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Research article

Effect of deficit irrigation on physiological, biochemical, and yield characteristics in three baby corn cultivars (Zea mays L.)

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ABSTRACT

The main problem in the production of crops in arid and semi-arid regions of the world is the lack of water and its effect on the plant in the form of drought stress. Cultivation of key crops such as corn, which also requires a lot of water, is not possible in these areas except by applying water consumption management methods. Among the most important of these methods is deficit irrigation. The effect of deficit irrigation on relative water content (RWC), malondialdehyde (MDA), compatible osmolytes (proline and soluble sugars), antioxidant enzymes, and yield was studied in three baby corn cultivars in a field experiment using a randomized complete block design (RCBD) with split-plots and three replications. Three levels of deficit irrigation (0, 20, and 40% deficit) constituted the main plots and three cultivars of baby corn (Challenger, Basin, and Passion) constituted the sub plots. Analysis of variance showed that deficit irrigation had a significant effect on all variables. Cultivar (Challenger, Basin and Passion) had a significant effect on proline (0%, 41.5% and 73.2%), carbohydrates (23.9%, 15.4% and 0%), and MDA content (0%, 26.1% and 41.2%), as well as peroxidase (POD) (0%, 136.1% and 227.9%) levels respectively. The interaction between deficit irrigation and cultivar had a significant effect on proline, carbohydrates, and POD. RWC decreased (26.9, 6.5 and 0%) with increasing irrigation deficit (0, 20 and 40%) respectively while proline (0, 23.7 and 64.8%), carbohydrates (0, 29.7 and 34.09%), catalase (CAT) (0, 20.8 and 70.1%), and POD (0, 55.05 and 113.2%) increased under the same conditions. Carbohydrate content was higher in the Basin and Challenger cultivars (21.71 and 19.07) and proline (145.9), POD (193.9), and MDA content (8.53) were higher in the Passion cultivar. Among the studied cultivars, the highest yield was achieved by the Passion cultivar (37.02 and 62.9% more than Challenger and Basin cultivars respectively). In general, the results showed that drought stress caused an increase in compatible osmolyte content and the activity of antioxidant enzymes. However, this increase could not offset the effects of drought stress on yield in the 40% deficit treatment.

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1. Introduction

The dominant and major cultivars cultivated in the region are these cultivars. More than 90% of the production in the region is related to the three studied cultivars. The production of this product in the region is over 35 tons. Which is of interest to farmers due to the high price of this product. Baby Corn is a dehusked ear of corn that is harvested before pollination, 2 or 3 days after the emergence of silk. This product is suitable for crop rotation because of its multiple uses as a vegetable in human nutrition, quality green fodder for livestock feed, and raw material for the canning and pickling industries [1,2]. Maize is a crop with almost high water requirements. Daily corn water use averages between 0.15 and 0.20 inches per day [3].

In recent years, demand for this product has been increasing in Iran. However Iran's arid climate and limited of water resources can put plants under drought stress [4].

Drought stress reduces yield through three mechanisms. First, drought stress reduces the absorption of photosynthetically active radiation (PAR) by the canopy through the reduction of leaf area [5]. Second, yield is affected due to reduced light utilization efficiency per unit of absorbed light, which is measured by measuring the dry matter accumulated per unit of absorbed light during a certain period of time [6]. Finally, drought lowers yield through an immediate decrease in the exchange of CO_2 per unit of absorbed light [7]. Water limitation has been shown to lead to reduced growth and yield in sesame [8,9].

Abiotic stress in plants can disrupt electron transport chains in organelles such as chloroplasts and mitochondria. Under these conditions, molecular oxygen (O_2) pairs with a free electron and initiates the accumulation of reactive oxygen spices (ROS). ROS such as the hydroxyl radical (OH^-), superoxide radical (O_2^-), and hydrogen peroxide (H_2O_2) are strong oxidizers and are therefore harmful to cellular integrity [10–12].

Antioxidants are molecules that destroy ROS and delay damage to cells [13]. Antioxidant activity varies from species to species, but the antioxidant system in all plants consists of an enzymatic antioxidant system and a non-enzymatic antioxidant system [14,15]. The most important components in the enzymatic antioxidant system include superoxide dismutase, catalase (CAT), ascorbate peroxidase (APX), and peroxides (POD) especially glutathione peroxide (GPX) [16].

One of the mechanisms employed by plants to respond to environmental stress is osmotic adjustment. Osmotic adjustment occurs as an adaptation to water stress through the accumulation of soluble substances inside cells to maintain turgor and the related processes at low water potentials. During abiotic stresses such as drought, organic molecules with lower molecular weight such as proline, proteins, betaine, and soluble sugars contribute to osmotic adjustment in the below- and above-ground parts of plants [17].

Drought stress is the most important type of abiotic stress experienced by crops in Iran, with most summer crops experiencing some degree of drought stress. Therefore, it is necessary to conduct experiments on the response of different cultivars under stress conditions. In this study, the effects of deficit irrigation on the activity of antioxidant enzymes and osmotic adjustment was investigated in three cultivars of baby corn.

2. Materials and methods

2.1. Study area

The effects of deficit irrigation on relative water content (RWC), malondialdehyde (MDA), compatible osmolytes, antioxidant enzyme activity, and yield of three baby corn cultivars were studied in a field experiment conducted at the Education-Research Farm of the Faculty of Agriculture, Mashhad Branch, Islamic Azad University (Mashhad, Iran) during the 2018–2019 growing season. The farm is located in a region with an arid and semi-arid climate (37°33′ N, 59°11′ E), at an elevation of 1176 m above sea level.

2.2. Experimental design

The experiment was carried out using a randomized complete block design (RCBD) arranged in split-plot with three replications. Deficit irrigation at three levels (0% deficit (i.e., full irrigation), 20 and 40% irrigation deficit) constituted the main plots and three cultivars of baby corn (Challenger, Basin, and Passion) constituted the sub plots.

2.3. Statistical analysis

Statistical data analysis and analysis of variance was conducted in SAS 9.1. Means were compared using Duncan's multiple range test at $p < 0.05$.

Table 1
The results of analysis of physicochemical properties of soil and manure of the study area.

Texture	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	Potassium (mg/kg)	Phosphorus (mg/kg)	Nitrogen (%)	Electrical conductivity	acidity
Sandy-loam	21.1	38.9	40	0.3	346	33	0.02	2.79	7.42

2.4. Soil properties and cultivation

Based on a soil test (Table 1) and local recommendations, mineral fertilization was performed using ammonium phosphate (200 kg/ha), potassium sulfate (200 kg/ha), and urea (300 kg/ha). Ammonium phosphate, potassium sulfate and 50% of the urea fertilizer were applied during the preparation of the cultivation beds and the remaining 50% of the urea fertilizer was applied as top dressing at the 10–12 leaf stage. Each sub-plot consisted of four 5-m rows spaced 60 cm apart. Thus, the dimensions of each sub-plot were 5 × 2.4 = 12 m^2. The distance between the rows was 60 cm and the distance on the row was 20 cm. According to the length of the rows of 5 m, 20 plants were planted in each row. More than 125 plants were cultivated in each sub-plot. Sub-plots and main plots were separated by one and two unplanted rows, respectively. The blocks (i.e., replications) were spaced 2 m apart. Seeds were sown in early May and were immediately irrigated.

2.5. Irrigation terms

The plants' water requirement was determined using the Netwat. Irrigation was performed through drip irrigation using a drip tape with a dripper spacing of 20 cm. The amount of water used in the treatments was calculated by placing a container under one of the drippers, calculating the output per unit of time, and multiplying the volume by the number of drippers per plot. During the growth of the plants, agricultural operations such as weeding and pest and disease control were carried out according to common practices.

2.6. Determination of relative water content (RWC)

To measure RWC, osmotic adjustment, and enzyme concentrations, young upper leaves were randomly selected from plants in the two middle rows of each plot. Leaves selected for RWC measurement were placed in plastic bags to prevent water loss and were immediately transported to the laboratory. The rest of the samples were stored at −70 °C until further analysis. RWC was measured according to Ref. [18] by taking 5 samples of the last developed leaves in each plot. Leaf sections with an area of 1 cm^2 were prepared and their fresh weight (FW) was determined to the closest 0.0001 g. Turgid weight (TW) was measured following 24 h of refrigeration at 4 °C in Petri dishes containing distilled water. Dry weight (DW) was measured following drying for 48 h at 70 °C in an oven. RWC was calculated using the following equation.

$$\text{RWC}\ (\%) = (\text{FW-DW/TW- DW}) \times 100$$

2.7. Determination of proline

The method proposed by Bates et al. was used to extract and measure proline [19]. For this purpose, 500 mg of leaf tissue was homogenized in 10 ml of sulfosalicylic acid 3% (w/v). Next, 2 ml of the resulting solution, 2 ml of Ninhydrin reagent, and 2 ml of glacial acetic acid were mixed in a test tube and placed in a water bath (100 °C) for 1 h. Then, 4 ml of toluene was adding to tube and stirred vigorously for 30 s, separating the contents of the tube into two phases. After 20 min, light adsorption of the solution was read at 520 nm and the concentration of proline (in $\mu g.\ g^{-1}$.FW) was calculated using the standard curve. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

$$[(\text{ug proline/ml x ml toluene}) / 115.5\ \mu\text{g/umole}]/[(\text{g sample})/5] = \mu\text{moles pro-line/g of fresh weight material}$$

2.8. Determination of glycine betaine

Glycine betaine was measured according to Ref. [20]. First, the samples were dried in an oven and 20 ml of distilled water was added to 0.5 g of dried leaf tissue and placed on a shaker for 48 h at 25 °C. Next, 1 ml of the resulting extract was mixed with 1 ml of 2 N sulfuric acid and placed in an ice water bath. Finally, 0.2 ml of potassium iodide and 0.2 ml iodine were added to the mixture. The mixture was centrifuged for 15 min at 0 °c at 10,000 rpm and light absorption was read at 365 nm.

2.9. Determination of carbohydrates

Carbohydrates (i.e., soluble sugars) were measured according to Ref. [21]. First, 0.2 g of leaf tissue and 10 ml of 95% ethanol were placed in a closed test tube and heated in a water bath at 80 °C for 1 h. After cooling, 1 ml of the sample was mixed with 1 ml of 0.5% phenol and 5 ml of 98% sulfuric acid. Finally, absorption was read at 483 nm, and the extracted carbohydrate content was calculated in µg glucose per g FW.

2.10. Determination of peroxidation of membrane lipids

Peroxidation of membrane lipids was measured based on the formation of a complex between malondialdehyde and thiobarbituric acid (TBA). Peroxidation was evaluated according to Heath and Packer [22] using malondialdehyde (MDA) concentration as an indicator of fatty acid peroxidation. MDA content was calculated (in $\mu g.g^{-1}$ FW) by measuring absorption at 792 and 244 nm.

2.11. Determination of catalase activity

The catalase activity was measured according to Ref. [23]. First, 100 μl of enzyme extract was poured into 28 ml of potassium phosphate buffer, and 30 μl of H_2O_2 was added. At a wavelength of 240 nm, the potassium phosphate buffer became zero, and 30 s after the first optical absorption reading, the second optical absorption of the samples was read again.

2.12. Determination of peroxidase content

Peroxidase (POD) content was measured according to Ref. [24] Three ml of a reaction mixture consisting of 50 mM sodium-potassium phosphate buffer (pH = 6.6), 1% guaiacol, 3% hydrogen peroxide, and 600 μl of enzyme extract was used for this purpose. Peroxidase activity was determined based on the oxidation rate of guaiacol at 470 nm, using an extinction coefficient of $\varepsilon = 26.6\ mM^{-1}Cm^{-1}$.

2.13. Determination of yield indices

Harvesting was done in the middle three rows of each plot and after excluding a 0.5 m margin the each end of the rows ($1.8 \times 4.2 = 7.2\ m^2$). A standard ear of baby corn had to be 1–2 cm in diameter and 10–12 cm in length.

3. Results and discussion

Soil drought that seriously restrict plant productivity, maize (Zea mays L.) is known for its sensitivity to abiotic stresses, which often results in substantial loss in crop productivity [25]. Drought tolerances mechanisms involving morphological adaptation, hydraulic control, hormonal regulation, osmotic adjustment, ion homeostasis, and antioxidant defense [26]. Yield components were highly sensitive to combined drought stress. Stomatal conductance is disrupted during drought stress and plays a major role in the final yield of ears, especially during the stage of ear filling [27].

3.1. Relative water content (RWC)

The effect of deficit irrigation on RWC was significant ($p < 0.01$). However, cultivar and the interaction between deficit irrigation and cultivar had no significant effect on this variable (Table 2). Comparison of means showed that RWC decreased significantly with increasing irrigation deficit (i.e., increased drought stress intensity). Compared to the no-deficit treatment, RWC decreased by 16 and 21% in the 20 and 40% deficit treatments, respectively (Table 3). Decreased RWC due to drought stress has been observed in several studies [28], which is consistent with our observations. RWC is as an important indicator in selecting cultivars for drought tolerance [29] due to the close relationship between leaf RWC and water potential, opening and closing of stomata, photosynthesis, biomass production, and yield [30,31].

3.2. Proline content assessment

The effect of deficit irrigation, cultivar, and the interaction of the two factors on proline content was significant ($p < 0.01$) (Table 2). Increased irrigation deficit lead to significantly higher proline content in all 3 cultivars. The highest proline content was observed in the Passion cultivar under 40% deficit and the lowest proline content was observed in the Challenger cultivar under 0% deficit (Table 5). Numerous studies have shown that plants accumulate different types of compatible osmolytes such as proline as an adaptation mechanism against drought tolerance [32]. An increase in proline content as a result of drought stress has been reported in corn cultivars [33], cotton cultivars [34] and two species of *Adonis* [35]. Proline stabilizes the three-dimensional structure of proteins, protects the photosynthetic system and cell membranes [36], contributes to osmotic adjustment [37], and deactivates ROS [32] under

Table 2

Analysis of variance for measured traits.

S.O.V	df	RWC	Proline	Carbohydrates	MDA	CAT	POD	Husked yield	Dehusked yield	Dehusked standard ear yield
Replication	2	ns	ns	ns	–	ns	ns	ns	ns	ns
Deficit irrigation (A)	2	**	**	*	**	**	**	**	**	*
Ea	4	–	–	–	–	–	–	–	–	–
Cultivar (B)	2	ns	**	**	**	ns	**	**	**	**
A × B	4	ns	**	**	ns	ns	**	ns	**	**
Eb	12	–	–	–	–	–	–	–	–	–
CV (%)		4.9	8.9	16.6	10.7	15	5.7	12.74	13.59	10.44

The *, * *, or ns indicate statistical differences at $p \leq 0.05$, $p \leq 0.01$, or non-significant, respectively.

Table 3
Effect of deficit irrigation on measured traits.

Deficit irrigation (%)	RWC (%)	Proline (µg. g^{-1} FW)	Carbohydrates (mg.g^{-1} FW)	MDA (µg. g^{-1} FW)	CAT (u. mg^{-1} FW)	POD (u. mg^{-1} FW)	Husked yield (kg/ha)	Dehusked yield (kg/ha)	Dehusked standard ear yield (kg/ha)
0	83.5a	90.1c	15.75b	5.79c	2.21b	83.84c	4373a	2708a	547.5a
20	70.1b	111.5b	20.44a	6.61b	2.67b	130b	3353a	1941 ab	486.1a
40	65.8b	148.5a	21.12a	9.8a	3.76a	178.8a	2062b	1162b	365.3b

Different letters in each column represent statistically significant differences at 5%.

Table 4
Effect of cultivar on measured traits.

Cultivar	Proline (µg. g^{-1} FW)	Carbohydrates (mg. g^{-1} FW)	MDA (µg. g^{-1} FW)	POD (u.mg^{-1} FW)	Husked yield (kg/ha)	Dehusked yield (kg/ha)	Dehusked standard ear yield (kg/ha)
Challenger	84.2c	21.71a	6.04c	59.12c	2947b	1453c	323.7a
Basin	119.2b	19.07 ab	7.62b	139.6b	3169b	1991 ab	437.2a
Passion	145.9a	16.52b	8.53a	193.9a	3672a	2367a	575.8b

Different letters in each column represent statistically significant differences at 5%.

Table 5
Effect of deficit irrigation and cultivar on proline, carbohydrates, POD, dehusked yield and dehusked standard ear yield.

Deficit irrigation (%)	Cultivar	Proline (µg. g^{-1}FW)	Carbohydrates (mg.g^{-1} FW)	POD (u. mg^{-1}FW)	Dehusked yield (kg/ha)	Dehusked standard ear yield (kg/ha)
0	Challenger	64.26c	14.98d	45.07f	2292.2c	435.1c
	Basin	106.1c	15.86d	89.24d	3068.4b	644.3b
	Passion	100.1cd	13.68d	117.21c	3344.9a	802.7a
20	Challenger	84.68d	24.49 ab	57.26f	1865.7c	335.8c
	Basin	104cd	18.22cd	127.3c	2176.2c	435.2c
	Passion	145.8b	16.39d	205.4b	2512b	577.7b
40	Challenger	103.9cd	25.64a	75.03e	1177.9d	200.2d
	Basin	149.6b	23.14abc	202.3b	1212.9d	230.4d
	Passion	191.8a	19.5bcd	259a	1577.8c	347.1c

abiotic stress. Verbruggen and Hermans state that the increase in proline in drought tolerant cultivars is greater than drought-sensitive cultivars [36]. This is closely related to increased drought tolerance and adaptation to dry conditions.

3.3. Sugar content evaluation

The effect of deficit irrigation ($p < 0.05$), cultivar ($p < 0.01$), and the interaction of the two factors ($p < 0.01$) was significant on carbohydrate content (Table 2(. Increasing irrigation deficit led to an increase in carbohydrate content in all three cultivars. The highest carbohydrate content was observed in the 40% deficit treatment for the Challenger cultivar, showing a significant difference compared to other cultivars and treatments except the Basin treatment under 40% deficit. The lowest carbohydrate content was observed in the Passion cultivar under non-deficit conditions (Table 4). The accumulation of soluble sugars in response to drought stress has been reported in different plants [38,39]. Drought stress increases soluble sugars by breaking down and reducing starch content through increased amylase activity [40]. The increase in soluble carbohydrate content prevents cell dehydration and thus maintains turgor pressure, protects cell membranes, and prevents the destruction of proteins under stress. In addition, the increased concentration of soluble sugars helps the plant survive under stress by supplying a supplementary source of energy [41].

3.4. Malondialdehyde (MDA)

The effect of deficit irrigation and cultivar on MDA content was significant ($p < 0.01$) but the interaction of the two factors had no significant effect on this trait (Table 2). Comparison of means showed that increased drought stress significantly increased MDA content; MDA content increased from 5.79 µg. g^{-1}.FW in the no-deficit treatment to 9.8 µg. g^{-1}.FW in the 40% deficit treatment, representing an increase of about 41% (Table 3). The difference between cultivars was also significant. The highest MDA content was observed in the Passion cultivar and the lowest in the Challenger cultivar (Table 4). We also observed increased MDA levels under drought stress. The cultivars responded differently in terms of MDA content under these conditions as well. Similar results have been observed in corn cultivars [33], two species of Adonis [35], and wheat cultivars [42], which indicates damage to cell membranes due to drought stress. Higher MDA content in the Passion cultivar indicates higher peroxidation of membrane lipids. Peroxidation of cell membrane lipids as a result of ROS activity is one of the most important negative effects of drought stress [43]. MDA is a product of cell membrane lipid peroxidation and is known to be an important indicator for assessing free radical damage to cell membranes in stress conditions [37]. MDA content was higher in the Passion cultivar, which may be due to increased peroxidation of membrane lipids.

3.5. *Catalase (CAT)*

Deficit irrigation had a significant effect ($p < 0.01$) on CAT activity but cultivar and the interaction of the two factors did not exhibit significant effects (Table 2). The 40% deficit treatment caused a significant increase in CAT activity. The highest CAT activity was observed in the 40% deficit treatment, which was significantly higher than the other two treatments (Table 3). Similar increases in catalase activity have been reported in corn cultivars [33], pea [44], and beans [45]. Catalase is an important antioxidant enzyme and an increase in its activity promotes resistance to stress. In this regard, Koca et al. observed that salinity stress increased catalase activity in two sesame cultivars [46]. Furthermore, the tolerant cultivar had more catalytic activity than the sensitive cultivar. Catalase plays an important role in reducing the destructive effects of ROS. This enzyme breaks down the hydrogen peroxide produced in peroxisomes into water and oxygen and thus prevents oxidative damage to cells [35,47].

3.6. *Peroxidase (POD)*

The effects of deficit irrigation, cultivar and the interaction of the two factors on POD activity were significant ($p < 0.01$) (Table 2). Drought stress significantly increased POD activity in all cultivars. The highest POD activity was observed under the 40% deficit treatment for the Passion cultivar followed by the Basin cultivar. We also observed significantly higher POD activity in the Challenger cultivar under 40% deficit irrigation compared to the no-deficit treatment, but the figures were smaller compared to the other two cultivars under the same treatment (Table 5). The presence of ROS accelerates the peroxidation of membrane lipids [48,49]. Lipid peroxidation and the increase in ROS concentration can lead to cell membrane rupture in plants under stress. Several studies have shown that increased peroxidase activity helps cells survival in stressful conditions due to the important role of the enzyme in breaking down hydrogen peroxide and neutralizing the destructive effects of ROS [50,51].

3.7. *Yield indices*

3.7.1. *Husked yield*

The effects of deficit irrigation and cultivar on husked yield were significant ($p < 0.01$) but their interaction had no significant effect on this trait (Table 2).

3.7.2. *Dehusked yield*

The effects of deficit irrigation, cultivar, and their interaction were significant on dehusked yield ($p < 0.01$) (Table 2). The highest dehusked yield was observed under the no-deficit treatment for the Passion cultivar, which was significantly higher than all other treatments except the Basin cultivar under no-deficit irrigation. The lowest dehusked yield was observed at 40% deficit for the Challenger cultivar (Table 5).

3.7.3. *Dehusked standard yield*

The effects of deficit irrigation ($p < 0.05$), cultivar and their interaction ($p < 0.01$) on dehusked standard ear yield were significant (Table 2). The highest dehusked standard ear yield was achieved under the no-deficit treatment for the Passion cultivar, which was significantly higher than all other treatments except the Basin cultivar under the no-deficit treatment. The Passion cultivar had the highest yield, likely due to the higher activity of catalase and peroxidase and increased levels of compatible osmolytes such as proline, which work together to reduce damage by ROS. The lowest dehusked standard ear yield was observed under the 40% deficit treatment for the Challenger cultivar (Table 5).

It has been reported that drought stress leads to a decrease in photosynthetic efficiency, which can affect plant performance and the rate of nutrient transfer to seeds [52] Compared to the control treatment, husked yield, dehusked yield, and dehusked standard ear yield respectively decreased by 23.5, 22, and 11% in the 20% deficit treatment and by 52, 53, and 33% in the 40% deficit treatment [53].

4. Conclusion

In this study, deficit irrigation (up to 40%) reduced RWC and increased MDA, compatible osmolytes (proline and carbohydrates), and the antioxidant activity of CAT and POD. This indicates that the plants were in a state of drought stress. The response of the cultivars to stress conditions was not uniform. In the Challenger and Basin cultivars, soluble sugars increased more markedly whereas the Passion cultivar showed a larger increase in proline and POD. MDA content was higher in the Passion cultivar. Despite the overall decrease in yield under water stress conditions, the Passion cultivar had the highest yield.

Author contribution statement

Golnaz Bazrgar, Mohsen Ghasemi and Saeid Khavari Khorasani: Conceived and designed the experiments; Performed the experiments.

Golnaz Bazrgar and Alireza Kelidari and Seyed Mohsen Nabavi Kalat: Wrote the paper.

Saeid Khavari Khorasani, Seyed Mohsen Nabavi Kalat and Mohsen Ghasemi: Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

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