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Reducing the effects of drought stress in fodder corn by using salicylic acid and abscisic acid

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KEYWORDS

water stress, fodder corn, abscisic acid, salicylic acid, corn yield, protein

ABSTRACT:

This experiment was carry out in order to evaluate the effect of salicylic acid and abscisic acid on the agricultural and biochemical characteristics of fodder corn under different moisture regimes. Water stress decreased plant height, number of rows per cob, thousand seeds weight and corn yield. However, the amount of total protein, soluble carbohydrate, proline and malondialdehyde increased significantly under moisture stress conditions. Foliar spraying of abscisic acid and salicylic acid had a significant positive effect on the plant height, the number of rows in the corn, the weight of thousand seeds and the free proline of leaves. Also, spraying salicylic acid led to a significant increase in cornlength. Spraying of salicylic acid and abscisic acid led to a significant reduction of malondialdehyde. In this experiment, irrigation based on 75 and 50% of available water caused a significant decrease in agricultural traits and an increase in some biochemical traits related to stress in fodder corn. The use of chemical compounds increased the plant's resistance to dehydration by significantly increasing the amount of soluble sugars and dehydrin proteins, so that the yield of corns increased and the amount of the stress biomarker, malondialdehyde, decreased significantly. According to the results of the present research, it is not recommended to plant drought-sensitive corn cultivars in dry areas due to very low yield, but it is possible to increase corn resistance to drought by applying a reduced level of recommended irrigation along with salicylic acid and abscisic acid foliar spraying. and as a result increased the production of corns.

Introduction

After wheat and barley, corn is considered the most important food product in the world. Because in addition to being used as human food, it is also used as fodder for livestock and also in fermentation industries and the preparation of various industrial products (Emam, 2004). Due to the special characteristics of this plant, which is four-carbon and thermophilic, it is more compatible with arid and semi-arid areas. Water shortage stress is one of the main limiting factors for corn production, which during the growth period of this plant causes a sharp decrease in its yield (Kaman et al., 2011). Because the reduction of water absorption by the root system of the plant leads to the reduction of stomatal conductance and photosynthesis, as well as disrupting the hormonal balance in the plant (Augé, 2015). Also, water shortage stress induces a

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wide range of physiological and biochemical changes in plants (Garg et al., 2012; Maksup et al., 2014), which include the accumulation of stress metabolites as well as the increase in the accumulation of oxygen free radicals (Noreen et al., 2018).

One of the effective ways to increase the resistance of corn plants to water stress is the use of plant growth regulators such as salicylic acid (Latif et al., 2016), which is a pseudo-hormonal and internal growth regulator compound with a phenolic nature that is produced by root cells and is involved in regulating the physiological processes of plants such as the rate of photosynthesis, stomatal conductance and transpiration (El-Taib, 2005). When the plant is subjected to various stresses such as drought, salicylic acid (SA) activates the antioxidant system. Antioxidants effectively reduce or stop oxidative damage caused by free radicals and thereby contribute to cell health. In addition, abscisic acid (ABA) is also a stress hormone that coordinates the complex meshes of stress response and its amount changes rapidly in plants under stress. Accumulation of abscisic acid in various plant tissues due to living and non-living stresses such as drought, salinity, cold, darkness and pathogenic factors confirms its undeniable role as a message transfer agent (Hare et al., 1999).

Note that to the fact that most of the corn cultivation areas are facing the stress of water shortage, our knowledge about the effects of this stress on the growth and physiology indicators of the plant and the yield of fodder corn in the country is relatively limited, therefore, while increasing the efficiency of using water resources, using methods to deal with drought, such using of different chemical compounds is important to mitigate the negative effects of water stress. Considering the antioxidant role of the two mentioned hormonal compounds, in this research the effect of abscisic acid and salicylic acid application on reducing drought stress as well as yield and growth and biochemical indices of fodder corn was investigated.

Methods and materials

Specifications of the test site

In order to evaluate the effect of salicylic acid and abscisic acid on the agronomic and biochemical characteristics of fodder corn S.C647 single cross variety under different moisture regimes, the experiment was carried out in Fars province, an agricultural farm in the Takht Jamshid region, 60 km from Shiraz in agricultural year 1401-1400 was implemented.

Specifications of the test plan

The experiment was carried out in a split plot design in the form of a basic randomized complete block design with three replications. The main terrace included moisture regimes at three levels: I_1) irrigation based on 100% of available water, I_2) irrigation based on 75% of available water, I_3) irrigation based on 50% of available water, and the subterrace on three levels included: 1) control, 2) application of salicylic acid, 3) usage of abscisic acid were implemented.

Agricultural operations and test procedures

After leveling the land, it was divided into terraces measuring 3.5 x 75 meters, where each plot had five rows of crops at a distance of 75 cm and the distance between the rows was 18 cm. Different irrigation regimes were applied after conducting soil analysis and determining the field capacity and wilting point of the soil sample. The dietary regime treatment started from the 5-leaf stage and continued until the end of the growth period. Foliar spraying was done twice at intervals of 7 and 14 days after applying stress. Salicylic acid was sprayed at the rate of 140 mg/liter (Shemi et al., 2021) and abscisic acid at the rate of 50 micromolar (Panahian et al., 2021). In order to increase the solubility of salicylic acid with water, ethanol is used at a ratio of one gram per 10 mg (Shemi et al., 2021).

Properties studied in the field

Harvesting of the product was done manually in the pulp stage of the seeds. From each terrace, 20 plants were selected completely randomly considering marginal effects, and morphological traits including plant height, ear length, number of rows in an ear, thousand seeds weight and corn yield were measured and recorded. In this experiment, the physiological traits including the amount of free proline, malondialdehyde, the amount of total soluble proteins and the amount of soluble carbohydrates were measured.

Free proline index in the leaf: 0.5 g of the fresh plant material of the leaf was grinded in 10 ml of 3% sulfosalicylic acid in a porcelain mortar and completely homogenized. In

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the next step, 2 ml of filtered extract was dissolved with 2 ml of ninhydrin reagent (1.25 mg of ninhydrin in 30 ml of glacial acetic acid in mild heat. Then 20 ml of 6 mular phosphoric acid was added to it and mixed completely) and 2 ml of glacial acetic acid was added to each tube and then the tubes were placed in a Bain-Marie at 100 degrees Celsius for one hour. After that, put the tubes in an ice bath to finish the mentioned reaction. After the end of the reaction, 4 ml of toluene was added to each tube and vigorously vortexed for 30 seconds. Keep the tubes still for a while until the solution inside is completely separated into two phases. The supernatant which was toluene and proline was separated from the aqueous phase and its absorbance was determined with a spectrophotometer at a wavelength of 520 nm (Bates et al., 1973).

The amount of malondialdehyde: 0.25 grams of leaves were ground in a porcelain mortar containing 5 ml of 0.1% trichloroacetic acid (TCA). The resulting extract was centrifuged for 5 minutes at 10,000 rpm (Hs 18,500R) (Heath and Packer, 1968). In the next step, 250 microliters of the supernatant solution obtained from the centrifugation was mixed with 1 milliliter of malondialdehyde solution containing 20% trichloroacetic acid and 0.5% thiobarbituric acid were mixed. The taken mixture was heated for 30 minutes at a temperature of 95 degrees Celsius in a bain-marie bath. Then it was immediately cooled in ice and the mixture was again centrifuged for 10 minutes at 10000 rpm. The absorption intensity of this solution was measured using a spectrophotometer (UV-Vis Lamdba 25) at a wavelength of 532 nm. The absorbance of other non-specific pigments was determined at 600 nm and subtracted from the resulting value.

Flavonoid measurement: First, 0.1 g of the frozen sample was weighed and poured into a porcelain mortar, and then 10 ml of acidic ethanol (ethanol and acetic acid in a ratio of 99:1) was added to it. The taken solution was centrifuged at 8000 rpm for 10 minutes. The filtered supernatant solution was placed in a spa bath with a temperature of 80 degrees Celsius for 10 minutes. The absorbance was read at three wavelengths of 300, 330, and 270 using a spectrophotometer (Krizek, 1993).

Soluble carbohydrates measurement: 0.1 ml of alcoholic extract was mixed with three ml of freshly prepared anthrone (150 mg of anthrone + 100 ml of 72% sulfuric acid). This solution was placed in a boiling water bath for ten minutes to make the reaction and color. Then its absorbance was read with a spectrophotometer at a wavelength of 625 nm and the amount of dissolved sugars was calculated (Paquin and Lessure, 1358).

Total soluble protein measurement: The Bradford method (Bradford, 1976) was used to determine the amount of soluble protein. The basis of this method is based on the binding of coumaxi briant blue dye in the acid reagent to the protein molecule. The protein concentration in the sample was obtained according to the spectrophotometer number and using the standard curve, which was drawn using bovine albumin.

Data analysis

After collecting statistical data, SAS (v.9.4) and Excel (2010) software were used to analyze data and draw graphs. The mean of the treatments was compared through Duncan's test (based on significance at the level of one percent and five percent). Before analysis of variance, the normality of the data was checked. Also, before variance analysis, Bartlett's test was performed for the traits under study in order to ensure the homogeneity of the variances.

Results and discussion

The results showed that the independent effect of irrigation regimes (I) and application of salicylic acid and abscisic acid (F) on plant height, number of rows in the corn, thousand seeds weight and corn yield were significant (Table 1). The length of the corn was not significantly affected by the irrigation regime, but the use of chemical compounds caused a significant difference in this index between the treatments (Table 1). The number of rows in the corn was not significantly affected by the application of chemical compounds (Table 1). Irrigation based on 50% of available water (I_3) led to a significant decrease in plant height and the number of rows in the corn compared to non-stressed conditions (Table 2). Lack of water disrupts the physiological processes of plants and changes in the metabolism of carbohydrates and nitrogen, changes in the structure of proteins and the activity of enzymes, the accumulation of proline and the reduction of growth enhancers (Levitt, 1980).

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It leads to morphological changes in seeds, seedlings, leaves and plant height, etc. (Kramer, 1992). The results of Moharramnejad et al.'s research (2016) showed a decrease in plant height, number of rows per cob, weight of 1000 seeds and yield of hybrid corn due to drought stress. The reduction of plant height, number of rows in the corn and weight of thousand seeds of fodder corn under the influence of water shortage stress has been reported in many studies (Moharramnejad et al., 2015, Mohammadi Behmadi et al., 2017).

The negative effect of water deficiency on cell division (Ge et al., 2012), reduced leaf emergence speed, premature senescence due to the transfer of water from old leaves to young leaves (Warren et al., 2011), less radiation absorption due to Loss of leaves (Ge et al., 2012), decrease in the efficiency of radiation consumption (Earl and Davis, 2012) leads to a severe disturbance in photosynthesis. Coincidence of the stages that determine the number of seeds with water deficit, which reduces the storage of photosynthetic materials in the stem tissue, reduces the transfer of the stored materials to the seeds and shortens the period of seed filling (Ge et al. 212) leads to a decrease in the number of seeds and the weight of the seeds in corn decreases as a result of the decrease in the number of seed rows and corn yield (Ghobadi et al., 2017). In this regard, based on the results of Seifzadeh et al.'s research (1400), the stress of water shortage led to a delay in the beginning of corn growth and a lack of photosynthetic materials for corn growth, which ultimately resulted in a decrease in yield and yield components of two hybrid corn varieties. So that the yield decreased by 26% under the stress of water shortage compared to normal irrigation.

The application of salicylic acid and abscisic acid led to a significant increase in plant height and corn length (Table 3). Salicylate plays a key role in plants affected by stress, such as the effect on the absorption of mineral elements, membrane stability and water relations, stomatal function, inhibition of ethylene synthesis and growth improvement (Rehbarian and Sardoei, 2014). Studies have shown that treatment with salicylic acid leads to an increase in plant growth, photosynthesis, and as a result plant height, thousand seeds weight, and yield (Hayat et al., 2012). And the performance is in line with the research results of Shemi et al. (2021) in corn. In this regard, the results of Sam et al.'s research (2020) in corn 704 showed that salicylic acid treatment reduced the damage by 27% and 9% respectively in mild stress and severe water stress treatments in terms of yield.

Table 1- Mixed variance analysis of the effects of irrigation regimes and salicylic acid and abscisic acid on the agricultural characteristics of fodder corn

		Average of squares				
Sources of changes	df	Plant height	Corn length	number of rows in a corn	weight of thousand seeds	yield
Irrigation regime (I)	2	4396.29**	0.72^{ns}	28.16**	42895.18**	78342744.1*
Application of SA and ABA (F)	2	247.84**	4.58**	2.38^{ns}	3767.98**	3921436.9**
I × F	4	8.33^{ns}	1.01^{ns}	0.46^{ns}	220.44^{ns}	840009.3^{ns}
Total error	96	115.9	1.96	1.01	118.13	191453.1
coefficient of variation (percentage)		5.8	6.9	6.8	14.4	5.9

ns, * and **: non-significant and significant at the statistical levels of 5 and 1%, respectively

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Table 2- Comparison of the mixed mean effect of salicylic acid and abscisic acid on the agricultural characteristics of fodder corn

Irrigation levels based on	Plant height (cm)	Corn length (cm)	number of rows in a corn	weight of thousand seeds (gr)	Yield (kg per hectare)
100% of available water	19.3.4a	19a	15.9a	270.7a	8889.9a
75% of available water	190.5a	18.9a	16a	235.2b	7575.5b
50% of available water	176.5b	19.1a	14.9b	218.7c	6172.9c

In each column the same letters indicate the absence of significant differences between the treatments based on Duncan's multi-range test.

Table 3- Comparison of the mixed average effect of salicylic acid and abscisic acid on the agricultural characteristics of fodder corn

Application of salicylic acid and abscisic acid	Plant height (cm)	Corn length (cm)	number of rows in a corn	weight of thousand seeds (gr)	Yield (kg per hectare)
Witness	184.3b	20.2b	14.4c	236.9b	6911.4c
Salicylic acid	187.8a	20.8a	14.9a	250ab	7044.3b
Abscisic acid	188.3a	20.3b	14.6b	252.4a	7233.4a

In each column the same letters indicate the absence of significant differences between the treatments based on Duncan's multi-range test.

Irrigation regimes also had a significant effect on total leaf protein, soluble carbohydrates, proline and malon in aldehyde of corn plants (Table 4). The stress of water shortage led to a significant increase in these indicators. The increase of these indicators was more noticeable in irrigation conditions based on 50% of available water. (Table 5).

It seems that the increase in the amount of total protein due to water shortage stress is due to the higher expression of stress proteins, based on the study of Riccardi et al., (1998) and Tida et al. (2006), drought stress can increase production of some proteins soluble dehydrin, which are known as stress proteins. The increase of soluble sugars during stress can be explained by the stoppage of growth or the synthesis of these compounds from non-photosynthetic pathways, as well as the destruction of insoluble sugars, which also causes the increase of soluble sugars (Draikewicz, 1994). Under environmental stress conditions, plants accumulate organic solutions with low molecular weight such as amino acids and sugars (Bajji et al., 2001). Kazemi et al.'s study (207) showed that lack of water led to a 55% increase in soluble corn plant sugar. The results of several studies indicated the accumulation of carbohydrates during drought stress (Luca and Osterhuis, 2013; Yooyongwech et al., 2013; Baslam and Goicoechea, 2012; Zafari et al., 2017).

One of the mechanisms of resistance of plants against drought stress is the mechanism of proline accumulation (Sanchez et al., 2008). When the plant is subjected to drought stress, the breakdown of proteins and as a result the increase of amino acids and amides accelerates. One of these amino acids is proline (Barker et al., 1993). As a soluble substance, proline regulates the osmotic pressure, reduces the loss of water from the cell, prevents the breakdown of different proteins, stabilizes the natural form of proteins, and as a result protects the membrane systems (Barker et al., 1993). Proline in the

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tissues of stressed plants is due to the increase in its synthesis by peproline-5-carboxylase synthetase and the decrease in the breakdown of proline oxidase enzyme. Based on the research carried out on different corn hybrids under similar water deficit conditions, it was observed that the amount of proline accumulation in them has a positive correlation with the grain yield stability (O'regan et al., 1993).

Oxidative stress during drought stress and the increase of free radicals by reducing the antioxidant defense leads to the damage of tissues, lipids, proteins and nucleic acids, and the concentration of biomarkers such as malondialdehyde increases (Jose et al., 1993). When many plants are placed in a dry environment, they are seriously damaged and their malondialdehyde content increases (Sanooka, 2004). There are reports about the reduction of malondialdehyde due to the preservation of membrane lipids from the damage induced by oxygen free radicals (Ozdemir et al., 2003) in a research on the level of the biomarker malondialdehyde increased due to water stress in corn (Shemi et al., 2021).

The results showed that the effect of using salicylic acid and abscisic acid (F) on the amount of free proline and malondialdehyde in leaves was significant (Table 4). The use of salicylic acid and abscisic acid led to a significant increase in the amount of proline and a decrease in malondialdehyde compared to the control (Table 5). In line with the results of the present study, treatment with salicylic acid led to a reduction in oxidative stress by improving the increase in proline levels under drought conditions (Rabieh et al., 2021). Under drought stress, the amount of malondialdehyde increased, and salicylic acid and abscisic acid foliar application led to a significant decrease in the malondialdehyde biomarker. Reducing the amount of malondialdehyde by spraying salicylic acid under water stress conditions is in line with the research results (Rabiea et al., 2021; Shemi et al., 1400; Maqsoudi et al., 2019) in corn.

Conclusion: In this experiment, irrigation based on 50 and 75% of available water caused a significant decrease in agricultural traits and an increase in some biochemical traits related to stress in fodder corn. Water shortage stress caused a decrease in corn yield and an increase in total protein, soluble carbohydrate, proline and malondialdehyde. But foliar application of abscisic acid and salicylic acid led to a significant increase in plant height, number of rows per corn, thousand seeds weight and free leaf proline compared to the control treatment (no foliar application). According to the above results, it can be said that the resistance of the studied corn variety to water shortage is low because the yield and growth indicators of corn showed a significant decrease against water stress. But the use of chemical compounds increased the plant's resistance to dehydration by significantly increasing the amount of soluble sugars and the amount of dehydrin proteins, because soluble sugars play a role in maintaining the osmotic balance of cells, maintaining the enzyme structure and protecting against free radicals, and increasing the proteins stress preserves the osmotic power of the plant (Shao et al., 2006), in addition, in the condition of dehydration, the superoxide radicals resulting from drought stress increased the rate of lipid peroxidation because the product of this reaction, which is malondialdehyde, increased, which with the application Chemical compounds showed a significant decrease in the production rate of this drought biomarker. Water shortage stress led to a decrease in yield and yield components of fodder corn variety. According to the results of the present research, it is not recommended to plant drought-sensitive corn cultivars in dry areas due to very low yield, but it is possible to increase corn resistance to drought by applying a reduced level of recommended irrigation along with salicylic acid and abscisic acid foliar spraying and as result, it increased the production of corns so that it is economical to plant in semi-arid areas.

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Table 4- Mixed variance analysis of the effects of irrigation regimes and salicylic acid and abscisic acid on the biochemical characteristics of fodder corn

		Average of squares			
Sources of changes	df	The amount of total protein	Soluble carbohydrates	Proline	Malondialdehyde
Irrigation regime (I)	2	414.57**	331.57^{ns}	76.53**	3.292**
Application of SA and ABA (F)	2	31.46 ^{ns}	27.23 ^{ns}	1.14*	1.95**
I × F	4	4.77^{ns}	3.56^{ns}	0.29 ^{ns}	0.015 ^{ns}
Total error	96	0.8	0.51	0.23	0.022
coefficient of variation (percentage)		8.6	9.5	8.4	10.2

ns, * and **: non-significant and significant at the statistical levels of 5 and 1%, respectively

Table 5- Comparison of the mixed mean effect of salicylic acid and abscisic acid on the agricultural characteristics of fodder corn

Irrigation levels based on	The amount of total protein	Soluble carbohydrates	Proline	Malondialdehyde
100% of available water	7.73c	5.68c	4.25c	1.2c
75% of available water	10.29b	8.84b	5.68b	1.49b
50% of available water	12.92a	10.27a	6.99a	1.71a

In each column the same letters indicate the absence of significant differences between the treatments based on Duncan's multi-range test.

Table 6- Comparison of the mixed mean effect of salicylic acid and abscisic acid on the agricultural characteristics of fodder corn

Application of salicylic acid and abscisic acid	The amount of total protein	Soluble carbohydrates	Proline	Malondialdehyde
Witness	9.88a	7.82c	5.48b	1.48a
Salicylic acid	11.03a	8.9a	5.9a	1.09c
Abscisic acid	10.03a	8.03bc	6a	1.26b

In each column the same letters indicate the absence of significant differences between the treatments based on Duncan's multi-range test.

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