

Corn (*Zea mays* **L.) Physiology and Yield Afected by Plant Growth Regulators Under Drought Stress**

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Abstract

It is important to fnd methods, which may improve corn physiology, including nitrogen (N) metabolism, and yield production under drought stress. The use of plant growth regulators (PGR) is among the methods, which has been found efective on the alleviation of drought stress on corn physiology and yield. However, in this research, some new PGR, which has been rarely investigated and may improve plant nitrogen (N) under drought stress including (A) control, (B) 6-benzyl adenine (10,000 mg/L), (C) proline (2.5 ml/L), (D) glutamine (1 ml/L), (E) $B+C$, (F) $B+D$, (G) $B+C+D$, and (H) superoxide dismutase (2.5 ml/L) were proposed and examined on corn (genotype Single Cross 640) physiology and yield components under feld conditions. The experiment was a split plot on the basis of a completely randomized block design with three replicates, and in addition to PGR (subplots) the main plots (drought stress) based on 70 (D1), 90 (D2) and 110 mm (D3) of evaporation from an evaporating pan were examined. Diferent corn physiology- and yield-related components including relative water (RW) and proline contents (Pro), weight of 100 grains (100GW), number of grains per corn (NGC), biological yield (BY), corn fresh yield (CFY), and grain yield (GY) were determined. According to the results, corn physiology and yield components were signifcantly afected by drought stress as Pro increased and RW and yield-related components decreased. However, interestingly the use of PGR (treatment G) signifcantly improved corn physiology and yield components by increasing RW (to a maximum of 63.81%), CFY (from a minimum of 80,542 kg/ha at control to a maximum of 100,263 kg/ha), and BY (from a minimum of 49,842 kg/ha at control to a maximum of 62,277 kg/ha). Although the efect of PGR was not statistically signifcant on GY, treatment G resulted in a 2500-kg increase compared with control. The interaction of drought stress and PGR signifcantly afected diferent corn physiology- and yield-related components except NGC and BY. The most efective PGR treatment on the alleviation of drought stress on corn physiology and yield production was treatment G containing 6-benzyl adenine, proline, and glutamine. It is possible to improve corn physiology and yield production under drought stress using the PGR tested in this research.

Keywords 6-Benzyl adenine · Biological yield · Glutamine · Grain number and weight · N metabolism · Proline · Relative water content · Super oxide dismutase

Abbreviations

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Introduction

Plant growth and yield production are negatively afected under drought stress. The tolerance of diferent crop plants difers under drought stress as some crop plants are more tolerant and some are semi-tolerant and some are sensitive. Plants by themselves can utilize diferent mechanisms including morphological (Mohammadi and Asadi-Gharneh [2018\)](#page-9-0) and physiological alteration, to enhance their tolerance under drought stress. The following are among the most important mechanisms utilized by plants under drought stress: (1) morphological alteration such as rolling the leaves, (2) production of diferent osmoprotectants such as proline, (3) activation of antioxidants including the enzymatic and non-enzymatic molecules, (4) production and alteration of diferent plant hormones, (5) activation of stress responsive genes, (6) activation of diferent signaling pathways, (7) regulation of plant stomata activity, and (8) exudation of root metabolites afecting the microbial activity as well as the symbiotic association of plant with the soil microbes (Sajedi et al. [2011](#page-9-1); Miransari et al. [2011a,](#page-9-2) [b;](#page-9-3) Khan et al. [2015](#page-9-4); Kong et al. [2017](#page-9-5); Nuccio et al. [2015](#page-9-6); Miransari and Smith [2019\)](#page-9-7).

The nitrogen metabolism is among the most important processes, which is negatively afected by drought stress, signifcantly decreasing plant growth and yield production. For example, Yang et al. [\(2018\)](#page-9-8) indicated that under drought stress, while in corn (*Zea mays* L.) plants, the concentrations of simple sugars and polyunsaturated fatty acids increased, the concentration of amines, poly amines, and dipeptides decreased. Additionally, the following processes were among the most important responses of corn plants subjected to oxidative stress caused by drought: (1) activation of urea and glutathione cycles, and (2) production of carbohydrates and lipids resulting in the cellular osmo- and antioxidant protection (Yang et al. [2018\)](#page-9-8). Although plants may utilize some mechanisms to alleviative the stress of drought on plant N metabolism, research has indicated that the exogenous use of plant products, which can infuence plant morphology and physiology under stress, may also be a favorable method to alleviate stress on plant growth and yield production (Sami et al. [2016](#page-9-9); Verma et al. [2016](#page-9-10)). Diferent plant growth regulators (PGR) have been examined on plant growth and activity under stress; however, we examined some PGR, which to our knowledge have not been previously much investigated including 6-benzyl adenine, proline, glutamine, and their combination as well as superoxide dismutase.

We recently proved it is possible to alter plant physiology (using PGR) in a way so that the environmental stresses including high temperature, drought, and cool conditions can be alleviated resulting in the enhanced plant growth and yield production (Tahaei et al. [2016;](#page-9-11) Shourbalal et al. [2019](#page-9-12); Zamani et al. [2020](#page-9-13)). Accordingly, Shourbalal et al. [\(2019\)](#page-9-12) indicated that the most suitable method to shorten vernalization in winter wheat and avoid the stresses of drought and cool conditions is the use of PGR including gibberellic acid (GA, 100 mg/l), kinetin (100 and 200 mg/l), and 6-benzyl adenine (BA6, 50 mg/l). Tahaei et al. ([2016\)](#page-9-11) found that using the same PGR it is possible to enhance the seed germination and seedling growth of Fennel (*Foeniculum vulgare* Mill).

With respect to the above-mentioned details and because there are not much data on the use of such PGR on corn growth under feld drought stress, this research was proposed and conducted. The objective was to investigate how such PGR may afect corn physiology and yield components under drought stress.

Materials and Methods

Experimental Site

The experiment was conducted in the Research Field of Islamic Azad University (Isfahan Branch), Isfahan, Iran, with the northern latitude and eastern longitude of 32° 40′ and 51° 48′, respectively, 1570 m above the sea level, and with the yearly rainfall of 150 mm. Soil physical and chemical properties were analyzed to the depths of 0–30 and 30–60 cm by the Research Complex of Kavosh, Isfahan, Iran using the standard methods (Table [1](#page-2-0)) (Miransari et al. [2008](#page-9-14)). Accordingly, the feld soil was not saline and was suitable for planting silage corn. Due to the relatively high amounts of available phosphorous (P) and potassium (K) in the soil, chemical fertilization was not applied. The analysis of the irrigation water using a well is also presented in Table [1.](#page-2-0)

Experimental Treatments

The corn seeds were planted in plots measuring 10×10 m using a seeder on the 21st of June 2016, with the seed and row distances of 10 and 75 cm, respectively. The experiment, conducted as a split plot on the basis of a completely randomized block design, investigated the efects of drought levels (main plots), established on the basis of evaporation from an evaporating pan, including control (D1, 70 mm), medium (D2, 90 mm), and severe (D3, 110 mm), and the foliar application (sprayed at fowering and at the time of photosynthates transfer to the grains) plant growth regulators (PGR) (subplots) including (A) control, (B) 6-benzyl adenine (10,000 mg/L), (C) proline (2.5 ml/L), (D) glutamine (1 ml/L), (E) $B + C$, (F) $B + D$, 9G) $B + C + D$, and 9H) superoxide dismutase (2.5 ml/L) on corn (genotype Single Cross 640) growth under feld conditions. A total of 96 plots, including 4 replicates, with the side distance of 5.1 m between the plots to avoid the interaction of water treatment were used for the experiment. The drought treatments were initiated at the V2–V4 growth stage and the PGR treatments were imposed at two diferent growth stages including (1) transition from the vegetative to the productive stage, and (2) the transition stage (Fig. [1\)](#page-3-0).

Sampling

The planting rows of 1, 2, 7, and 8 were randomly selected for sampling, ignoring the 1-m distance of the two sides of each row. The samples were collected at the V6–V8, V8–V12, the milky and the maturity stages, and diferent parameters includ ing plant proline content (Pro), relative water (RW), number of grains per corn (NGC), weight of 100 grains (100GW), biological yield (BY), corn fresh yield (CFY), and grain yield (GY) were determined.

Relative Water Content (RW)

RW was measured according to the following. The leaf sam ples were randomly collected at 11–12 a.m. and were placed in paper bags containing dry ice, and were transferred to the lab. The fresh weight of the samples was weighed and then the samples were cut into 2-cm pieces and immersed in distilled water for 6–8 h at room temperature. The saturated weight of the samples was determined, and the samples were then oven dried at 70 °C for 72 h and the dry weight of the samples was determined. RW was calculated using the following formula:

RW $(\%) = ((\text{ fresh weight} - \text{dry weight})$ $/$ (total weight – dry weight)) × 100.

Proline Content (Pro)

Proline content was measured according to the follow ing (Bates et al. [1973\)](#page-9-15). Sulfosalicylic acid was prepared by increasing the volume of 30 mL acid to the fnal volume of 1 L using distilled water. Ninhydrin indicator was prepared by dissolving and heating 1.25 g ninhydrin in 30 mL acetic acid and 20 mL phosphoric acid 6 M. The proline standard was prepared by dissolving 100 mg pure proline in 1 L distilled water, which was then used to prepare the standards of 1, 2, 5, 10, and 20 mg/L. Plant samples (0.5 g), which had been stored in a freezer, were smashed by a crucible, and were homog enized with 10 mL sulfosalicylic acid 3%. The solution was then centrifuged at 2000× *g* for 10 min, 2 mL of which was treated with 2 mL ninhydrin and 2 mL acetic acid. The tubes containing the solution were placed in a bain-marie with the temperature of 100 °C for one hour, and the reaction was terminated by putting the tubes in an ice bain-marie. The tubes were then mixed with 4 mL toluene for 30 s and the absorption of the color phase was determined by a spectrophotometer at the wavelength of 520 nm; proline concentration was calcu lated using the following formula:

Proline = (absorption value \times consumed toluene /115.5)

∕ (sample weight ∕ 5) .

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Fig. 1 Diferent stages of the experiment

Statistical Analyses

Data were subjected to analysis of variance and the signifcance of the experimental treatments and their interactions

on the above-measured parameters were determined using SAS. Means were compared using Duncan's multiple range test at $P = 0.05$. Using Proc Plot, the presented plots were drawn.

Results

Analysis of Variance

According to the analysis of variance, the effects of drought treatments were significant on different corn yield components excluding GY. However, PGR significantly affected RW, BY, and GFY. The interactions of irrigation and PGR were also significant on different corn components excluding NGC and BY (Table [2](#page-4-0)).

RW

Different drought treatments significantly affected plant RW, and the highest one was resulted by D1 (0.77 a), followed by D2 $(1.05 b)$ and D3 $(1.72 c)$. The effects of PGR were also significant on RW, and treatment G (benzyl adenine $6 +$ proline + Glutamine, 1.24 a) resulted in the highest RW higher than the other PGR treatments including treatment C (proline, 1.198 ab)) and treatment H (super oxide dismutase, 1.20 a) (Table [3\)](#page-5-0).

Pro

The effects of drought treatments significantly affected corn proline content (ranging from 32.16 at D1 to 63.36 mg/g fresh weight at D3) as D2 (58.24 g/g fresh weight) and D3 significantly increased this component, compared with D1. However, the effects of PGR were not significant on plant proline content ranging from 49.28 (B) to 53.30 g/g fresh weight (A). The interaction of drought treatments and PGR significantly affected plant proline content indicating that the effects of PGR on the alleviation of drought stress differ at different levels of drought stress (irrigation treatments) (Fig. [2a](#page-6-0), Table [3](#page-5-0)).

NGC

The statistical analysis indicated the signifcant efects of irrigation treatments and PGR, and not their interaction, on NGC. D1 had the highest NGC (538.74a) followed by D2 (451.57b) and D3 (379.91c). The least and the highest NGC was resulted by A $(414.1b)$ and G $(517.8a)$, respectively (Fig. [2b](#page-6-0), Table [3](#page-5-0)).

100GW

The effects of drought treatments including D1 (27.97 g a) , D2 (23.70 g b), and D3 (20.05 g c) and its interaction with PGR were signifcant on the weight of 100 grains. However, the effects of PGR were not significant on the weight of 100 grains ranging from 22.0[3](#page-7-0) g (A) to 25.70 g (G) (Fig. 3a, Table [3](#page-5-0)).

CFY

Corn fresh yield was also signifcantly afected by both the irrigation treatments and PGR as the highest grain fresh yield was resulted by D1 (109,048.0 kg/ha) signifcantly higher than D2 (92,695.0 kg/ha) and D3 (69,584.0 kg/ha). The use of PGR also significantly increased corn fresh yield from 80,542 kg/ha (A) to 100,263 kg/ha (G) (Fig. [3b](#page-7-0), Table [3](#page-5-0)).

BY

The effects of irrigation treatments and PGR, and not their interaction, signifcantly afected corn biological yield, and D1 resulted in the highest biological yield (67,868.0 kg/ ha) signifcantly higher than the other treatments including D2 (54,257.0 kg/ha) and D3 (48,107.0 kg/ha). However, PGR was able to signifcantly alleviate the drought stress by increasing corn biological yield from a minimum of 49,842 kg/ha (A) to a maximum of $62,277$ kg/ha (G) (Fig. [4a](#page-8-0), Table [3\)](#page-5-0).

Table 2 Analysis of variance indicating how diferent experimental treatments afected corn yield and yield components

M.S								
S.V	d.f	RW	Proline	NGC	100 GW	BY	CFY	GY
Rep	3	0.0045	54.38	3848.42	22.26	14,665,509	201, 707, 525	1,763,903
Irig	2	$7.656**$	9201.75**	202,408**	$502.71**$	3,272,350,012**	12.581.222.123**	5,053,091
Error A	6	0.0133	169.76	7196.06	5.69	33,261,111	241, 221, 681	5,455,389
Foli	7	$0.0663**$	29.941	20.372.28*	14.70	162.291.109**	470.366.114**	8.802.655
Irig. x Foli	14	$0.3203**$	52.19	5427.36	$76.52**$	39.651.910	153,775,534**	85,902,929**
Error	63	0.0230	27.35	9522.6	19.19	47,210,347	50,297,115	6,640,586

S.V. source of variation, *d.f.* degree of freedom, *RW* relative water content, *NGC* number of grains per cob, *100GW* weight of 100 grains, *BY* biological yield, *CFY* corn fresh yield, *GY* grain yield

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SD standard deviation, *RW* relative water content, *NGC* number of grains per cob, *100GW* weight of 100 grains, *BY* biological yield, *CFY* corn fresh yield, *GY* grain yield

Fig. 2 The efects of stress and PGR on: **a** proline, and **b** the number of grains per corn; diferent letters indicate signifcant diferences using Duncan's multiple range test at $P=0.05$. D1, D2, and D3 rep-

GY

The effects of irrigation treatments, ranging from 19,385.3 kg/ha (D3) to 20,178.7 kg/ha (D1), and PGR, ranging from $18,640$ (A) to $21,139.0$ kg/ha (G), were not significant on grain yield; however, their interaction signifcantly afected grain yield (Fig. [4](#page-8-0)b, Table [3](#page-5-0)).

Discussion

The alleviation of drought stress on plant growth and yield production may contribute to the increased food production, worldwide. In this research, the efects of diferent PGR, which has been rarely examined, on the physiology and yield of corn under drought stress was investigated. This has been in the continuation of our previous research in which the use of PGR on shortening vernalization in wheat and increasing seed germination in fennel was determined (Tahaei et al. [2016](#page-9-11); Shourbalal et al. [2019](#page-9-12)). This research indicated that although the efects of PGR were not signifcant on grain yield, it resulted in an increase of 2500 kg/ha compared with control under drought stress. However, RW, BY, and CFY

resent 70, 90, and 110 mm evaporation from the pan, respectively. **a** Control, **b** 6-benzyl adenine, **c** proline, **d** glutamine, **e** B+C, **f** B+D, **g** B+C+D, and **h** superoxide dismutase

were signifcantly increased by PGR. This indicates PGR are more effective on the increase of plant fresh tissues, which is mainly by afecting plant N metabolism. In this research, the single and combined use of Pro (amino acid), Glu (amino acid), and BA6 (plant hormone) as well as the single use of SOD (antioxidant enzyme) were tested.

Alam et al. (2016) (2016) investigated the effects of exogenous proline (sprayed at the stages of vegetative and tasseling) on corn growth under salt stress. Although salinity stress signifcantly decreased plant growth and negatively afected plant biochemical properties, the exogenous use of proline improved corn growth under the stress. Accordingly, the use of proline at 25 mM signifcantly enhanced the growth of the stressed corn by increasing the ratio of $K + /Na +$ and plant nutrient uptake, especially phosphorus.

The effects of PGR including paclobutrazol, uniconazole, propiconazole, and gibberellic acid were tested on the yield of maize plants subjected to the single or combined effects of drought stress, nitrogen deficiency, and high plant density (Stutts et al. [2018\)](#page-9-17). They indicated the response of maize plants to alleviate the stress is by altering the hormonal balance afected by genotypic diferences. The PGR were able to alleviate the single and the

Fig. 3 The effects of stress and PGR on: **a** the weight of 100 grains, and **b** grain fresh yield; diferent letters indicate signifcant diferences using Duncan's multiple range test at $P=0.05$. D1, D2, and

D3 represent 70, 90, and 110 mm evaporation from the pan, respectively. **a** Control, **b** 6-benzyl adenine, **c** proline, **d** glutamine, **e** B+C, $f(B+D, g(B+C+D, and h)$ superoxide dismutase

combined efects of the tested stresses by increasing maize seed yield; however, it was a function of the stress type, environment, and plant genotypic properties.

Stutts et al. [\(2018](#page-9-17)) suggested fnding and altering the biochemical pathways, which regulate plant hormonal balance and subsequent plant response under stress, may be a favorite method for the production of resistant maize plants in diferent stresses. The research is also interesting because the tested PGR were able to make a balance between N metabolism (under N defcient conditions) and plant resistance when maize was planted at high number. It is because high N levels increase maize growth resulting in plant lodging and subsequent yield reduction. However, the PGR were able to make a hormonal balance, so N defciency was alleviated and the plants were not lodged. Such a consequence may require further research on how the tested PGR are able to make such a biochemical balance.

Nitrogen metabolism is an important process, afecting plant tolerance under drought stress. However, N metabolism is negatively afected under drought stress and increased N uptake can enhance plant tolerance under drought stress. Wang et al. (2017) (2017) (2017) investigated the effects of N metabolism on maize tolerance under drought stress. The authors accordingly determined the expression of the genes regulating N uptake and assimilation, plant photosynthesis, and nutrient uptake in diferent maize tissues subjected to drought stress. The response of diferent tissues was signifcantly diferent under drought stress, as the stress signifcantly increased activation of root genes regulating N uptake and assimilation. Accordingly, such gene activities resulted in increased N uptake and quick accumulation of amino acids indicating that such root genes may increase maize tolerance under drought stress (Zhang et al. [2016](#page-9-19); Kant, [2018](#page-9-20)).

Fig. 4 The efects of stress and PGR on **a** biological yield and **b** grain yield; diferent letters indicate signifcant diferences using Duncan's multiple range test at $P=0.05$. D1, D2, and D3 represent 70, 90, and

The higher efficiency of the combined treatments tested in this research, compared with their single use, indicates that they are synergistic and not antagonistic and each PGR can alleviate some of the negative efects of drought stress on corn growth. When plant is N deficient, for example under drought stress, diferent signaling pathways are activated in plant to alleviate the stress among which the production of cytokinins is one of the most important ones. Such plant hormones are derived from adenine (in diferent parts of the plant) determining N availability (Gu et al. [2018\)](#page-9-21). This may be considered as of the main reasons indicating why the use of 6-benzyl adenine can regulate N metabolism and subsequent plant growth under drought stress.

The absorption of BA6 by plant affects the molecular pathway of cytokinins and cellular division. The absorbed BA6, which is converted to some other organic molecules such as 6-benzylamino-9-glucopyranosylribosyl-purine, can afect the activity of plant cells and the subsequent

110 mm evaporation from the pan, respectively. **a** control, **b** 6-benzyl adenine, **c** proline, **d** glutamine, **e** $B + C$, **f** $B + D$, **g** $B + C + D$, and **h**) superoxide dismutase

plant growth by reducing the rate of internal cytokinins (Zhang et al. [2010](#page-9-22)). Similar to the exogenous use of proline, glutamine can also alleviate drought stress on corn physiology and yield components by stimulating plant N metabolism. The exogenous use of SOD can also regulate plant growth under stress by scavenging reactive oxygen species and our results indicate that such a molecule has also been efficient on the alleviation of the stress.

The main reason for the highest impact of the combined PGR is because the three tested amino acids are required for plant growth and metabolism, and N uptake by plant results in the production of such amino acids, which can (1) regulate plant metabolic activities under diferent conditions including stress, and (2) can have structural roles by being incorporated in diferent protein structures including enzymes (Stutts et al. [2018\)](#page-9-17). Accordingly, the combination of the two tested PGR can be more efficient on the alleviation of stress than the single one.

Conclusion

The drought response of corn plants (corn physiology and yield components), under feld conditions, as afected by different PGR including the single and combined use of N-containing molecules including proline, glutamine, 6-benzyl adenine as well as the single use of super oxide dismutase (antioxidant enzyme) was investigated. The main reasons for the selection of such PGR were due to the efects of drought stress on N metabolism and according to our recent research (Tahaei et al. [2016;](#page-9-11) Shourbalal et al. [2019](#page-9-12)). The results indicated that the stress signifcantly afected corn physiology (relative water content and proline) and yield components. However, the use of PGR, especially their combined use, signifcantly improved plant water content as well as corn fresh and biological yields under drought stress. Although the efects of PGR (their combined use) were not statically signifcant on corn grain yield under stress, they increased corn grain yield by 2500 kg/ha compared with control. Such molecules are able to alleviate drought stress on corn growth by the activation of the N-stimulating genes and metabolism-related signals, which can enhance corn tolerance under drought stress. Such molecules are of economic and environmental signifcance.

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Data Availability The authors are not allowed to share their data.

Compliance with Ethical Standards

Conflict of interest The authors do not have any confict of interest.

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