Study of some parameters of vegetative growth and stomata in wheat by the effect of Nano silica and levels of salt stress

Fatima Al-Zahra Mahdi AL-Hassnawi [1]*, Fouad Razzaq Al-Burki [1]

[1] **Field Crops Department, College of Agriculture, Al-Muthanna University, Al-Muthanna, Iraq.**
***Corresponding author email:** agrpl.grad.fatima1@mu.edu.iq

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Abstract
A field experiment was conducted at the second agricultural experiment station of College of Agriculture- Al-Muthanna University during the agricultural season 2021-2022 to study some physiological and phenotypic parameters of three cultivars of bread wheat (Furat, Mawaddah, and Russi) by the effect of foliar apply of Nano silica (100 ppm) and irrigation with three levels of salinity (2.5, 5, 10 ds m-1). The experiment was carried out using the split-split plot design with three replications, the salinity factor (S) was included in the main plot, the Nano silica (N) was included in the secondary plot, and the sub-secondary plot were for cultivars (V). The results of statistical analysis showed that some traits recorded a significant decrease, such as flag leaf area, which decreased by 22.5%, root length, decreased by 16.83%, and the dry weight, by 19.2%, due to the influence of salinity. The results indicated that although Nano silica had a positive effect on root length, which increased by 10.89%, but it had a negative effect on stomata width on the lower surface of leaves, which decreased by (8.75%). The Furat cultivar is superior to the other cultivars in terms of root length, dry weight, stomata width in the upper surface and stomata length in the lower surface.

Keywords: Wheat, Salinity, Nano silica, Stomata, Root length

Introduction
Wheat crop (*Triticum aestivum* L.) is at the forefront of cereal crops, and one of the oldest and most significant crops known to humankind because it serves as the foundation of his diet and a source of energy needed by his body because it contains a high percentage of carbohydrates rich in calories [1]. In terms of global cultivable land, it comes in first place, and in second place after corn in terms of global grain production. According to [2], the annual global production of it was 749,467,531 tons, while the estimated cultivated area in Iraq for the winter of 2021 was 9464 thousand dunams, with a production of 4234 thousand tons [3]. Wheat is significant because it includes the gluten protein that serves as the foundation of the bread industry [4]. Salinity stress has a negative impact on plant growth and results in considerable losses in grain yield [5]. Stomata typically regulate the exchange of water vapor and carbon dioxide between the inner blade of the leaf and its outer circumference.

The modification of the stomata can affect the progress of photosynthesis in plants. The salinity-tolerant cultivars have higher water content, lower stomata density, and less leaf osmosis. Stomata characteristics include: size, density, and leaf osmosis [6], The agricultural production sector has a number of challenges and issues, particularly in our developing nations. Among these challenges are the detrimental impacts of abiotic stress factors, which affect crop growth and yield by generating physiological stress. The increase in wheat production is significantly impacted by salt, the second-most significant abiotic stress after water stress. This problem will worsen in the up-coming years due to the excessive salinity of irrigation water and agricultural fields, as well as other factors. To reduce the negative effects of this problem, researchers and plant breeders have recently adopted a salt-tolerant cultivars and methods and strategies to improve plant performance under conditions of severe salinity, including screening the current cultivars and developing more tolerant cultivars to salt stress [7], or adding fertilizers and Nano-fertilizers, including potassium or silica, due to their small size and large surface area lead to an increase in the absorption surface [8] and reduce the effects of plant stress and to control the stimulation or delay of the maturation and aging processes in the plant [9]. Silica nanoparticles have been shown to have a positive impact on crops' physiological characteristics, reducing the negative effects of irrigation with salt water, due to its nano scale size, which enables it to penetrate leaf tissue and cause changes in physical and chemical reactions in the cell and its contents [10].

The Iraqi wheat cultivars suffer from the issue of their sensitivity to salinity, which significantly reduced their growth rates and production. Our study set out to investigate some physiological and phenotypic parameters of cultivars of bread wheat by the impact of spraying with Nano silica and irrigation with salt water.

Materials and Methods

Executing the experiment

A field experiment was carried out at the second agricultural research station, during the winter season (2021-2022) on 17 November.

Field preparation and agricultural operations

to study some physiological and morphological parameters of cultivars of bread wheat by the effect of foliar application of Nano silica and irrigation with salt water. The study included three factors, which is the salinity of irrigation water: (10, 5, 2.5) ds m-1 , 2.5 ds m-1 is irrigation with river water (control: by calculating the rate during the growing season), and the treatment was started with saline water by watering the plants, a month after the first irrigation. the second factor: Nano-silica. A concentration of (50 ppm) was used, in addition to the control treatment (spraying with distilled water), it was prepared by adding 50 ppm of Nano silica powder (which contains 11-13 nm silica, which is supplied by the Iranian company Alfa). Per 1 liter of water and sprayed on the leaves of the plant in two stages (the tillering stage and the booting stage). The third factor is three varities of wheat: (Furat, Mawadda and Russi)

A Split-split plot design with three replications and random complete plot design (R.C.B.D) was used. The salinity factor was assign to whole plots, then the Nano silica factor to subplots within the application of the salinity factor, then the experimental units used for Nano silica factor were split into sub-subplots to receive cultivar factor (three cultivars) with three replications to form 54 experimental units The experimental land was plowed by two orthogonal plows, after carrying out the (irrigation) process for it then it was leveled, smoothed, and divided into panels with an area of (2 m x 1 m = 2 m2) in accordance with the design used, the secondary panels were separated from each other by a distance of 0.5m, and the experimental units had 10 lines with a length of 1m for each line and a distance of 20 cm between each line. Before planting, the soil was examined in the U science Laboratory as shown in Table No. (1). Wheat seeds were then sown at a rate of (2.4) grams per line in order to obtain a plant density of 120 kg ha-1 [11]. Fertilization procedures were carried out in accordance with the fertilizer advice [12]. Operations for weeding and irrigation were also done as needed.

Table (1): Some chemical and physical properties of the experimental soil before planting

chemical properties	(pH)	ECe (ds m^{-1})	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})
	8.07	16.13	48.02	20.67	186.33
physical properties	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	Soil texture	
	50.47	40.21	9.31	Loam	

Attributes and measurements studied during the experiment phenotypic traits

At the end of the flowering stage, the flag leaf area was measured using a ruler using the formula leaf length x maximum width x correction factor 0.95 [13]. Root size: It was measured in the blossoming stage with a ruler.

Weight of the shoot when wet: A sensitive scale was used to weigh the plants once they had reached the blossoming stage.

Shoot dry weight: The plants were removed once flowering was finished to be dried in an oven. When the weight is stabilized, shoot dry weight was measured using a sensitive scale at a temperature of 65 C.

Analyses of anatomy Additionally, samples were taken from the third leaf of the plant [14]. The samples were then examined under an Olympus compound optical microscope with an X40 lens and a graded lens at an X7 magnification. Measurements were made using an Ocular micrometer and the graduated lens was calibrated using 0.1 mm micrometric slides. The samples were captured on camera using the microscope's built-in camera.

Results and Discussion

Effect of salinity levels and apply with Nano silica and cultivars on vegetative growth characteristics

According to Table (2)'s findings, salinity had a substantial impact on the leaf area characteristic, with the second salinity level producing the lowest average for this trait (19.99) cm2 and the first salt concentration producing the greatest average (25.78) cm2. One of the critical adaptive processes associated with avoidance of salinity is the rate of growth of the flag leaf, which may explain why there is a discrepancy in these results [15].

There were also notable changes in root length, with the first salt concentration having the highest average (12.42 cm) and the third salinity level having the lowest average (10.33 cm). Also, the reason for the decrease in some characteristics may be due to the toxic ionic effect caused by high salinity, which disturbs the ionic balance of plant cells and reduces the amount of water available for cell growth and expansion. [16]. The average the wet weight was lowest at the second salt level (3371.53) gm m-2,but there were no significant variations in this characteristic, and it was highest at the first salt concentration (4016.61) gm m-2 .

In terms of dry weight, salinity had a substantial impact, with the second salt level S2 producing the lowest average (959.01) gm m-2and the first salt concentration S1 producing the greatest average (1198.76) gm m-2. An increase in NaCl uptake may be an increase in NaCl uptake may be resulting in a toxic potential of reactive oxygen species (ROS)., which disrupts the plasma membrane and causes an ionic imbalance that inhibits development and metabolism [17].

Table (2): Effect of salinity levels on some vegetative growth characteristics

Salinity levels	Leaf Area (cm^2)	root length (cm)	wet weight (gm m^{-2})	dry weight (gm m^{-2})
S1 (2.5) ds m^{-1}	25.78	12.42	4016.61	1198.76
S2 (5) ds m^{-1}	19.99	11.92	3371.53	959.01
S 3(10) ds m^{-1}	20.95	10.33	3574.53	989.75
L.S.D (0.05)	3.306	1.052	N.S	66.6

According to table (3), Nano silica had no discernible impact on the leaf area, as evidenced by the measurements of treatment N1 (22.27) cm2 and treatment N0 (22.21) cm2.

In contrast to the N0 treatment, which produced an average root length of 10.89 cm, N1 treatment gave an average of 12.22 cm. Nano-silica is beneficial because it enhances root length and growth [18].

Additionally, The N1 treatment provided an average of (3491.44) gm m-2 of wet weight while the N0 treatment provided an average of (3817.01) gm m-2, therefore its effect on the wet weight characteristic was not noteworthy .

Additionally, it was not significant in dry weight, with N1 providing an average of (1010.22) gm m-2compared to N0's average of (1088.12) gm m-2.

Table (3): Effect of Nano silica on some vegetative growth traits

Nano silica	Leaf Area (cm^2)	root length (cm)	wet weight (gm m^{-2})	dry weight (gm m^{-2})
0 ppm	22.21	10.89	3817.011	1088.12
100 ppm	22.27	12.22	3491.448	1010.22
L.S.D (0.05)	N.S	0.400	N.S	N.S

Table (4) revealed that there was no statistically significant difference between the cultivars' leaf areas average, as the Mawadda cultivar gave the lowest average of (20.39) cm2, while the Russi cultivar recorded the highest average of (24.31) cm2.

While it showed a significant difference in root length, the Furat cultivar gave the highest average of (13.33) cm, and Mawaddah cultivar gave an average of (10.58) cm, and the Russi cultivar recorded (10.75) cm. It may be due to the genetic differences between cultivars.

As for wet weight, the Furat cultivar recorded the lowest average of (3643.46) gm m-2 and the Russi cultivar gave the greatest average of (3661.41) gm m-2, therefore its variance in the wet weight characteristic was not noteworthy.

The Russi cultivar had the lowest average of dry weight of (969.85) gm m-2, while the Furat type gave the highest average of (1171.84) gm m-2. Dry weight varied greatly. The mismatch may result dry weight from the varying capacities of the cultivars to accumulate dry matter and the differences in the relative values of their constituent parts [19].

Table (4): Effect of cultivars on some vegetative growth characteristics

Cultivar	Leaf Area (cm^2)	root length (cm)	wet weight (gm m^{-2})	dry weight (gm m^{-2})
V1 (FURAT)	22.02	13.33	3643.46	1171.84
V2 (MAWADA)	20.39	10.58	3657.81	992.98
V3 (RUSSI)	24.31	10.75	3661.41	969.85
L.S.D (0.05)	N.S	0.573	N.S	110.1

Effect of salinity levels and apply with Nano silica and cultivars on the stomata of the upper surface of the leaf

The results of Table (5) demonstrated that the characteristic of stomata length was not significantly affected by salinity, with the highest average (1.54) µm being attained in the third salinity level and the lowest average (1.47) µm in the first salinity level. Also, in the width of the stomata, salinity had no significant effect, as it reached the lowest average (0.71) µm at the second and third salinity level, and the highest average (0.73) µm at the first salinity level .The stomata length reached its maximum mean (0.78) µm in the first salinity level (control) and its lowest mean (0.73) µm in the third salinity level, salinity had no significant effect on it .Also, its effect was not significant in the width of the stomata opening, as it reached the highest average (0.31) µm in the first salinity level and the lowest average (0.30) µm in the second and third salinity levels.

Table (5): Effect of salinity levels on the physiological characteristics of the upper surface of the leaf

Salinity levels	stomata length	stomata width	stomata aperture length	stomata aperture width
S1 (2.5) ds m^{-1}	1.47	0.73	0.78	0.31
S2 (5) ds m^{-1}	1.50	0.71	0.75	0.30
S 3(10) ds m^{-1}	1.54	0.71	0.73	0.30
L.S.D (0.05)	N.S	N.S	N.S	N.S

Table (6) demonstrates that the characteristic of stomata length is unaffected by Nano-silica, with the highest average for this trait in the non-addition treatment of N0 being (1.54) µm and the lowest average for this trait in the treatment with Nano silica N1 being (1.47) µm.

Additionally, it was not significant for stomatal width, reaching its greatest mean (0.73) µm in the N0 treatment and its lowest average (0.70) µm in N1 treatment.Additionally, the length of the stomata opening did not differ significantly between treatments N0 and N1, with treatment N0 having the highest mean (0.77) µm and treatment N1 having the lowest mean (0.74) µm.Also, its effect was not significant in the width of the stomata opening. Non-treatment N0 averaged (0.30) µm and treatment N1 averaged (0.31) µm.

Table (6): Effect of spraying with Nano silica on the physiological characteristics of the upper surface of the leaf

Nano silica	stomata length	stomata width	stomata aperture length	stomata aperture width
N0 (0) ppm	1.54	0.73	0.77	0.30
N1(100) ppm	1.47	0.70	0.74	0.31
L.S.D (0.05)	N.S	N.S	N.S	N.S

The results of Table (7) showed that the cultivars did not have a significant difference in the characteristic of the stomata length, as the highest mean was (1.55) µm in Furat cultivar and the lowest average was (1.46) µm in the Mawaddah cultivar. Also, in the width of the stomata, it was not significant, as the Mawadda cultivar gave the highest average of 0.73 and the Furat and Russi cultivar gave the lowest average of 0.71. It also did not significantly affect the length of the stomata opening, as it reached the highest average (0.80) µm in the Furat cultivar and the lowest average (0.72) µm in the Russi cultivar.While the cultivars showed a significant difference between them in the width of the stomata opening, as the Furat cultivar reached an average of (0.33) µm, the Mawaddah cultivar averaged (0.32) µm and the Russi cultivar reached an average of (0.27) µm .this discrepancy may be due to the nature of the genetic structure.

Table (7): Effect of cultivars on the physiological characteristics of the upper surface of the leaf

Cultivar	stomata length	stomata width	stomata aperture length	stomata aperture width
V1 (FURAT)	1.55	0.71	0.80	0.33
V2 (MAWADA)	1.46	0.73	0.74	0.32
V3 (RUSSI)	1.50	0.71	0.72	0.27
L.S.D (0.05)	N.S	N.S	N.S	0.03376

Effect of salinity and Nano silica and cultivars on the physiological characteristics of the lower surface of the leaf

The stomata length of plants grown in the first salinity level was (1.23) µm, and at the second and third salinity levels gave an average of (1.41) µm, demonstrating that salinity had no significant effect on the stomata length.In the width of the stomata, it had non-significant effect, as the first salinity level (control) recorded the highest average of (0.84) µm, and the lowest average of (0.72) µm was in the second and third salinity levels.

Salinity had no significant effect on the pore length characteristic, as it recorded the highest average (0.67) µm in the third salinity level and the lowest average amounted to (0.63) µm in the first and second salinity levels. There was no significant effect of salinity on the pore aperture, as it recorded the highest average (0.31) µm at the first salinity level and the lowest average decrease to (0.30) µm at the second and third salinity levels.

Table (8): Effect of salinity levels on the stomata of the lower surface of the leaf

Salinity	stomata length	stomata width	Pore length	Pore aperture
S1 (2.5) ds m^{-1}	1.23	0.84	0.63	0.31
S2 (5) ds m^{-1}	1.41	0.72	0.63	0.30
S3(10) ds m^{-1}	1.41	0.72	0.67	0.30
L.S.D (0.05)	N.S	N.S	N.S	N.S

Table (9) demonstrated that the stomata length characteristic was not significantly affected by Nano silica, with the non-treated N0 recording an average of (1.33) µm and the treatment N1 providing an average of (1.36) µm. while it had a significant effect on the characteristic of the stomata width on the lower surface of the leaf, as it gave the highest average (0.8) µm when not treated N0, while the lowest average was (0.73) µm at N1 .This increase in stomata width may be due to the effect of nano-silica in forming a double cuticle layer on the leaves, as a result of which the water loss is reduced (20) or the effect of silica may have a moisture-retaining effect State in plants. Thus, aiding in cell division (21) and then increasing the length of stomata. Also, its effect was not significant for the characteristic of the pore length, as the non-treatment N0 recorded an average of (0.64) µm, and the treatment N1 gave an average of (0.65) µm. However, it was not significant in the characteristic of the pore aperture, as the non-treatment N0 recorded an average of (0.31) µm, and the treatment N1 gave an average of (0.30) µm.

Table (9): Effect of Nano silica spraying on the stomata of the lower surface of the leaf

Nano silica	stomata length	stomata width	Porelength	Pore aperture
0 ppm	1.33	0.80	0.64	0.31
100 ppm	1.36	0.73	0.65	0.30
L.S.D (0.05)	N.S	0.0521	N.S	N.S

The Russi cultivar had the highest mean stomata length (1.40 µm), while the Mawaddah cultivar had the lowest average (1.31 µm). Table (10) demonstrated that the cultivars had a significant effect on this characteristic. this discrepancy may be due to the nature of the genetic structure.

The width of the stomata did not significantly differ among the Furat, Mawadda, and Russi cultivars, which each recorded an average of 0.73, 0.76 , and 0.78, µm respectively. The cultivars had no significant effect on the Porelength characteristic, as the cultivar Firat gave an average of (0.65) µm, and the two cultivars Mawadda and Al-Russi had an average of (0.64) µm. The cultivars had no significant effect on the characteristic of the Pore aperture, as the Furat cultivar gave an average of (0.30) µm, the Mawadda cultivar had an average of (0.31) µm, and the Russi cultivar gave an average of (0.29) µm.

Table (10): The effect of cultivars on the physiological characteristics of the lower surface of the leaf

Cultivar	stomata length	stomata width	Pore length	Pore aperture
V1 (FURAT)	1.33	0.73	0.65	0.30
V2 (MAWADA)	1.31	0.76	0.64	0.31
V3 (RUSSI)	1.40	0.78	0.64	0.29
L.S.D (0.05)	0.0554	N.S	N.S	N.S

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