]. Priming is a controlled wetting process of the seeds to the necessary metabolic activities for germination and the emergence of the root system occur. It may be due to the induction of physiological processes such as hydration and imbibition, and the activity of enzymes and germination with the speed that causes germination, due to the improved metabolic activities during and before emergence. Activation caused an increase in the final high percentage in the treatment of seeds with ascorbic acid [5]. Lukatkin [20] showed an increase in vegetative and root growth in stimulation with acid Ascorbic acid in maize improved germination speed and antioxidant activity of active enzymes as a result of rapid and simultaneous emergence of maize seedlings.

A foliar spray of boron showed a significant effect on the early growth of wheat components compared to the control. These results agreed with the findings of [17] that indicated the foliar applications of micronutrients had positive effects on plant growth and yield indicators. The effect of boron on plant height is attributed to the important role of boron in stimulating meristematic tissue division and increasing the concentration of the hormone Cytokines that contribute to cell division and elongation [8].

**Table (2):**The impact of seed soak by ascorbic acid and foliar spray with boron on the height of plant (cm) of wheat

Boron concentration (mg.L <sup>-1</sup> )	Concentrations of ascorbic acid mg.L <sup>-1</sup>				Mean
	0	10	20	40	
0	91.333	93.000	95.333	96.000	93.917
3	97.000	97.333	98.000	98.000	97.583
5	98.000	100.000	100.000	102.667	100.167
<b>LSD 0.05</b>	0.7431				0.3715
<b>Mean</b>	95.444	96.778	97.778	98.889	
<b>LSD 0.05</b>	0.4290				

### Branches in a plant (branch. plant<sup>-1</sup>)

Table 3 shows that there was a significant increase in the number of branches in the plant due to the effect of the ascorbic acid treatments. The highest number of branches 11.667 branches. Plant<sup>-1</sup> was at a concentration of 40 mg.L<sup>-1</sup>, followed by the number of branches recorded at a concentration of 20 mg.L<sup>-1</sup> and 10 mg.L<sup>-1</sup>, which were 11.111 branches. Plant<sup>-1</sup> and 10.667 branches. plant<sup>-1</sup> respectively. There are highly significant differences among the number of branches in the plant at tested boron concentrations, as they reached 13.000, 10.417, and 9.167 branches. plant<sup>-1</sup> at concentrations 0, 3, and 5 mg.L<sup>-1</sup> sequentially. The highest significant difference was recorded at 40 mg.L<sup>-1</sup> of ascorbic acid and 5 mg. L<sup>-1</sup> of boron, as the number of branches was 14.000 branches. Plant<sup>-1</sup> compared to the control treatment, in which the number of branches per plant was 8.333 branches. Plant<sup>-1</sup>.

**Table (3):** The impact of seed soak by ascorbic acid and foliar spray with boron on the Branches in a plant (branch. plant<sup>-1</sup>) of wheat

Boron concentration (mg.L <sup>-1</sup> )	Concentrations of ascorbic acid (mg.L <sup>-1</sup> )				Mean
	0	10	20	40	
0	8.333	9.000	9.333	10.000	9.167
3	10.000	10.000	10.667	11.000	10.417
5	11.667	13.000	13.333	14.000	13.000
LSD 0.05	0.6280				0.3140
Mean	10.000	10.667	11.111	11.667	
LSD 0.05	0.3626				

### Leaf area (cm<sup>2</sup>)

Table 4 showed a highly significant increase in the leaf area of the plant according to the increase of ascorbic acid concentrations, which were 49.09, 51.56, 53.84, and 57.41 cm<sup>2</sup>. The results presented in this table show highly significant differences among the leaf areas as a result of the effect of boron spraying treatments, which were 42.66, 52.69, and 63.58 cm<sup>2</sup> at concentrations 0, 3, and 5 mg.L<sup>-1</sup>, respectively. The leaf area was 68.55 cm<sup>2</sup> at 40 mg.L<sup>-1</sup> of ascorbic acid and 5 mg.L<sup>-1</sup> of boron with a significant difference as compared with the control treatment 38.54 cm<sup>2</sup>.

The percentage of germination (increased due to seed priming with ascorbic acid, which improved seed growth by repairing DNA, RNA, and protein damage [21] and improved the effectiveness of metabolic activity in the activation of seeds [2] or increased the activity of antioxidant enzymes. Seed stimulation is a strategic efficacy for increasing seed vitality, synchronizing germination, and improving germination rate in wheat [22], the possible reason for this increase is due to increased cell division within the tops of roots and stems in seedlings, which led to an increase in plant growth including branches numbers and leaf area. The increase in the leaf area is ascribed to the role of boron in the development of the growing apex, the increase in the growth rate of meristematic tissues, the increase in the length of the internodes, the spacing of

nodes, and its contribution to the activation of cell membranes, which causes an increase in the leaf area [3]. These results agreed with [23] indicating that boron foliar spray on sunflower plants increased the leaf area and the number of branches per plant.

**Table (4):** The impact of seed soak by ascorbic acid and foliar spray with boron on the leaf area (cm<sup>2</sup>) of wheat plant

Boron concentration (mg.L <sup>-1</sup> )	Concentrations of ascorbic acid( mg.L <sup>-1</sup> )				Mean
	0	10	20	40	
0	38.54	41.40	42.97	47.71	42.66
3	50.31	51.62	52.83	55.99	52.69
5	58.41	61.66	65.70	68.55	63.58
LSD 0.05	2.291				1.145
Mean	49.09	51.56	53.84	57.41	
LSD 0.05	1.323				

### Total chlorophyll (mg.gm<sup>-1</sup>)

The results in Table 5 showed that the highest rate of total chlorophyll was 4.196 mg.gm<sup>-1</sup> at the concentration of 40 mg.L<sup>-1</sup>, while the total chlorophyll was 3.836 mg.gm<sup>-1</sup> at the concentration of 20 mg.L<sup>-1</sup>, and 3.608 mg.L<sup>-1</sup> at the concentration of 10 with significant differences as compared with the control treatment, which was 3.323 mg.gm<sup>-1</sup>. The results indicated that there are significant differences between total chlorophyll contents due to the effect of boron treatments. The total chlorophyll was 4.524 mg.gm<sup>-1</sup> at the concentration of 5 mg.L<sup>-1</sup>, then gradually decreased with the decrease in boron concentration to reach 3.759 and 2.939 mg.gm<sup>-1</sup> at 3 and 0 mg.L<sup>-1</sup> respectively. The total chlorophyll at the concentration of 40 mg.L<sup>-1</sup> of ascorbic acid and 5 mg.L<sup>-1</sup> of boron was 5.227 mg.gm<sup>-1</sup> with a significant difference compared with the control treatment which was 2.381 mg.gm<sup>-1</sup>.

The application of ascorbic acid increases the total chlorophyll by promoting its accumulation and / or preventing its degradation, because the effective role of antioxidants in stabilizing enzyme's active sites complicated in photosynthesis process, as well as their role in eliminating the felled ROS that may disrupt the chlorophyll pigments [24]. Furthermore, Chloroplast produce ROS in plant, but it deficient catalase enzyme that can hunt ROS. Alternatively, ascorbic acid considered as a substrate for ascorbate peroxidase (APX) to scavenge ROS create in the thylakoid membranes [25]. Smirnoff and Wheeler [26] reported that ascorbic acid acts as a cofactor for violaxanthin de-epoxidase, an enzyme involved in xanthophyll cycle-mediated photoprotection, and in the Mehler peroxidase reaction with ascorbate peroxidase to regulate the redox state of photosynthetic electron carriers. The foliar spray with boron caused an increase in the concentration of boron within the plant tissues, which reflected positively on the leaf area, chlorophyll content, and dry weight of the plant, with the effect of photosynthesis, which leads to the provision of suitable conditions for plant growth [7].

**Table (5):** The impact of seed soak by ascorbic acid and foliar spray with boron in the determination of total chlorophyll ( $\text{mg}\cdot\text{gm}^{-1}$ ) of leaves of wheat plant

Boron concentration ( $\text{mg}\cdot\text{L}^{-1}$ )	Concentrations of ascorbic acid ( $\text{mg}\cdot\text{L}^{-1}$ )				Mean
	0	10	20	40	
0	2.381	2.824	3.224	3.327	2.939
3	3.444	3.698	3.860	4.033	3.759
5	4.145	4.301	4.426	5.227	4.524
LSD 0.05	0.3525				1.145
Mean	3.323	3.608	3.836	4.196	
LSD 0.05	0.2035				

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