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ROMANIAN AGRICULTURAL RESEARCH, NO. 40, 2023
First Online: January, 2023. DII 2067-5720 RAR 2022-65

THE EFFECT OF PLANTING DATE AND IRRIGATION REGIMES ON YIELD AND CHLOROPHYLL CONTENT, OSMOLYTES AND ANTIOXIDANT ENZYMES IN SWEET CORN (*Zea mays* L. var saccarata)

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ABSTRACT

The occurrence of drought stress in most areas of Iran with limited water reserves, the need to use agricultural techniques and adaptation measures with the aim of helping crop producers to choose the appropriate planting date and optimal use of irrigation water to increase corn production is necessary. In order to investigate the yield, chlorophyll content, osmolytes and the activity of antioxidant enzymes in sweet corn (cv. Amylopop) in response to drought stress and delay in planting, an experiment was conducted as split plots based on a randomized complete block design with three replications during two growing seasons (2018 and 2019) at Razavi Khorasan Province, Iran. The treatments included the irrigation as the main factor in three levels [100% water requirement, 80% water requirement (mild stress), 60% water requirement (severe stress)] and planting date as a sub-plots in three levels (16 May, 26 May and 5 June). The findings showed that grain yield and leaf chlorophyll content decreased by 62% and 40% by delay in planting and increased drought stress caused by low irrigation regime, respectively, compared to the control (no stress and timely cultivation), and also, glycine betaine, proline, soluble sugars and antioxidant enzymes contents increased. Therefore, according to the climatic conditions of the Mashhad region, water loss through surface evaporation is much less at the beginning of the growing season, so it is necessary to choose the well-timed planting date and not to use a low irrigation strategy to achieve the maximum grain yield.

Keywords: antioxidant enzymes, irrigation regime, osmolyte, planting date, sweet corn.

INTRODUCTION

Corn (*Zea Mays* L.) is one of the most important crops, which ranks third among cereals after wheat and rice (Kikakedimau et al., 2022). The area under maize cultivation in the world is reported to be more than 130 million hectares, and Iran's share of this is estimated at 234 thousand hectares (Iran's Ministry of Agriculture, 2020). Maize is one of the crops that is very sensitive to drought, and its yield decreases by more than 50% under drought stress conditions (Alvi et al., 2022). Various physiological, molecular and genetic approaches have been used to deal with drought stress and increase the yield of agricultural products. Other methods include conventional and molecular breeding. However, all these approaches are very costly, time-consuming and sometimes do not even help to develop desirable drought tolerance traits (Ahmar et al., 2020). One of the effective measures to save agricultural water consumption is to use the strategy of low irrigation and planting date. In this situation, the plant is given less water than the actual need, but reducing the amount of water allocated for plant irrigation happens with a special logic and management, so that even the least possible stress is applied to the plant and the amount of crop reduction is insignificant compared to the benefit of the amount of water storage. The success of this strategy will be when the farmer is fully

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Received 5 October 2022; accepted 6 December 2022.

aware of his plant's response to lack of irrigation in all stages of the growth period and the appropriate planting date for the region (Cao et al., 2022; Palash et al., 2021).

In a study in the Kermanshah region, west of Iran, on corn production reported that the 40% less water consumption caused a 69% decrease in grain yield compared to the no water stress (Palash et al., 2021). Mirshakarnejad et al. (2020) investigated the effect of planting date and different irrigation regimes on grain yield and water consumption efficiency in grain maize. The results indicated that choosing an early planting date and avoiding excess irrigation according to the plant's requirements are important to achieve the maximum grain yield and improving water consumption efficiency. In another study, Karimi et al. (2019) evaluated the effect of interruption of irrigation on corn grain yield and physiological characteristics. They found that during the pollination and seed filling stages, even two weeks of interruption of irrigation causes significant damage to grain yield, and the crop should not face the problem of water shortage or delay in irrigation.

The chlorophyll content and photosynthetic pigments are among the most important factors affecting the photosynthetic capacity of plants, because they directly affect the photosynthesis rate and ultimately the amount of biomass (Rahemi Karizaki et al., 2020). In general, grain photosynthetic assimilates are the result of two sources, the first is current photosynthesis and the second is remobilization of assimilates stored in the stem and other parts of the plant before pollination (Zhang et al., 2019). There is a high correlation between leaf chlorophyll content and photosynthesis rate, so that low chlorophyll content under drought stress conditions is a sign of oxidative stress that may cause pigment photo-oxidation and chlorophyll degradation (Yusefi et al., 2017). Khayatnezhad and Gholamin (2020) in a study on durum wheat cultivars reported a decrease in the chlorophyll content with increasing drought stress. Also, a decrease in the corn leaves chlorophyll content under severe drought stress conditions (40% of crop capacity) has been reported (Alvi et al., 2022).

In drought stress conditions, plants increase the production of osmolytes or osmotic protectors such as proline, soluble sugars and glycine betaine as a defense mechanism (Shafiq et al., 2021). Glycine betaine is a quaternary ammonium compound that is synthesized in chloroplasts in response to abiotic stress factors such as drought and salinity (Nazar et al., 2020). Glycine betaine not only acts as an osmotic regulator, but also stabilizes the structure and activity of enzymes and protein complexes and maintains the integrity of membranes against the damaging effects of drought (Shafiq et al., 2021).

Another effect of drought stress is the Reactive Oxygen Species (ROS) production during various abiotic stresses. During drought stress, many ROS are produced such as singlet oxygen, hydroxyl ions, superoxide radicals and hydrogen peroxide. These have destructive effects on the membrane system of plants (De Vasconcelos, 2020). Antioxidants prevent oxidative damage caused by ROS through inhibition mechanism. These antioxidants are classified into two categories of enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), polyphenol oxidase (PPO), and ascorbate peroxidase (APX). Non-enzymatic antioxidants include α-tocopherol, beta-carotene, cysteine, glutathione and ascorbic acid (Soares et al., 2019). All these antioxidants collectively work to neutralize ROS and thus play a defensive role during oxidative stress under drought stress (Farooq et al., 2009). Yusefi et al. (2017) in a two-year study on two varieties of corn found that drought stress caused a significant decrease in grain yield and leaf chlorophyll content compared to non-stressed conditions, while drought stress caused a significant increase in glycine betaine, proline, soluble sugar levels and catalase enzyme activity. Shafiq et al. (2021) in a study on two varieties of corn under drought stress conditions caused by shortage of irrigation (60 and 75% of crop capacity) compared to the control (100% crop capacity) found that under drought stress conditions, antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POD), osmolytes such as proline and glycine betaine had a significant increase in the leaves. In contrast, the concentration of chlorophyll (a, b and total) had a significant decrease.

The occurrence of drought stress in most areas of Iran with limited water reserves, the need to use agricultural techniques and adaptation measures with the aim of helping the crop producers in choosing the appropriate planting date and optimal irrigation water use to increase production and productivity is inevitable; Therefore, the current research was carried out in order to determine the appropriate planting date and control the water requirement of the plant with the aim of minimizing the risk of grain yield reduction and greater water productivity in sweet corn in the cold and dry climate of Eastern Iran.

MATERIAL AND METHODS

This study was performed at training and research farm of the Islamic Azad University, Mashhad branch, Razavi Khorasan Province, Iran (36°33´N, 59°11´E, 1176 m.a.s.l). The split plot experimental design was based on randomized complete blocks with 3 replications.

Before conducting the experiment, soil samples were taken from the depth of 0 to 30 cm and the physical and chemical properties of the soil were determined. Based on the results, the soil texture was loamy sand (Table 1). The experimental treatments include irrigation regime as the main factor in three levels [100% water requirement, 80% water requirement (mild stress), and 60% water requirement (severe stress)] and planting date as a sub-plots factor in three levels (16 May, 26 May and 5 June) which were implemented during the two growing seasons (2018 and 2019). Cv. amylopop was selected.

Table 1. Physiochemical properties of the soil of the experimental site (0-30 cm depth)

Soil texture	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	K_2O (mg/kg)	P_2O_5 (mg/kg)	Nitrogen (%)	EC (ds/m)	pH
Sandy-loam	21.1	38.9	40	0.3	346	33	0.028	2.79	7.42
Sandy-loam	20.8	37.8	38	0.23	357	34.10	0.024	2.84	7.33

The desired field was prepared every year with a plow and a disk. Each plot included 4 planting rows with 75 cm distance between the rows and 18 cm on the row, and the row length was 5 m. 3 no planting rows were considered between two sub-plots and two main plots. The distance between both blocks was about 2 m. The plant density was 75000 plants per hectare in both growing season. Plant water requirement was calculated based on NETWAT software. In this experiment, the irrigation method was drip irrigation with a plate-type tape, the distance between the drippers was 30 cm, and the water amount in each irrigation was calculated using a volumetric meter. Additional plants were thinned at the stage of 4-6 leaves. In both growing season, 200 and 100 kg/ha of phosphorus and potash were added to the land at the beginning of land preparation and before planting, respectively. Also, 300 kg of urea fertilizer was used, so that one third was used at the beginning of planting and the rest was used in two stages in the middle of the vegetative period. Also, weed control was done manually and no special poisons were used due to the absence of pests and diseases.

To measure grain yield, the two side rows of each plot, half a meter from the beginning and end of each planting line were removed as removing the marginal effect, and the rest of the plot was harvested. Also, samples from the upper young leaves of the plants were also selected to measure quality traits such as compatible osmolytes (proline, soluble sugars and glycine betaine) and antioxidant enzymes.

Chlorophyll a, b and total were measured according to Arnon (1949). The soluble sugars content was measured by the method of Kochert (1978). Proline and glycine betaine contents were measured according to the methods of Bates et al. (1973) and Grieve and Grattan (1983), respectively. The method of Nakano and Asada (1981) was also used to measure ascorbate peroxidase enzyme. Measuring catalase and superoxide dismutase were based on the methods of Aebi (1984) and Giannopolities and Ries (1977), respectively.

Before performing any analysis, the test of uniformity of variances was performed and it was observed that the error variance between two different growing seasons is not significant. Therefore, the experiment was analyzed in the form of a combined analysis design using SAS software, and the means comparison was done by LSD test at the level of 5% (Sousa et al., 2018).

RESULTS AND DISCUSSION

Weather conditions

A significant difference was observed in the comparison of rainfall between two growing seasons, so that growing season 2019 (72.03 mm) was wetter than 2018 (47.94 mm). Also, the highest rainfall occurred in May in both growing seasons. The average minimum temperatures in 2018 and 2019 growing seasons were from 10.84 to 19.40°C and 11.64 to 19.97°C, respectively.

Indeed, the average maximum monthly temperature during 2018 and 2019 growing seasons were from 25.45 to 36.43°C in May and from 26.45 to 33.56°C in July, respectively, but almost no significant difference was observed between the two growing seasons in terms of temperature (Table 2).

Table 2. Average minimum and maximum temperature and total monthly rainfall related to the growth period of corn in Mashhad weather conditions during two growing seasons 2018 and 2019

Month/Year	Precipitation (mm)		Mean of maximum temperature (ºC)		Mean of minimum temperature (ºC)	
	2018	2019	2018	2019	2018	2019
May	38.11	50.72	25.45	26.45	11.22	12.68
June	9.22	21.2	31.97	32.13	16.33	15.37
July	0	0.1	36.43	36.09	19.40	19.97
August	0	0	32.84	32.69	16.38	16.18
September	0.61	0.01	28.82	28.49	10.84	11.64

Grain yield

The results of combined variance analysis showed that the effect of year (at 5% level), irrigation regime, planting date and planting date × irrigation regime on grain yield (at 1% level) were significant (Table 3). Mean comparison showed that the highest grain yield was observed in 2019 (Table 4). The findings revealed that the highest grain yield (5968 kg/ha) was obtained from the planting date of 16 May with no stress condition, and the lowest grain yield (2284 kg/ha) was obtained from severe stress condition (providing 60% of the water requirement). In such a way, this grain yield decrease was significant and about 62%. On the other hand, in other planting dates compared to the first planting date the grain yield reduction was observed by 16, 8.62 and 28% in each stress level, respectively. Therefore, planting date could reduce the effects of drought stress to some extent.

Table 3. Combined variance analysis of grain yield, osmolytes, antioxidant enzymes, photosynthetic pigments, sweet corn soluble sugars under the influence of planting date and low irrigation treatments

S.O.V	df	GY	Chla	Chlb	TChl	SS	Pro	GlBe	APX	SOD	CAT
Y	1	3563310*	55780**	37059**	183773**	0.047**	0.854**	465**	0.001*	1036**	1.21**
R (Y)	4	192298^{ns}	8745^{ns}	1853^{ns}	17969^{ns}	0.005^{ns}	0.004^{ns}	49.87^{ns}	0.001^{ns}	25.740^{ns}	0.008^{ns}
DI	2	21182439**	211637**	44608**	450356**	0.924**	3.767**	1320**	0.005**	158^{ns}	25.467**
Y×DI	2	101154^{ns}	366^{ns}	108^{ns}	585^{ns}	0.002^{ns}	0.011*	19.61^{ns}	0.001^{ns}	1.609^{ns}	0.099**
Eror	8	63747	780	2738	3093	0.003	0.002	20.76	0.001	40.849	0.005
SD	2	5602131**	71833**	97828**	336303**	0.159**	0.295**	571**	0.001**	154 **	8.849**
Y×SD	2	16960^{ns}	6241^{ns}	245^{ns}	8964*	0.002^{ns}	0.001^{ns}	23.74^{ns}	0.001^{ns}	0.154^{ns}	0.029^{ns}
DI×SD	4	1480195**	43634**	37672**	136844**	0.323**	1.190**	481**	0.001**	170**	26.519**
Y× DI× SD	4	124281^{ns}	313^{ns}	213^{ns}	811^{ns}	0.004^{ns}	0.004^{ns}	26.93^{ns}	0.001^{ns}	0.379^{ns}	0.108**
Error	24	62993	2336	587	2232	0.002	0.004	36.72	0.001	31.694	0.013
CV	-	6.09	5.71	5.26	3.61	7.22	3.26	5.61	6.70	8.06	4.63

ns, *, **, respectively: Non Significant, Significant at 5% and 1% probability; Y, Year; R, Replication; DI, Deficit Irrigation; SD, Sowing Date; GY, Grain Yield; Chla, Chlorophyll a; Chlb, Chlorophyll a; TChl, Total chlorophyll**;** SS, Soluble sugar; Pro, Proline; GlBe; Glycine betaine; POD, Peroxidase; APX, Ascorbate peroxidase; SOD, Superoxide dismutase; CAT, Catalas.

Table 4. Mean comparison of grain yield, osmolytes, antioxidant enzymes, photosynthetic pigments, soluble sugars of sweet corn between two growing seasons 2018 and 2019

Year		GY	TChl	Chlb	Chla	SS	Pro	GlBe	APX	CAT	SOD
2018		3863b	1249b	434b	815 b	0.683a	2.075a	111a	0.040a	2.566a	74.246a
2019		4377a	1365a	487a	879 a	0.624b	1.823b	106b	0.038b	2.267b	65.483b
DI	SD	GY	TChl	Chlb	Chla	SS	Pro	GlBe	APX	CAT	SOD
100	1	5968a	1599a	602a	996a	0.423d	1.697d	89.91e	0.0223f	0.9485g	58.19c
	2	4452c	1402bc	487c	915b	0.415d	1.611e	99.23d	0.0248e	1.283f	71.74a
	3	5549b	1443b	457d	986a	0.340e	1.339f	94.57b	0.0180g	0.9532g	72.15a
80	1	4057d	1457b	544b	912b	0.352e	1.738d	103.3cd	0.0291d	2.875c	69.83ab
	2	3911d	1408c	525b	882b	0.955a	1.563e	110.4bc	0.0360c	1.680e	72.47a
	3	3503e	1359cd	507c	852c	0.974a	2.250b	101.4d	0.0496b	4.139b	65.00b
60	1	3966d	1327d	479cd	848c	0.874b	2.014c	111.8b	0.0491b	1.913d	73.85a
	2	2284f	1205e	4218e	783d	0.830b	3.042a	127.5a	0.0708a	6.688a	74.94a
	3	3395e	985f	357f	289f	0.715c	2.287b	113.7b	0.0486b	1.268f	70.62ab

In each column means with similar letters had not significant difference at 5% probability; GY, Grain Yield (kg/ha); Chla, Chlorophyll a (μg. g^{-1}.fw); Chlb, Chlorophyll a (μg. g^{-1}.fw); TChl, Total chlorophyll (μg. g^{-1}.fw)**;** SS, Soluble sugar (mg/g wfl); Pro, Proline (μ mol/gr); GlBe; Glycine betaine (μg/gr dwl); APX, Ascorbate peroxidase (μmol H_2O_2 $min^{-1}mg^{-1}$ protein); CAT, Catalas (μmol H_2O_2 $min^{-1}mg^{-1}$ protein); SOD, Superoxide dismutase (Unit/min g fw).

Chlorophyll content

The findings illustrated that the effects of year, irrigation regime, planting date, and planting date × irrigation regime on chlorophyll a, b and total chlorophyll contents were significant at the level of 1%, while the effect of planting date × year was significant only on total chlorophyll at the level of 5%. Other interaction effects on these traits were not significant (Table 3). The comparison between two growing seasons showed that the chlorophyll contents (a, b and total) in the second growing season was significantly different from the first year (Table 4). In general, the mean comparison of the effects of irrigation regime × planting date indicated that the chlorophyll content in the plant leaves decreased by the delay in planting and amount of water required reduction. So that the highest chlorophyll a and b total chlorophyll content were observed in the common planting date of the region and in the conditions of full water supply, and the lowest in the third planting date and in the conditions of 60% water supply. This reduction rate compared to the control (16 May and the condition of providing the full water requirement) for chlorophyll a, b and total was 71, 40 and 41%, respectively (Table 4).

In both growing seasons of the experiment, the chlorophyll content on the first planting date was higher than other planting dates. However, the chlorophyll content in the first planting date in the second growing season was higher than the first growing season at the level of 5%. The lowest total chlorophyll content was related to the second planting date in the first growing season. Although it was not significantly different from the third date in this growing season at the 5% level (Figure 1).

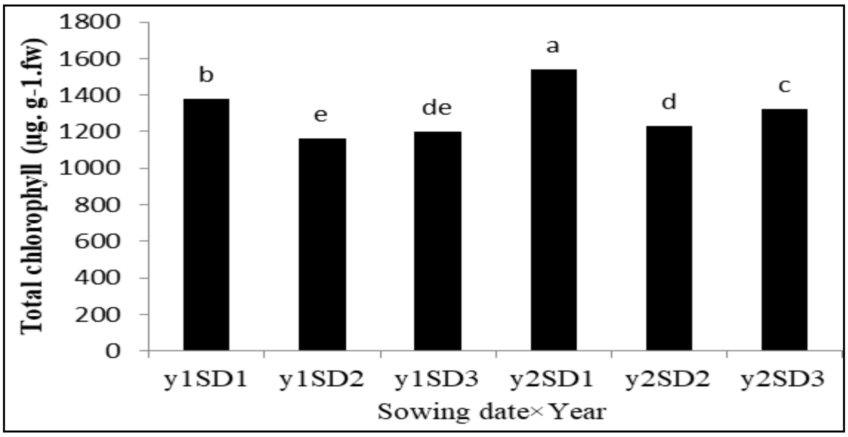

Figure 1. Interaction of year × planting date for total chlorophyll content (µg/g fresh weight); Y1SD1, planting date 16 May in the first year, Y1SD2, planting date 26 May in the first year, Y1SD3, planting date 5 June in the first year, Y2SD1, planting date 16 May in the second year, Y2SD2, planting date 26 May in the second year, Y2SD3, planting date 5 June in the second year.

Soluble sugars

The results showed that except the effect of year, irrigation regime, planting date and planting date × irrigation regime, which were significant on the f soluble sugars content at the level of 1%, other interaction effects on this trait were not significant (Table 3). Although the accumulation of soluble sugars in the first growing season was higher than the second growing season, it was not significant (Table 4). Also, a statistically significant difference was observed between 60% and 80% irrigation regimes, but there was a significant difference compared to full irrigation.

Antioxidant enzymes

The effect of year, planting date and planting date × low irrigation on all studied antioxidant enzymes were significant at 1% level. Also, the effect of dehydration on the component of superoxide dismutase enzyme on other antioxidant enzymes was significant at the level of 1% (Table 3).

The results showed that the amounts of antioxidant enzymes were higher in the first year of the experiment than in the second year of the experiment (Table 4). Also, the highest amount of antioxidant enzymes was observed in severe drought stress caused by providing 60% of water requirement and in the conditions of the second planting date, which is most likely due to the exposure of the plant to high temperatures during the growing season. In general, the change in catalase and ascorbate peroxidase enzymes was much higher compared to superoxide dismutase enzyme, and in general, drought stress caused an increase of approximately 2.5 times in the amounts of APX and SOD enzymes and three times in CAT enzyme compared to non-stress conditions (Table 4). and the highest soluble sugars content were observed in providing full and 80% water requirements, respectively (except for the first planting date). So that the soluble sugars content in low irrigation regime was almost double compared to full irrigation regime (Table 4).

Proline and glycine betaine

The effect of year, irrigation regime, planting date and planting date × irrigation regime on the proline and glycine betaine contents were significant at the 1% level. Also, the interaction effect of low irrigation × year was significant only on proline content at 5% level. While other interaction effects on these traits were not significant (Table 3).

The results showed that the accumulation rate of proline and glycine betaine osmolytes was higher in the leaves of corn in the first growing season than the second growing season (Table 4). In this experiment, planting delay at each irrigation regime did not have a significant effect on content of proline and glycine betaine osmolytes, although they were statistically placed in different groups at the 5% level (Table 4), but in general, with the increase of drought stress caused by lack of irrigation in each growing season, the content of these osmolytes, especially proline, has increased in corn leaves under conditions of 60% water supply (Figure 2).

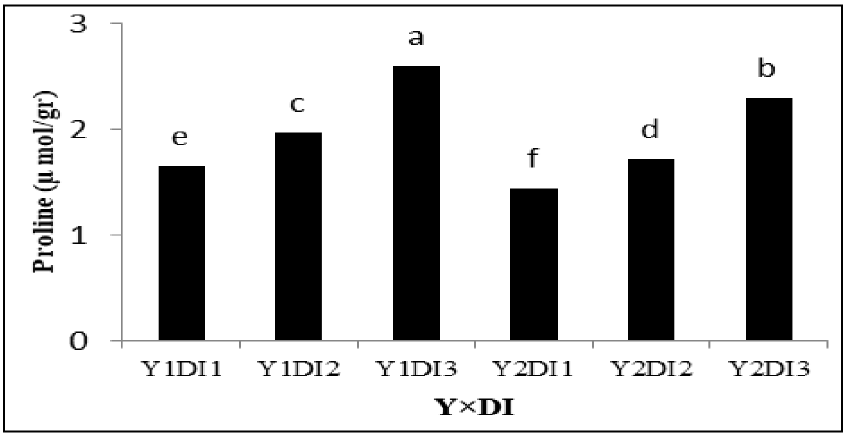

Figure 2. Interaction of year in irrigation regime for proline (µmol/g wet weight); Y1DI1, 100% water supply in the first year, Y1DI2, 80% water supply in the first year, Y1DI3, 60% water supply in the first year, Y2DI1, 100% water supply in the second year, Y2DI2, 80% water supply in the second year, Y2DI3, 60% water supply in the second year.

Antioxidant enzymes

The effect of year, irrigation regime, planting date and planting date × irrigation regime were significant on all studied antioxidant enzymes at 1% level. Also, the effect of low irrigation on antioxidant enzymes except superoxide desmutase enzyme was significant at 1% level (Table 3).

The results showed that the antioxidant enzymes content were higher in the first growing season than the second growing season (Table 4). Also, the highest antioxidant enzymes content was observed in severe drought stress caused by providing 60% of water requirement at the second planting date, which is most likely due to the exposure of the plant to high temperatures during the growing season. In general, the change in catalase and ascorbate peroxidase enzymes was much higher compared to superoxide dismutase enzyme. In general, drought stress caused an increase of approximately 2.5 times in ascorbate peroxidase and superoxide dismutase enzymes and three times in catalase enzyme compared to non-stress conditions (Table 4).

In areas where water shortage is a limitation in agriculture, if the negative effects of water stress on crop yield are not significant, low irrigation strategy can be recommended to save water consumption and increase the cultivated area of crops with saved water. But due to the significant difference between grain yields at different of irrigation regime and 62% decrease in grain yield due to 40% reduction in water supply compared to full irrigation, and on the other hand, a significant decrease (about 32%) in grain yield on the first planting date in stress levels compared to the first planting date in full irrigation, it can be said that probably with temperature increasing the rate of evaporation will be increase. As a result, when corn is planted on the common planting date of the region (16 May), the temperature, evaporation and transpiration reach their maximum value after 75 days from the planting date (Table 4). Under these unfavorable conditions, the increase in stress of humidity and heat in the pollination sensitive stage with disruption in the reproductive organs function and the decrease in the pollination percentage in florets have been increasingly involved in the production of cobs with seedless heads, which has ultimately led to a decrease in yield. However, facing the late ripening period in June 15 planting date with cool temperatures at the beginning of the autumn season caused the little opportunity to compensate the decrease in grain yield through current photosynthesis (Mirshakarnejad et al., 2020). In such a way that the results of this research are in accordance with the results of other researchers in Kermanshah region, west of Iran (Palash et al., 2021). Therefore, if the goal is to harvest the grain of corn, the low irrigation strategy is not recommended in the Mashhad region in eastern Iran, because changing the planting date will not compensate for this decrease in yield.

One of the methods of evaluating and predicting the tolerance of crops to drought stress is to study the amount of changes that occur in leaf chlorophyll synthesis due to water shortage. Stomata of leaves are partially or completely closed during drought stress and this natural process disrupts photosynthesis. In this research, low irrigation and delay in planting date compared to the full irrigation conditions and timely planting date caused about 40% reduction in corn leaf chlorophyll. Therefore, drought stress has a direct effect on the reduction of leaf chlorophyll index in plants. So that the stems are the most important source of carbohydrates during the grain filling, the these carbohydrates content is reduced by photosynthesis reduction in the delay in planting date and drought stress, and while affecting the stem diameter, it has a negative effect on the grain filling, as a result, the grain weight and grain yield will be decrease (Yusefi et al., 2017). These results agree with the observations of Khayatnezhad and Gholamin (2020) on Durum wheat and Alvi et al. (2022) on corn.

The results of this experiment showed that the accumulation of soluble sugars increased about two times in drought stress compared to the control. Soluble sugars are among compatible osmolytes, which increase under stress conditions, and their accumulation causes the protection and stability of membranes and. Because the increase of soluble sugars is one of the mechanisms of increasing the osmotic pressure inside the cell, the plant tries to neutralize the osmotic pressure of the environment and absorb more water from the soil. This mechanism causes stability of cell membrane, proteins, increase of photosynthesis and drought resistance (Gaffney et al., 2021).

In this experiment, drought stress caused by low irrigation regime increased glycine betaine and proline (Table 4 and Figure 2). Glycine betaine can destroy free radicals only by activating antioxidants, but proline, in addition to activating antioxidants, can also directly inhibit free radicals (Nazar et al., 2020), in addition, glycine betaine can protect photosynthetic activities including photosynthetic enzymes, proteins and lipids in the thylakoid membrane and electron flow in the combination of photosystem II (Shafiq et al., 2021). Studies on corn under drought stress conditions reported the increase in osmolytes such as soluble sugars, proline and glycine betaine (Shafiq et al., 2021; Yusefi et al., 2017).

Drought stress is one of the main factors in reducing crop production. This stress causes oxidative stress by disrupting the balance between the reactive oxygen species production and the plant's antioxidant defense activities. The results showed that drought stress caused a significant increase in catalase and ascorbate peroxidase enzyme antioxidants compared to the control (Table 4). Findings showed that the planting date did not have a significant role in increasing the activity of these enzymes. Catalase and ascorbate peroxidase act as antioxidant enzymes and play an important role in removing and scavenging hydrogen peroxide produced in peroxisomes and reducing the destructive effects of reactive oxygen species. The increase in these two enzymes activity under stress conditions indicates that these enzymes play a key role in cleaning reactive oxygen species (De Vasconcelos, 2020). The increase in ascorbate peroxidase and catalase activity under drought stress conditions has been reported in many studies (Soares et al., 2019; De Vasconcelos, 2020; Shafiq et al., 2021).

Also, drought stress did not cause a significant increase in superoxide dismutase enzyme, although it was statistically significant (Table 4). The decrease in superoxide dismutase activity enzyme is the imbalance of defense mechanisms components, which causes their poor function. In this way, the key points of metabolism and defense mechanisms of the cell are attacked by the accumulation of active forms of oxygen, and these factors have caused a reduction in the superoxide dismutase enzyme activity (Soares et al., 2019). It can also be said that in this experiment, this enzyme did not play an important role in the defense mechanisms under severe stress conditions, and other enzymes and antioxidant factors play a defensive role for the plant in these conditions. For this reason, it can be said that different antioxidant enzymes coordinate with each other for proper homeostasis in high concentrations of H_2O_2.

This means that, different categories of enzymes are activated in different ranges of drought stress (Shafiq et al., 2021), and which probably does not play an important defensive role in the studied corn (cv. Amylpop) under severe drought stress. Antioxidants play an important role in maintaining the oxidative balance of cells, because these metabolites have the ability to react directly with reactive oxygen species and collect them, so that the decrease in the activity of some antioxidant enzymes is compensated by the increase in the activity of other enzymes (Farooq et al., 2009).

CONCLUSIONS

The results showed that with the delay in planting date and the increase of drought stress due to low irrigation regime, the grain yield and leaf chlorophyll content decreased significantly compared to the control (no stress and timely cultivation). Also the glycine betaine, proline, soluble sugars and antioxidant enzymes content increased. Therefore, according to the climatic conditions of the Mashhad region in eastern Iran, water loss through surface evaporation is much less at the beginning of the growing season, so it is necessary to choose the planting date on time and not to use a low irrigation strategy to achieve the maximum grain yield in sweet corn.

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