Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 9, No. 2, 2023
https://jcns.ahvaz.iau.ir/ OPEN ACCESS

The Influence of Growth-Promoting Bacteria and Salicylic Acid on Leaf Area Index, Grain Yield and Harvest Index of Corn (*Zea mays* L.) under Non-irrigation Conditions at Different Growth Stages

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RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *30 Mar. 2023*
Received in revised form: *1 May. 2023*
Accepted Date: *10 Jun. 2023*
Available online: *30 Jun. 2023*

To Cite This Article: Gholam Esmaeilvandi, Aslan Egdernezhad. The Influence of Growth-Promoting Bacteria and Salicylic Acid on Leaf Area Index, Grain Yield and Harvest Index of Corn (*Zea mays* L.) under Non-irrigation Conditions at Different Growth Stages. ***J. Crop. Nutr. Sci.,*** 9(2): 53-73, 2023.

ABSTRACT

BACKGROUND: Salicylic acid exerts significant effects on plant morph physiology, playing a pivotal role in stimulating protective mechanisms and enhancing resistance against both biotic and abiotic stresses.

OBJECTIVES: Current research was done to assess effect of biofertilizers and salicylic acid under non-irrigation conditions at different growth stages at corn crop production.

METHODS: The study was done as a split-plot experiment, using Randomized Complete Block Design (RCBD) with three replications. The main plots consisted of different irrigation conditions at various growth stages (including full irrigation (control), non-irrigation from the flowering stage to the end of the growth period and non-irrigation from the beginning of the grain filling stage to the end of the growth period) and various combinations of biological fertilizers and salicylic acid treatments were implemented in the sub-plots (including the control treatment, 1 mM salicylic acid, nitroxin + biophosphorus bacteria grain rub and nitroxin + biophosphorus bacteria grain rub along with salicylic acid spraying).

RESULT: The results indicated significant effects of irrigation treatments, biological fertilizers and salicylic acid, as well as their interactions, on leaf area index, yield and yield components. Non-irrigation from the flowering stage resulted in a substantial 58.3% reduction in grain yield compared to the control. However, the combined application of biological fertilizers and salicylic acid, under both non-irrigation and full irrigation conditions, led to a noteworthy increase in yield and yield components. In the non-irrigation treatment starting from the flowering stage and grain filling stages, the application of nitroxin + biophosphorus + salicylic acid bacteria treatment resulted in a significant increase in grain yield by 22.7% and 19.3%, respectively, compared to the control treatment. Moreover, the highest grain yield (with an average of 6552 $kg.ha^{-1}$) was achieved through the combined effect of full irrigation and nitroxin + biophosphorus + salicylic acid foliar application.

CONCLUSION: The application of non-irrigation during the grain filling stage, along with the combined use of nitroxin + biophosphorus grain treatment and salicylic acid foliar application, not only provided optimal conditions for the corn plant but also effectively mitigated the adverse effects of drought stress, resulting in yields equivalent to those achieved under normal irrigation conditions.

KEYWORDS: *Biofertilizer, Grain filling, Growth indices, Nutrition, Seed yield.*

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1. BACKGROUND

The corn cultivation belt in the world due to the special characteristics of this plant, including belonging to the C4 group and especially its thermophilicity, has a close match with arid and semi-arid regions (Lack *et al*., 2006). Lack of water as an important factor in most stages of the growth of crops in arid and semi-arid regions by limiting growth makes it difficult to achieve high yield, so that the reduction in growth due to lack of water is far more than other environmental stresses (Liu *et al*., 2005). The corn plant needs a lot of water during the growth period and does not have a high tolerance for dehydration. If it is possible to reduce water consumption using a method, more land can be cultivated and the efficiency of water use can be increased (Jensen *et al*., 2007). In sustainable agricultural systems, the use of the biological fertilizers, especially in soils poor in nutrients, is particularly important in increasing production and maintaining soil quality (Sharma, 2003; Abraham *et al*., 2007). According to the available reports, in water shortage conditions, the use of biological fertilizers can also be effective in reducing the negative effects of water shortage (Ehteshami *et al*., 2013). Biofertilizer is made of one or more beneficial microorganisms along with preservatives or their metabolic products, which are used with the purpose of providing nutrients to plants (Vessey, 2003). These microorganisms affect plant growth by producing plant hormones, fixing nitrogen, facilitating the absorption of elements from the soil, and producing biological control agents against plant pathogens (Gharib *et al*., 2008). The use of biological fertilizers reduces the use of chemical fertilizers and produces high quality products without harmful chemicals for human health (Mahfouz and Sharaf-Eldin, 2007). Researchers showed that the inoculation of agricultural plants with growth promoting bacteria under the environmental stress conditions stimulates plant growth by reducing the absorption of toxic ions, increasing the production of auxin hormone and stress-specific proteins (Han and Lee, 2005). Silicon (Si) is the second most abundant element on earth after oxygen. Despite the abundance of this substance on the surface of the earth, due to its association with other elements, it is out of the plant's reach and the plant is only able to use its salicylic acid form, i.e. $Si(OH_4)$. It has been reported that silicon protects the plant against environmental stress by reducing the amount of ROS in plant cells (Epstein, 1994). Several studies reported the effect of Si is negligible in suitable growth conditions, while it is obvious and significant in stress conditions. Si-deprived plants are usually weaker and more sensitive than other plants (Al-Aghabri *et al*., 2004).

2. OBJECTIVES

This research was done to assess effect of biological fertilizers under non-irrigation conditions at different growth stages on improving corn yield and also investigating the possibility of increasing drought resistance.

3. MATERIALS AND METHODS

3.1. *Field and Treatments Information*

3.1.1. *Implementation time and geographical location of the research place*

This research was conducted in Izeh city during the beginning of August in the 2016 cropping year. Izeh city is situated at 31 degrees and 50 minutes north latitude, and 49 degrees and 52 minutes east longitude, relative to the Greenwich meridian, and it is situated at an elevation of 760m above sea level.

3.1.2. *Weather condition*

Izeh city is located in Khuzestan province and is considered one of the semi-arid regions in terms of climate. The average annual rainfall in this province is very low and at the same time very irregular. In terms of climate, Izeh is the coolest city in Khuzestan province. The region temperature in winter sometimes reaches below 0^0C and its average temperature in summer is 24^0C.

3.1.3. *Physical and chemical properties of farm soil*

In order to check the physical and chemical characteristics of the field soil, before planting, six parts of the field soil were sampled from a depth of 0-30 cm and after crushing the clods, the samples were passed through a two mm sieve and finally a composite sample was obtained. The samples were evaluated in the soil science laboratory in terms of some physical and chemical characteristics, the results of which are presented in Table 1.

Table 1. Physiochemical properties of farm soil before planting

Soil depth (cm)	Soil texture	Clay (%)	Silt (%)	Sand (%)	EC ($dS.m^{-1}$)	pH	OC (%)	N (%)	K (ppm)	P (ppm)	SP (%)
0-30	Clay loam	44	38	18	1.1	6.7	0.4	0.09	233	12.2	47

3.1.4. *Statistical model of research*

The study was done as a split-plot experiment, using Randomized Complete Block Design (RCBD) with three replications. The main plots consisted of different irrigation conditions at various growth stages [including full irrigation (I_1,control), non-irrigation from the flowering stage to the end of the growth period (I_2) and non-irrigation from the beginning of the grain filling stage to the end of the growth period (I_3)] and various combinations of biological fertilizers and salicylic acid treatments were implemented in the sub-plots [including the control treatment (F_1), 1 mM salicylic acid (F_2), nitroxin + biophosphorus bacteria grain rub (F_3) and nitroxin + biophosphorus bacteria grain rub along with salicylic acid spraying (F_4)].

3.2. Farm Management

3.2.1. *Land preparation operations*

Before the planting operation, the land was first irrigated. In August, the land preparation process began with fertilization. This process involved using a plow with a depth of 30 cm and two disks perpendicular to each other. Basic fertilizers were applied to the soil before second disk, and they were mixed with soil as the disk worked through it. Finally, field was leveled using a trowel.

3.2.2. *Required fertilizer consumption*

The required amount of fertilizer was determined based on the soil test. All the phosphorus, potassium, and half of the nitrogen fertilizer were added to the field. The used fertilizer included 150 $kg.ha^{-1}$ of pure phosphorus from the triple superphosphate source, $120kg.ha^{-1}$ of pure potassium from the potassium sulfate source, and $250kg.ha^{-1}$ of nitrogen from the urea source. To minimize leaching and wastage, the nitrogen fertilizer was applied in two stages. Fifty percent of the nitrogen was applied at the time of planting, and the remaining fifty percent was applied at the 6-leaf stage. This approach was adopted to ensure the efficient utilization of the nitrogen fertilizer and to reduce the risk of nutrient loss from the soil.

3.2.3. *Planting operations and applying treatments*

In the treatments involving biological fertilizer, the grains were inoculated with the specified biological compounds before planting, and the grain planting process was carried out manually. Before planting, the selected grains were soaked in 1 liter per hectare of nitroxin biological fertilizer, which contained Azotobacter and Azospirillum, for 20 minutes. Subsequently, the grains were spread on a flat surface and left to dry for ten minutes before planting. After the soaking process, the grains were immediately inoculated with 1 liter per hectare of biophosphorus biological fertilizer (Pseudomonas) (all these steps were conducted in the shade). Subsequently, the treated grains were planted. Each experimental plot consisted of six cultivation rows, each 5m in length. The distance between the rows was 75 cm, and the spacing between grains within the same row was 20 cm. The planting depth for the grains was set at 5 cm. A distance of 2m was maintained between each experimental block (repetition). Moreover, the distance between the main plots and subplots was set to be four no-till lines apart. The first irrigation was performed after grain planting and continued until the emergence of male inflorescences in all treatments. Additionally, foliar application of salicylic acid (1 mM) was carried out at the 8-10 leaf stage. Subsequently, from the stage of the appearance of male inflorescences and the beginning of the grain filling stage until the stage of full ripening, the specific irrigation treatment was implemented. However, in the control treatment, irrigation continued as usual until the ripening stage. Irrigation intervals for the different treatments were determined based on the customary practices of the region and in accordance with the control treatment. Manual weeding was diligently performed to control weed growth in all plots. Throughout the entire growth period, necessary precautions were taken to prevent and manage pests and diseases effectively. Fortunately, no specific pests or diseases were observed during course of the study.

3.3. Measured Traits

3.3.1. *Leaf Area Index* (*LAI*)

Leaf area index was measured in three stages (tasseling, beginning of flowering and grain filling). At each stage, the surface of each leaf was calculated using the following equation (Sobhani, 2000):

Equ.1. S = 0.46 (L×W) + 0.00046 (L×W) R2 ≥ 0.98**

Where S is the surface of the leaf, L and W are the maximum length and width of the green corn leaf, respectively. After measuring the surface of all the leaves, the leaf area index was calculated at different experimental treatments.

3.3.2. *Seed Yield and its components*

The ripeness of the grains was determined by observing the formation of a black layer at the base of the grains and the final harvest was carried out manually. To calculate the biological yield, fully ripened plants were carefully collected from the two middle rows in the center of each plot, covering an area of 3m². After removing any marginal lines, the collected plants were dried for 48 hours in an oven set at 70^{o}C. Subsequently, the dried plants were weighed using a digital scale with an accuracy of 0.01g and the yield was calculated in grams per square meter. To assess the yield components, including the number of rows of grains per cob, the number of grains per row and the 1000-grain weight, 10 cobs were randomly selected from each plot and analyzed accordingly. To measure the 1000 grain weight, the grains are separated from the cob and counted in two groups of 500 grains each. These groups are then weighed using a digital scale with an accuracy of 0.001g. If the difference in their weight is less than 5%, their total weight is considered as the 1000 grain weight for each treatment (Tahmasebi and Dolatmand Shahri, 2017). The harvest index (HI) was also calculated based on the following equation (Fageria, 1992):

Equ.2. HI = GY/BY×100

GY= grain yield and BY = biological performance

3.4. Statistical Analysis

The data was statistically analyzed by SAS software (Ver.8). Mean comparison was done by Duncan test. Additionally, the required graphs were created using Excel software.

4. RESULT AND DISCUSSION

4.1. *Leaf Area Index (LAI)*

The leaf area index was significantly affected by the irrigation treatment and the combination of growth-stimulating bacteria and salicylic acid treatment at different growth stages (Table 2). With the non-irrigation from the flowering stage to the end of the growth period, the leaf area index decreased significantly compared to full irrigation. The lowest leaf area index in the two stages of grain formation and grain filling, with an average of 2.7 and 1.4, respectively, was attributed to the treatment of non-irrigation from the flowering stage to the end of growth period (Table 3). The use of salicylic acid and growth-stimulating bacteria significantly increased the leaf area index. The highest leaf area index at the three stages of tasseling, beginning of flowering and grain filling was obtained with an average of 5.7, 4.6 and 3.8 from nitroxin + biophosphorus + salicylic acid treatment, respectively. The lowest leaf surface index at the stages of tasseling, beginning of flowering and grain filling was assigned to the control treatment with an average of 4.6, 3.5 and 2.7, respectively (Table 3). The leaf area index was significantly affected by the interaction effect of the treatment of non-irrigation and the use of salicylic acid and growth-promoting bacteria (Table 4). The treatment of non-irrigation at different growth stages caused a significant decrease in the leaf area index, on the other hand, the treatment combination of growth-promoting bacteria and salicylic acid improved the leaf area index significantly. In the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2), the leaf area index at three stages of tasseling, beginning of flowering and grain filling was 4.6, 2.8, and 1.4, respectively, and with nitroxin + treatment Biophosphorus + salicylic acid increased with an average of 5.5, 3.5 and 2.9, respectively. Until the stage of beginning of flowering, no significant difference was observed between the two treatments of complete irrigation and non-irrigation from the grain filling stage in terms of leaf area index. At grain filling stage, the maximum leaf area index (with an average of 3.85) was related to the full irrigation treatment (control) and the application of nitroxin + biophosphorus + salicylic acid (Table 4). The decrease in leaf area index under drought stress was consistent with the results of researchers in the same field (Islam *et al*., 2011; Mao *et al*., 2011; Jalilian and Mohsennia, 2013). Salicylic acid in combination with growth bacteria led to the improvement of the leaf area at different stages of sampling compared to the control by preventing the falling of leaves, delaying senescence, increasing greenness and vigor of the plant. Similarly, the decrease in the leaf area index in the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) compared to full irrigation (control) (I_1) and non- irrigation from the stage of grain filling to the end of the growth period (I_3) may be due to the reduction cell division, accelerating the aging and shedding of plant leaves and preventing its spread in vegetative growth stages (Cakir, 2004). The reason for the increase in the leaf area index in the F_4 treatment may be due to the increase in the development and expansion of the plant roots, followed by the increase in access to water and nutrients and the increase in the leaf area index.

Table 2. Result of analysis of variance effect of treatment on studied traits

S.O.V	df	LAI Tasselling stage	LAI Beginning of flowering	LAI Grain filling	No. row per ear	No. grain per ear
Replication (R)	2	0.98 ^{ns}	1.43^{ns}	1.26^{ns}	6.55 ^{ns}	10.44 ^{ns}
Irrigation (A)	2	19.12**	21.82**	11.35**	135.75 **	194.61 **
E_a	4	4.19	2.37	3.28	8.62	13.78
Fertilizer (B)	3	14.75**	17.47**	10.81**	85.81**	124.45**
A×B	6	11.95 **	13.7 **	5.14 **	65.74 **	95046**
E_b	18	2.94	1.66	2.37	5.32	7.49
CV (%)		11.28	12.02	7.85	10.82	11.23

^{ns}, * and **: non significant, significant at 5% and 1% of probability level, respectively.

Continue table 2.

S.O.V	df	1000 Grain weigh	Grain yield	Biological yield	Harvest index
Replication (R)	2	30.15 ^{ns}	3465 ^{ns}	5579 ^{ns}	13.63^{ns}
Irrigation (A)	2	1985.2 **	65372 **	113295 **	398.1**
E_a	4	78.25	6527.7	9417.2	15.26
Fertilizer (B)	3	1376.12 **	41297**	97826**	212.8 **
A×B	6	763.19 **	37588 **	85479**	169.4 **
E_b	18	33.55	3815.29	4918.61	7.38
CV (%)		8.54	11.43	7.69	6.45

^{ns}, * and **: non significant, significant at 5% and 1% of probability level, respectively.

Treatment with salicylic acid and growth promoting bacteria in normal conditions and in drought stress conditions has increased plant tolerance and the ability to absorb water and elements to its highest level. The researchers also found that the drought stress during the vegetative period led to the shrinking of the leaves and reduced the leaf area index during the ripening period and the amount of light absorption by the plant. In severe stress the stomata were closed, carbon dioxide absorption and dry matter production decreased and the continuation of the stress caused a sharp decrease in the intensity of photosynthesis (Gardner *et al*., 1984).

4.2. *Number of row per ear*

By non-irrigation at different groth stages, the number of row per ear decreased. The highest number of row per ear (with an average of 14 rows) was related to full irrigation treatment (control) (I_1), although there was no significant difference between this treatment and the treatment of no irrigation from the grain filling stage to the end of the growth period (I_3). The lowest number of rows with an average of 9 rows belonged to the non-irrigation from the flowering stage to the end of the growth period (I_2) (Table 5). The average number of rows per ear was different in the treatment of combination of growth promoting bacteria and salicylic acid. The highest number of row per ear with an average of 16 rows was obtained from nitroxin + biophosphorus + salicylic acid treatment. No significant difference was observed between nitroxin + biophosphorus treatment and salicylic acid treatment in terms of the number of row per ear (Table 5). With the usage of salicylic acid and growth-promoting bacteria, the number of rows per ear was the same in the two treatments of full irrigation (control) (I_1) and non-irrigation from the grain filling stage to the end of the growth period (I_3) and no significant difference was observed between them. By applying drought stress in the middle of the period, especially by non-irrigation from the flowering stage to the end of the growth period (I_2), a significant decrease in the number of row per ear was observed. The usage of salicylic acid and growth-promoting bacteria in drought stress conditions led to an increase in the number of row per ear. So, with nitroxin + biophosphorus + salicylic acid treatment, the number of row per ear increased from 8 to 10 rows in the treatment of no-irrigation from the flowering stage to the end of the growth period (I_2) (Table 6). Drought stress reduced the number of row per ear by reducing the growth of various vegetative and reproductive organs, especially ear. With the combined treatment of growth-promoting bacteria and salicylic acid, a significant increase in the growth of the ear and the increase in the number of rows per ear were observed. The results of this experiment were consistent with the results of similar experiments in the same field (Mansouri Far *et al*., 2010 and Mao *et al*., 2011). Drought stress, especially in the stages of non-irrigation from the flowering stage to the end of the growth period (I_2) with the reduction of chlorophyll content and leaf area index, caused a reduction in photosynthesis and the accumulation of photosynthetic materials and also created a limitation in the source. The decrease in the production and accumulation of photosynthetic materials has caused that during the graining time, the ear is limited under conditions of drought stress, and as a result, due to the increase in competition between the ears, the number of rows per ear has decreased significantly. Since the non-irrigation was from the stage of grain filling to the end of the growth period (I_3) after the stage of row formation per ear, therefore it did not have a negative effect on the number of row per ear. But since final number of grain rows per ear is determined before other yield components on ear development area, it is likely to compete with combined treatments of growth promoting bacteria and salicylic acid per treatment of non-irrigation and full irrigation, per row formation stage, competition between physiological sinks for assimilates is reduced and number of rows per cob increases (Tohidi Moghadam, 2016).

Table 3. Mean comparison effect of different level of irrigation and fertilizer on Leaf area index (LAI)

Treatment	LAI Tasselling stage	LAI Beginning flowering	LAI Grain filling
Irrigation			
I_1	4.9 a*	3.8 a	2.7 a
I_2	4.8 a	2.7 b	1.4 b
I_3	4.9 a	3.8 a	2.8 a
Fertilizer			
F_1	4.6 c	3.5 b	2.7 c
F_2	5.2 b	3.5 b	3.2 b
F_3	5.6 a	4.4 a	3.2 b
F_4	5.7 a	4.6 a	3.8 a

*Mean which have at least once common letter are not significant different at 5% level using (DMRT).
I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth. F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biophosphorus, F_4: Nitroxin bacteria grain rub + Biophosphorus along with salicylic acid foliar application.

4.3. *Number of grain per row*

The highest number of grain per row with the average of 23 grain among the different treatments of irrigation belonged to full irrigation (control) (I_1). The lowest number of grain per row with an average of 12 grains was observed per treatment with non-irrigation from the flowering stage to the end of the growth period (I_2) (Table 5). The number of grain per row increased significantly per treatment of combination of growth-stimulating bacteria and salicylic acid. The maximum number of grain per row (27) belonged to nitroxin + biophosphorus + salicylic acid and the lowest belonged to the control treatment (Table 5). The maximum number of grain per row (26) was for control and nitroxin + biophosphorus + salicylic.

Table 4. Mean comparison of interaction effects of different level of irrigation and fertilizer on Leaf area index (LAI)

Treatment		LAI Tasselling stage	LAI Beginning flowering	LAI Grain filling
I_1	**F_1**	4.95 cd	4.11 c	3.24 cd
	F_2	5.43 b	4.81 ab	3.40 bc
	F_3	5.31 bc	4.82 ab	3.63 ab
	F_4	5.82 a	4.95 a	3.85 a
I_2	**F_1**	4.65 d	2.83 f	1.41 g
	F_2	4.85 cd	3.22 ef	2.42 f
	F_3	5.28bc	3.43 de	2.81 e
	F_4	5.55ab	3.5 de	2.95 e
I_3	**F_1**	4.85 cd	4.31 bc	2.41 f
	F_2	5.45 b	4.93a	2.83e
	F_3	5.28 bc	4.84ab	3.45 bc
	F_4	5.83 a	4.93 a	3.65ab

*Mean which have at least once common letter are not significant different at 5% level using (DMRT).
I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth. F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biophosphorus, F_4: Nitroxin bacteria grain rub + Biophosphorus along with salicylic acid foliar application.

The minimum number of grain per row with an average of 11 grains belonged to the treatment of non- irrigation from the flowering stage to the end of the growth period (I_2) and not using salicylic acid and growth bacteria (Table 6). Although in this experiment, the non-irrigation at different growth stages caused a decrease per yield components, including the number of grain per row, but growth-promoting bacteria and salicylic acid in a combination, while increasing the number of grain per row under drought stress conditions, also increased this trait under full irrigation conditions. The decrease per number of grain per row under drought stress was consistent with the results of the experiments (Tohidi Moghadam, 2016; Mao *et al*., 2011 and Fazeli Rostampour *et al*., 2010). Considering that the number of grain per row plays the main role in determining the yield among the components of grain yield, identifying the physiological mechanisms that are related to the ability to produce more grains under drought stress conditions should have a high priority per programs to improve the condition of corn yield. It seems that in areas where corn cultivation is a priority and water is limited, determining a suitable irrigation schedule that balances the growth of vegetative and reproductive components and coincides with the critical period of growth with less moisture restriction is a good solution to increase efficiency. Production in these conditions, because moisture stress at different stages will cause a sharp drop in corn yield (Islam *et al*., 2011). Nitroxin + biophosphorus + salicylic acid treatment provided better conditions for increasing yield components by creating suitable conditions for the plant during the growth period. Non-irrigation from the flowering stage caused the greatest decrease in the growth and yield components of corn, which may be due to insufficient plant growth and reduced photosynthetic capacity to produce sap and transfer it to the ear for ear growth and grain formation. In fact, creating the optimal physiological balance between the production source and storage sink of photosynthetic materials is one of the important factors in having yield and yield components such as the number of grain per row (Fernado *et al*., 2012). Because the number of grains per row is determined after pollination, it is likely that the decrease in the number of grains in the treatment of non-irrigation from the flowering stage and not using salicylic acid and growth-stimulating bacteria is due to the lack of sufficient moisture in this stage. Despite non-uniform environmental conditions, use of plant hormones such as salicylic acid and growth-promoting bacteria by reducing negative effect of stress and creating a balance in corn causes the appearance of silk to be delayed much less compared to appearance of crown flower and number of fertilized eggs (Grains) increase, in other words, the storage capacity of the sink increases and the ratio of sterile florets decreases and the number of grain per row increases. The researchers reported that non-irrigation and occurrence of moisture stress from vegetative stage and pollination affected the seeding process in corn by reducing the photosynthesis of the leaves, and it reduces the number of grains per row due to the increase in the production of sterile pollen grains (caused by the lack of assimilate) (Setter *et al*., 2001). Reducing number of grain per row is one of the most common effects of drought stress reported by other researchers (Fernado *et al*., 2012; Fazeli Rostampour *et al*., 2010).

Table 5. Mean comparison effect of treatment on studied traits

Treatment Irrigation	No. row per ear	No. grain per ear	1000 Grain weigh (g)	Grain yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Harvest index (%)
I_1	14a	22a	238a	5214a	13110a	39.77a
I_2	9b	12c	144.2c	2173c	6896c	31.51c
I_3	14a	20b	181.4b	3348b	10638b	35.23b
Fertilizer						
F_1	13c	21c	235.2b	5190d	13615c	38b
F_2	15b	24b	245.5a	5640c	14482b	39ab
F_3	15b	24b	245.2a	5982b	14610b	41.2a
F_4	16a	27a	249a	6433a	15765a	41a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth.

F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biosphere, F_4: Nitroxin bacteria grain rub + Biosphere along with salicylic acid foliar application.

Mentioned researchers considered increase of assimilate for growth of grains and reduction of time interval between pollination and appearance of silks in treatment with stress-reducing agents as main reasons for increase number of grain per row.

4.4. *1000 grain weight*

The 1000 grain weight was significantly affected by the treatment of irrigation, the combination of growth-promoting bacteria and salicylic acid treatment their interaction (Table 2). Non-irrigation from the flowering stage caused the greatest decrease in the 1000 grain weight through the reduction of vegetative and reproductive growth of corn plants. In this experiment, the highest 1000 grain weight with an average of 238 grams was obtained from the full irrigation treatment (control) (I_1). The treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) had the lowest 1000 grain weight with an average of 144.5 grams (Table 5). The combination of growth-stimulating bacteria and salicylic acid not only increased the vegetative and reproductive growth, but also significantly increased the 1000 grain weight compared to their non-use (Table 5). The 1000 grain weight was different due to the interaction effect of non-irrigation treatments and the combination of growth-stimulating bacteria and salicylic acid. Drought stress caused a decrease in the 1000 grain weight following the decrease in the vegetative and reproductive growth of corn plants in different stages. In treatments of non-irrigation from the flowering stage to the end of the growth period (I_2) and non-irrigation from the grain filling stage to the end of the growth period (I_3), the application of nitroxin + biophosphorus + salicylic acid increased the 1000 grain weight by 37 and 34 grams, respectively. The highest 1000 grain weight with an average of 251.5 grams was obtained from the interaction effect of full irrigation (control) (I_1) and nitroxin + biophosphorus + salicylic acid treatment. The lowest 1000 grain weight with an average of 148 grams was obtained from the non-irrigation from the flowering stage to the end of the growth period (I_2) and not using salicylic acid and stimulating bacteria treatment (Table 6). Reduction in the 1000 grain weight in the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) and non- irrigation from the stage of grain filling to the end of the period was consistent with the results of similar experiments in the same field (Tohidi Moghadam, 2016; Lack *et al*., 2006; Salemi *et al*., Mosharaf, 2006). The decrease in the 1000 grain weight in drought stress is due to the disruption of grain filling, lack of dry matter or lack of material transfer to the grain. The researchers stated that drought stress reduces the number of grains and grain weight through the production of small grains even if the pollen grain is sufficient and proper fertilization takes place in the ovary (Li *et al*., 2014). The 1000 grain weight in corn depends on the ability of the plant to provide growing materials for the sinks and also environmental conditions such as the availability of moisture and nutrients when the grains are filled (Lack *et al*., 2006). The more the access to moisture and nutrients around the roots under drought stress conditions, the more the share of each tank of the available nutrients increases with the production of assimilated, and as a result, the grains become larger and the 1000 grain weight shows a significant increase (Mao *et al*., 2011). Lack *et al*. (2006) reported that the lower carbohydrate storage in the stems before pollination and the decrease in current photosynthesis due to the decrease in the durability of the leaf surface after flowering and the high respiration rate in the treatments of non-irrigation caused a decrease in the 1000 grain weight.

Table 6. Mean comparison of interaction effects of tratments on studied traits

Treatment		No. row per ear	No. grain per ear	1000 Grain weigh (g)	Grain yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Harvest index (%)
I_1	F_1	13 c*	21 de	237.5 b	5304 d	12988 d	40.8 de
	F_2	15 ab	24 bc	240.3 b	5672 c	14974 b	37.7 bc
	F_3	15 ab	25 ab	243.7 b	5913 b	14256 c	41.4 ab
	F_4	16 a	26 a	251.5 a	6552 a	15943 a	41.4 a
I_2	F_1	8 e	11 h	148.1 i	2010 k	6913 l	29.5 g
	F_2	9 de	13 g	160.6 h	2254 j	7324 jkl	30.7 fg
	F_3	9 de	14 fg	175.8 g	2341 ij	7599 jk	30.8 de
	F_4	10 d	14 fg	185.4 ef	2598 h	7745 ij	33.54 cde
I_3	F_1	14 bc	20 e	181.3 fg	3375 g	10655 h	30.6 ef
	F_2	15 ab	23 c	187.5 ef	3862 f	11432 fg	33.7 ef
	F_3	15 ab	25 ab	209.7 d	3798 f	11942 f	31.5 cd
	F_4	16 a	25ab	215.2 cd	4182 e	12261 e	34.1 b

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

I_1: full irrigation (control), I_2: non-irrigation from the flowering stage to the end of the growth period, I_3: non-irrigation from the grain filling stage to the end of the growth.

F_1: control, F_2: 1 mM salicylic acid, F_3: Nitroxin bacteria grain rub + Biophosphorus, F_4: Nitroxin bacteria grain rub + Biophosphorus along with salicylic acid foliar application.

The number of grains per ear and the 1000 grain weight are the most important traits effective in reducing corn yield during drought stress. In irrigation management, the usage of stress-reducing agents is a method that, while reducing severe damage to plants due to drought stress, also saves the amount of irrigation water (Li *et al*., 2014; Salemi and Mosharaf, 2006).

4.5. *Grain yield*

Drought stress caused a decrease in the yield components, after which the grain yield also decreased significantly (Table 2). Non-irrigation from the flowering stage to the end of the growth period (I_2) caused the maximum decrease in grain yield. The highest grain yield with an average of 5214 $kg.ha^{-1}$ belonged to the full irrigation treatment (control) (I_1). The lowest grain yield with an average of 2173 $kg.ha^{-1}$ belonged to the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) (Table 5). The effect of combination of growth-promoting bacteria and salicylic acid on grain yield was statistically significant (Table 2). The highest and lowest grain yield with an average of 6433 and 5190 $kg.ha^{-1}$ belonged to nitroxin + biophosphorus + salicylic acid treatment and control treatment, respectively (Table 5). The interaction effect of irrigation and combination of growth-promoting bacteria and salicylic acid had a significant effect on grain yield. The decrease in the 1000 grain weight and other yield components, including the number of rows per ear and the number of grains per row, caused a significant decrease in grain yield by non-irrigation and creating drought stress. The maximum grain yield was obtained with an average of 6552 $kg.ha^{-1}$ in full irrigation treatment (control) (I_1) and using nitroxin + biophosphorus + salicylic acid. Non-irrigation at the stages of flowering and grain filling showed a significant decrease in grain yield. The minimum grain yield with an average of 2010 $kg.ha^{-1}$ was achieved by non-irrigation from the flowering stage to the end of the growth period (I_2) and not using salicylic acid and growth-stimulating bacteria. However, with nitroxin + biophosphorus + salicylic acid treatment, grain yield increased from 2010 $kg.ha^{-1}$ to 2600 $kg.ha^{-1}$. Also, in the treatment of non- irrigation from the stage of grain filling to the end of the growth period (I_3) and the use of nitroxin + biophosphorus + salicylic acid, the grain yield increased by 907 $kg.ha^{-1}$ (Table 6). Following the reduction of the yield components such as the 1000 grain weight, the length and diameter of the cob, the number of rows and the number of grains per row in the condition of non-irrigation, the grain yield also decreased significantly, which was consistent with the results of other researchers in the same direction (Gardner *et al*., 1984 and Shoae Hoseini *et al*., 2008). Under the conditions of non-irrigation at different growth stages and full irrigation, nitroxin + biophosphorus + salicylic acid treatment showed a significant superiority in terms of grain yield compared to other treatments. The drought stress before flowering and during the flowering period caused non-synchronism of the emergence of male and female organs of corn and increased the interval of the emergence of corolla and silks, and as a result, with the decrease in the number of rows, the number of grains and the weight of grains, the yield of corn decreased. The occurrence of drought stress at the same time as the meiotic division in the corolla caused the pollen grains to become sterile, it prevented the elongation of the silks, and by reducing the number of rows per ear, the number of grains per row and the 1000 grain weight, it caused a sharp decrease in yield (Rashidi, 2005), as a result of any stress in these periods by reducing the number of grains per row, the number of rows per cob and the weight of the grain, it causes a sharp decrease in the grain yield. Grain yield potential at the time of flowering is a function of the previous growth of the plant, so the plants that faced drought stress from the flowering stage with nitroxin + biophosphorus + salicylic acid treatment have grown in less competitive conditions for moisture absorption than the control. As a result, by improving yield components, they had more yield potential. Drought stress and non- irrigation in corn causes a sharp decrease in egg fertilization and as a result, the number of grains per cob and then the grain yield decreases. Basically, in limited growth species such as corn, reproductive growth stages show great sensitivity to drought stress (Shoae Hoseini *et al*., 2008). The researchers reported that drought stress by non-irrigation reduces corn yield in three major ways. First, it reduces the total absorption of photosynthetically active radiation by the plant canopy due to the limitation of leaf development, temporary wilting, tuberization or premature senescence during periods of severe drought stress. Second, it reduces the efficiency of light consumption, which is an important limiting factor in corn yield under stress. Third, it lowers grain corn yield by reducing harvest index (the proportion of crop dry matter allocated to grain) (Earl and Davis, 2003). The increase in grain yield with the usage of salicylic acid and the combination with growth-promoting bacteria can be due to the increase in the 1000 grain weight, the reduction of disturbance in pollination and the photosynthetic stages of the plant and the transfer of materials to the grains (Kuiper *et al*., 1990). The increase in the 1000 grain weight in the combined treatment of bacteria and salicylic acid may be due to one of two reasons: 1- increasing the amount of photosynthetic substances introduced into the cob or 2- increasing the length of the grain filling period. One of the goals of plant hormones spraying is to increase the green period and photosynthetic activity of leaves to transfer more photosynthetic materials to grains (Garsia and Honowy, 2006). Poneleit *et al*., (2008) reported that corn grain weight is one of the effective components in yield regulation, but it has less sensitivity than other yield components. According to the report of Gardner *et al*., (1984) the hormones resulting from the activity of biological fertilizers and their foliar application by affecting the formation, growth and destruction of flowers and grains have an important effect on the relationship between the origin and destination of plants and it is possible through the effect indirectly affect the transfer speed on the need of the sink. Considering the physiological yield of these treatments, it is evident that these compounds can effectively enhance the targeted processes, mitigate the aging process and reduce plant sensitivity (Xie *et al*., 2003).

4.6. *Biological yield*

The effects of irrigation treatments and combination of growth-promoting bacteria and salicylic acid and their interaction on biological yield were significant at the 1% of probability level (Table 2). The highest biological yield with an average of 13110kg.ha^{-1} was obtained in the full irrigation treatment (control) (I_1). The lowest biological yield with an average of 6896 kg.ha^{-1} was obtained from the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2). The biological yield in the treatment of non-irrigation from the grain filling stage to the end of the growth period (I_3) was significantly higher than the flowering stage (Table 5). Following the increase in grain yield, the biological yield also increased significantly with the application of salicylic acid and growth-stimulating bacteria. The highest biological yield with an average of 15765kg.ha^{-1} was obtained from nitroxin + biophosphorus + salicylic acid treatment. The lowest biological yield with an average of 13615 kg.ha^{-1} was observed in the control treatment. No significant difference was observed between nitroxin + biophosphorus treatment and salicylic acid treatment in terms of biological yield (Table 5). With the non-irrigation and the creation of drought stress, following the reduction of vegetative and reproductive growth and yield of corn plants, the biological yield also decreased significantly. In two treatments of non-irrigation from the flowering stage to the end of the growth period (I_2) and non-irrigation from the grain filling stage to the end of the growth period (I_3), nitroxin + biophosphorus treatment and salicylic acid treatment increased the biological yield by 832 and 1606 kg, respectively. The highest biological yield with an average of 15943 kg.ha^{-1} was obtained from the interaction effect of full irrigation (control) (I_1) and nitroxin + biophosphorus treatment and salicylic acid treatment. The lowest biological yield with an average of 6913 kg was obtained from the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) and not using salicylic acid and growth-stimulating bacteria (Table 6). The decrease in grain yield under drought stress conditions and the lack of use of growth-promoting bacteria and salicylic acid also resulted in a decrease in biological yield. Nitroxin + biophosphorus treatment and salicylic acid treatment caused a significant increase in biological yield compared to other treatments under drought stress conditions. Non-irrigation treatments caused a decrease in yield components through the reduction of growth and production of assimilate, which was clearly evident in the reduction of biomass, and ultimately resulted in a decrease in grain and biological yield. In favorable and non-irrigation conditions, the application of nitroxin + biophosphorus and salicylic acid treatment, through more expansion and better continuity of the leaf surface, created a strong and sufficient physiological source for using the received light as much as possible and producing dry matter. The researchers stated that the stress during the vegetative period led to the shrinking of the leaves and reduced the leaf surface index during the ripening period and the amount of light absorption by the plant. In severe stress, the stomata were closed, in severe stress, the stomata were closed, carbon dioxide absorption and dry matter production decreased, and the continuation of stress caused a severe decrease in the intensity of photosynthesis (Gardner *et al*., 1984). These findings were consistent with the experimental results of Ma *et al*. (2014) Abedini and Sajedi (2014). Setter *et al*. (2001) also reported that the non-irrigation and the occurrence of moisture stress during the pollination stage and before that affected the process of grain formation in corn by reducing the photosynthesis of leaves. Non- irrigation significantly reduced biological yield by reducing the growth and accumulation of photosynthetic materials, as well as increasing the production of sterile pollen grains (caused by the lack of assimilates). Paknejad *et al.* (2010) reported drought stress had a significant effect on grain and biological yield and all yield components and caused a significant decrease in traits including biologic yield.

4.7. *Harvest index*

The drought stress, simultaneously with the non-irrigation in the stages of flowering until the end of the growth period (I_2) and the grain filling stage until the end of the growth period (I_3), significantly reduced the harvest index. The highest harvest index was observed in the full irrigation treatment (control) (I_1) with an average of 39.8%. The lowest harvest index with an average of 31.51% was obtained from the treatment of non-irrigation from the flowering stage to the end of the growth period (I_2) (Table 5). The treatment combination of growth-promoting bacteria and salicylic acid not only improved the yield and yield components, but also significantly increased the harvest index due to increasing the plant's access to nutrients, increasing the plant's drought tolerance and increasing growth. Among the different levels of treatment, the maximum harvest index with an average of 41% was obtained in nitroxin + biophosphorus + salicylic acid treatment and nitroxin + biophosphorus treatment (Table 5). The highest harvest index with an average of 41% belonged to the interaction effect of full irrigation (control) (I_1) and nitroxin + biophosphorus + salicylic acid treatment and nitroxin + biophosphorus treatment. In treatments of drought stress, including the non-irrigation from the flowering stage to the end of the growth period (I_2) and the non-irrigation from the grain filling stage to the end of the growth period (I_3) with the use of nitroxin + biophosphorus + salicylic acid, the harvest index increased, So that the harvest index in I_2 treatment was four percent higher than I3 treatment. The lowest harvest index with an average of 29.5% was obtained from the interaction effect of non-irrigation treatment from the flowering stage to the end of the growth period (I_2) in the condition of not using salicylic acid and growth-stimulating bacteria (Table 6). The length of the period and the speed of grain filling are important factors that determine the final weight of the grain. Since the process of transferring photo-synthetic materials from leaves or sharing them in grains is reduced under the influence of environmental stresses, especially drought and salinity, it may reduce the demand of the sink, therefore, in this way, the production of dry matter and grain yield are reduced, and then the harvest index also decreases (Mahdi Nezhad *et al*., 2015). Based on these results, it was found that with the interruption of irrigation, the decrease in grain yield is mainly affected by the yield components; therefore applying stress by non-irrigation at each stage of corn growth caused a decrease in yield, which also decreased the harvest index. The increase in the harvest index in the treatment of growth-promoting bacteria and salicylic acid under normal conditions and non- irrigation can be seen as the result of the increase in grain yield per unit area, which can be expressed as the number of rows per ear, the number of grains per row, and the 1000 grain weight. Nisar *et al*. (2016) and Farre and Fasi (2009) studied the drought stress on corn plants in different growth stages and concluded that the harvest index decreased with the decrease in grain yield. So that if drought stress and non-irrigation occurs in the vegetative growth and flowering stage, a greater decrease in grain yield and harvest index is observed. Memar and Mojdam (2015) and Shear Baf Khojasteh and Ahmadi (2017) concluded that drought stress caused a decrease in leaf area index and corn harvest index. In this research, non-irrigation from the flowering stage had the greatest effect on reducing the harvest index. The stress caused by the treatment of non-irrigation from the flowering stage reduced the grain yield to a greater extent than the biological yield, as a result of which the harvest index decreased significantly. However, the use of salicylic acid and growth-promoting bacteria, while increasing the yield of corn grains, also significantly increased the harvest index in both conditions of no irrigation and full irrigation. The high harvest index in nitroxin + biophosphorus + salicylic acid treatment means that the share of grains from the total dry matter produced by the plant has increased. In other words, a large amount of photosynthetic materials have been transferred to the grains. Because these materials are stored in the stems and sheaths of leaves (before pollination), facilitating their transfer increases the harvest index. In dry conditions, due to the reduction of transpiration, the transfer of materials takes place slowly. Therefore, the treatments that have a high harvest index under the condition of non-irrigation have performed better in the transfer of materials.

5. CONCLUSION

Non-irrigation from the flowering stage had the most adverse impact on the yield and yield components of grain corn, resulting in a substantial 59% reduction in grain yield compared to the control treatment. All yield components experienced a significant decrease under the influence of non-irrigation from the flowering stage, leading to a severe reduction in grain yield. The Nitroxin+biophosphorus+salicylic acid treatment resulted in the highest grain yield .The significant increase in grain yield observed in this combined treatment, under full irrigation conditions, can be attributed to several factors. Firstly, the treatment led to an improvement in the photosynthesis process, enhancing the overall productivity of the plants. Secondly, there was an increased accumulation of assimilates, providing more nutrients for the growth and development of the plants. As a result, the treatment positively influenced the number of grains per row, the number of rows per ear, and the 1000-grain weight, all contributing to the enhanced grain yield. The significant decline in grain yield observed in the treatment with non-irrigation from the flowering stage and the absence of salicylic acid and growth-stimulating bacteria application was primarily due to a considerable drop in the leaf area index and yield components. Under favorable irrigation conditions, Nitroxin + biophosphorus + salicylic acid treatment exhibited the highest grain yield.

ACKNOWLEDGMENT

The authors thank all colleagues and other participants, who took part in the study.

FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

FUNDING/SUPPORT: This study was done by support of Department of Agronomy, Islamic Azad University, Ahvaz Branch.

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