Plant growth regulators affecting wheat (*Triticum aestivum* L.) growth and seed components and subsequent vernalization under drought stress

Sayed Komeil Sayed Shourbalal[1], Ali Soleymani[1&2]*, and Hamid Reza Javanmard[1]

1. Department of Agronomy and Plant Breeding, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran
2. Plant Improvement and Seed Production Research Centre, Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran

Abstract

Plant physiology, specifically nitrogen (N) metabolism affecting plant phenology (vernalization), may be affected under stress. Plant growth regulators (PGR), planting dates, and cold stratification affecting vernalization of wheat under drought stress were investigated. The PGRs used for priming seeds or spraying plants (at the double bridge stage, V4-V6 stage) consisted of the single and the combined use of gibberellins (GA_3, GA_4, and GA_7) benzyl adenine 6 (BA6), and kinetin at 100, 200, and 50, mg/l, respectively. Plant morphological properties and seed components were determined in a split plot experiment on the basis of a completely randomized block design with four replicates. Priming, spraying, and their interaction significantly affected the measured parameters. Autumn planting and spraying with GA_{4+7} + BA6 resulted in the highest root length (15.75cm). Interestingly, seed components were more affected by the treatments in spring planting as the highest spike dry weight was resulted by spraying with GA3+4+7 + kinetin + BA6 (3.97g). Spraying at spring planting was also more affective on grain N percentage, as spring planting without priming, using GA3+7 and kinetin, and cold stratification resulted in the highest GNP (3%). The obtained results are another confirmation to our recent experiment indicating it is possible to regulate wheat phonology (vernalization) and improve its growth and seed quality using the tested PGR under drought conditions.

Keywords: benzyl adenine 6, gibberellins, grain moisture, grain N, kinetin, wheat N metabolism

Sayed Shourbalal, S. K., A. Soleymani, and H. R. Javanmard. 2023. 'Plant growth regulators affecting wheat (*Triticum aestivum* L.) growth and seed components and subsequent vernalization under drought stress'. *Iranian Journal of Plant Physiology 13*(4), 4729-4739.

Introduction

Wheat (*Triticum aestivum* L.) is among the most important crop plants feeding a large number of people worldwide. It is a source of carbohydrate, proteins, and nutrients and is used in different food products (Abbas et al., 2010). It is planted both under irrigated (higher yield) and rainfed conditions. Different stresses including drought can significantly affect wheat growth and yield, which is mainly due to the negative effects of the stress on plant nitrogen (N) metabolism (Dwivedi et al., 2012; Gupta and Thind, 2019; Gupta et al., 2014).

Although wheat is not a drought resistant crop plant, it can tolerate the stress up to a certain

---

** Corresponding Author*
E-mail Address: a_soleymani@Khuisf.ac.ir
Received: December, 2022
Accepted: April, 2023

level. Different methods have been used to alleviate drought stress on wheat growth and yield production including the use of tolerant wheat species, genetic modification, the use of soil microbes such as arbuscular mycorrhizal (AM) fungi, plant growth promoting rhizobacteria (PGPR), and the use of plant growth regulators (PGR) (Chandra et al., 2019; Daei et al., 2009; Dwivedi et al., 2018; Li et al., 2018; Shourbalal et al., 2019).

Lv and coworkers (Lv et al., 2018) investigated the molecular mechanisms which may regulate wheat tolerance under drought stress using two different wheat genotypes (sensitive and tolerant) under field irrigated and drought stress conditions. They investigated the genes expressed under the stress and found that 377 genes were expressed in both the sensitive and tolerant wheat species and that such genes may regulate wheat response in the stress conditions. Genetic analysis indicated that in the tolerant species, the expressed genes resulted in the activation of different signaling pathways and the activity of mitogen-activated protein kinase while in the sensitive genotype such genes regulated the processes of evapotranspiration, photosynthesis, and membrane protein complex. They also further found that RNA editing can also significantly affect wheat tolerance under drought stress.

Plant physiological responses to environmental conditions including light and temperature are functions of planting dates and plant genotype (Kamali et al., 2020). Vernalization is one important growth process in wheat production, required for transition from the vegetative to the reproductive stage, and subsequent yield production in winter wheat. However, under drought stress conditions, because the plant may not be able to absorb suitable amounts of water and nutrients for its growth (Zamani et al., 2020), it may not be able to face the vernalization stage effectively, resulting in the subsequent yield reduction. Although strengthening wheat plants by the above-mentioned methods may improve wheat growth under drought stress, one interesting method, which to our knowledge has not been previously investigated, is the use of PGR to regulate wheat vernalization under the stress (Shourbalal et al., 2019). PGRs are able to alter plant physiology in a way so that the plant can tolerate the stress more efficiently (Zafari et al., 2020a; Zafari et al., 2020b)and the process of vernalization may not be required for the production of wheat yield. It is possible to enhance wheat growth and yield production under drought stress by shortening the vernalization stage (Shourbalal et al., 2019). These researchers found some important results along the following lines: 1) it is possible to plant winter wheat as spring wheat (without vernalization), with optimum yield, using the tested PGRs, which is of significance due to the important issue of global warming, 2) reduce vernalization in winter wheat, which is especially important under drought stress conditions, and 3) improve wheat grain quality by increasing grain gluten and protein. They also found that the tested PGRs were more effective on the reduction of vernalization than the cold stratification treatment.

Since there is no data on the alleviation of wheat growth and seed components subjected to drought stress by the regulation of the vernalization process, the present study was conducted shed light on this. The objective was to investigate the effects of seed priming and plant spraying with PGRs (gibberellins, kinetin, and 6-benzyl adenine), planting dates, and cold stratification on wheat vernalization which in turn affects plant growth and seed components under drought conditions.

Materials and Methods

The research site

The experiment was conducted in 2017-2018 in the Research Field of Khorasgan Islamic Azad University, Isfahan, Iran. The field is located in the Eastern longitude of 51° and 47′ and the Northern latitude of 32° and 40′, 1552.9 m above the sea level. The climate of the region according to Koppen climate classification is dry and warm during the summer with semi-cold winter and the yearly average rainfall and temperature equal to 125 mm and 15.6 ℃, respectively. Physical and chemical properties of the experimental soil, including salinity (EC = 3.23 ds/m), pH (7.85), organic carbon (O.C. = 0.14%), total N (0.11%), available phosphorous (65 mg/kg), available potassium (910 mg/kg), sand (12.6%), clay (39.6%), silt (47.85) were determined using the standard methods (Miransari et al., 2008).

The experimental treatments

The experiment was a split plot on the basis of a completely randomized block design with four replicates. Each plot consisted of 4 rows (4 m long) with a 20 cm interspacing. The field was planted by hand at the plant density of 400 plants/m^2 during the autumn (2016/11/1) and winter (2017/2/10). The genotype used for the experiment was Mihan (Bkt/90-Zhong 87).

The main plots consisted of a combination of different planting dates and priming with plant growth regulators (PGRs) including P1 (autumn planting), P2 (spring planting without priming), P3 (spring planting using priming with 100 mg/l gibberellic acid (GA)$_3$ + 100 mg/l GA$_{4+7}$), P4 (spring planting using priming with 100 mg/l GA$_3$ + 100 mg/l GA$_{4+7}$ + 100 mg/l kinetin + 50 mg/l benzyl adenine 6 (BA6)), and P5 (spring planting + cold and moisturized stratification of seeds) (Shourbalal et al., 2019). The subplots consisted of the following PGR spraying treatments (at the double bridge stage, V4-V6 stage): S1 (control, without spraying), S2 (spraying with water), S3 (100 mg/l GA$_3$ + 100 mg/l GA$_7$), S4 (100 mg/l GA$_4$+$_7$), S5 (50 mg/l BA6), S6 (200 mg/l kinetin), S7 (100 mg/l GA$_{3+7}$ + 50 mg/l BA6, S8 (100 mg/l GA$_{4+7}$ + 50 mg/l BA6), S9 (100 mg/l GA$_{3+7}$ + 200 mg/l kinetin), and S10 (100 mg/l GA$_{4+7}$+ 200 mg/l kinetin) (Shourbalal et al., 2019).

Cold stratification of the seeds was conducted as follows: the seeds were moisturized with water for 18 h and were placed in the sterilized plastic bags for 30 d at the temperature of 4 ℃ (Tahaei et al., 2016). The seeds were sterilized with 2 mg/l fungicide Tebaconazol 12% DS. In treatments P3 and P4, the seeds were treated with the above-mentioned PGRs for 8 h and were then spread on the ground to get dried.

Agronomical practices

The experiment was conducted in the Research Field with an area of 1540 m^2 (70 x 22), which was prepared according to the farmer's practices in the region. The field which was uncultivated the year before was cultivated in the summer to the depth of 30 cm using a cultivator and was then smoothed. Field fertilization was done according to soil tests including 50 kg/ha triple super phosphate and 100 kg/ha urea (46% N), before planting. The other practices including irrigation, spraying, pest and weed control, and N fertilization were also conducted during the season. The field was irrigated (surface irrigation) for the first time right after planting. The other irrigations were done according to the plant requirement and rainfall in the region, which was usually with the interval of 7-10 d. N fertilization was done at 60 kg/ha urea during the stem elongation in the beginning of the spring and at 30 kg/ha during flowering. Wide-leaf weeds were controlled using 2, 4-D + MCPA 67.5% SL at 1.5 l/ha. During the experiment, there was not any disease or pest in the field.

Sampling and measurements

Spraying was done using a manual sprayer. The samples were collected from the 1 x 1 m areas and were stored in paper bags. Plant morphological properties including root length (RL), plant height, peduncle length, spike dry weight, grain nitrogen (N), and moisture percentage were determined. Root length and plant height (PH) were measured by randomly selecting 10 plants from each plot. The spike dry weight (SDW) was determined by randomly selecting 20 plants from each plot. Paper bags were then placed in an oven at 75 ℃ for 72 h, and their weights were determined using a digital scale. Grain N percentage (GNP) was determined using Kjeldahl method (1983), grain moisture percentage (GMP) was measured according to AACC44-14a using the following formula:

grain moisture percentage = (fresh grain weight/dry grain weight) / 100.

Statistical Analysis

Data were subjected to analysis of variance using MSTAT-C. Means were compared using Duncan multivariate test at P<0.05.

Table 1
Analysis of variance indicating the effects of priming and spraying treatments on wheat morphology and yield components

S.V.	d.f.	RL	PH	SDW	GNP	GMP
Rep.	3	0.018^{ns}	0.893^{ns}	0.006^{ns}	0.017^{ns}	0.004^{**}
Priming (P)	4	63.392^{**}	3217.550^{**}	16.866^{**}	1.875^{**}	0.317^{**}
Error A	12	0.206	0.560	0.013	0.015	0.001
Spraying (S)	9	15.861^{**}	126.411^{**}	1.425^{**}	0.074^{**}	0.095^{**}
P * S	36	15.279^{**}	86.114^{**}	2.072^{**}	0.145^{**}	0.360^{**}
Error B	135	0.220	0.760	0.013	0.015	0.0001
C.V. (%)		4.79	1.13	3.28	4.95	0.18

S.V.: source of variation, d.f.: degree of freedom, RL: root length, PH: plant height, SDW: spike dry weight, GNP: grain N percentage, GMP: grain moisture percentage, C.V.: coefficient of variation, ns: not significant, * and **: significant at 5 and 1% of probability, respectively.

Table 2
The single effects of different priming and spraying treatments on wheat morphology and yield components

Treatment	RL	PH	SDW	GNP	GMP
P_1	11.82a	92.13a	3.74c	2.14d	8.72c
P_2	9.25c	71.72d	2.39e	2.57b	8.9a
P_3	10.13b	77.43b	3.91b	2.5c	8.75b
P_4	9.175c	75.05c	3.97a	2.7a	8.7d
P_5	8.6d	69.18e	3.46d	2.61b	8.68d
S_1	8.95de	76.75d	3.12e	2.44bc	8.69h
S_2	8.85e	76.15e	3.11e	2.37c	8,72f
S_3	10.3b	79.85ab	3.52c	2.49ab	8.79b
S_4	11.75a	80.3a	3.67b	2.56a	8.76d
S_5	10.35b	78.2c	3.91a	2.55a	8.71g
S_6	9.5c	79.7b	3.43d	2.56a	8.92a
S_7	9.4c	76.95d	3.85a	2.52ab	8.72f
S_8	10.35b	75.85e	3.41d	2.48ab	8.69h
S_9	9.25cd	72.15g	3.44d	2.55a	8.78c
S_{10}	9.25cd	75.1f	3.48cd	2.51ab	8.74e

RL: root length, PH: plant height, SDW: spike dry weight, GNP: grain N percentage, GMP: grain moisture percentage; values in each column followed by the same letter are not statistically different at P<0.05 using Duncan's multiple range test. The P treatments (P_1-P_5) are P_1 (spring planting without priming) to P_5 (the stratification (wet chilling) of the seeds), and the S treatments (S_1-S_{10}) are S_1 (control without spraying) to S_{10} (GA_4 and GA_7 at 100 mg/l and kinetin at 200 mg/l).

Results

Root length

The single effects of priming and spraying and also their interaction significantly affected root length (RL) at the physiological maturity (P< 0.01) (Table 1). The highest RL, affected by priming, was related to autumn planting (11.75 cm) and the least (8.06 cm) was related to winter planting, treated with moisturized stratification (Table 2). The highest RL, affected by spraying, was related to GA4 + GA7 (11.75 cm) and the least one was related to spraying with water (8.85 cm) (Table 2). The interaction of priming and spraying indicated that the highest RL resulted from autumn planting and spraying with GA_{4+7} + BA6 (15.75 cm), and the shortest length resulted from winter planting without priming + spraying with water (6.25 cm) (Table 3).

Plant height

The single and the interaction effects of priming and spraying significantly affected plant height (PH) (P< 0.01) (Table 1). Autumn planting resulted

Table 3
The interaction of effects of priming and spraying on wheat yield and yield components

Priming	Spraying	RL	PH	SDW	GNP	GMP
P_1	S_1	10.75fgh	84.25g	3.23t-w	1.96pq	8.5x
P_1	S_2	11efg	85.75f	3.76jkl	1.99pq	8.53vw
P_1	S_3	8.25lm	97a	3.18vw	2.12opq	8.95ij
P_1	S_4	11.75e	94.75b	2.91x	2.15op	8.32b’
P_1	S_5	15b	91.75de	4.05fgh	2.23no	8.48y
P_1	S_6	9.75ij	97a	3.47n-r	2.23no	9.25b
P_1	S_7	13.5c	93.75bc	4.71b	1.94q	8.93k
P_1	S_8	15.75a	91.25e	5.15a	2.2no	8.66r
P_1	S_9	11.25ef	93cd	3.33q-v	2.2no	9.1d
P_1	S_{10}	11.25ef	92.75cd	3.61lmn	2.36i-n	8.48y
P_2	S_1	7o	76.75nop	1.38c’	2.31l-o	8.84m
P_2	S_2	6.25p	73.75s	1.51c’	2.33k-o	8.85l
P_2	S_3	9.75ij	75.5pqr	2.28z	2.51g-l	9.24c
P_2	S_4	12.75d	81.5h	3.2uvw	2.48h-m	8.92k
P_2	S_5	7.5mno	79.75i	3.37p-u	2.69c-h	9.09d
P_2	S_6	11.25ef	74.5qrs	3.54m-p	2.61d-h	9.01g
P_2	S_7	7.5mno	70.75t	3.26s-w	2.63d-h	8.48y
P_2	S_8	10.5gh	61.75x	1.76a’b’	2.85abc	8.97h
P_2	S_9	9.75ij	58.25y	1.7b’	3a	9.07e
P_2	S_{10}	10.25hi	64.75w	1.88a’	2.29mno	8.58t
P_3	S_1	11.75e	74.75qrs	3.4o-t	2.37i-n	8.94j
P_3	S_2	11.75e	73.5s	2.86x	2.38i-n	8.97h
P_3	S_3	12.75d	78.75ijkl	3.43n-s	2.535f-j	8.46z
P_3	S_4	13.25cd	81h	4.3de	2.7b-g	8.96hi
P_3	S_5	11.75e	81.25h	4.36d	2.67c-h	8.82n
P_3	S_6	7o	78jklmn	2.87x	2.57e-j	8.26c’
P_3	S_7	8.25lm	77.75klmn	4.66bc	2.73b-f	9.01g
P_3	S_8	9.25jk	78.5ijkl	3.86ijk	2.28no	8.52w
P_3	S_9	8.25lm	77mno	5.06a	2.29mno	8.54v
P_3	S_{10}	7.25no	73.75s	4.3de	2.52g-k	9.05f
P_4	S_1	7.5mno	79ijk	3.51n-q	2.57e-j	8.66r
P_4	S_2	7.5mno	79.25ij	3.57mno	2.577e-j	8.69p
P_4	S_3	12.75d	74.25rs	4.53c	2.53f-j	8.56u
P_4	S_4	11.75e	77.5lmn	4.81b	2.67c-h	8.97h
P_4	S_5	8.25lm	73.5s	3.92g-j	2.51g-k	8.42a’
P_4	S_6	9.25jk	77.5lmn	3.35q-v	2.86abc	9.52a
P_4	S_7	9jk	75.5pqr	4.03f-j	2.77b-e	8.23d’
P_4	S_8	8lmn	69.5u	3.99f-j	2.79bcd	8.47yz
P_4	S_9	8lmn	68.75u	3.7klm	2.9ab	8.52w
P_4	S_{10}	9.75ij	75.75opq	4.31de	2.79bcd	8.94j
P_5	S_1	7.75mno	69u	4.06fg	3a	8.52w
P_5	S_2	7.75mno	68.5u	3.85ijk	2.57e-j	8.56u
P_5	S_3	8lmn	73.75s	4.15ef	2.78bcd	8.77o
P_5	S_4	9.25jk	66.75v	3.13w	2.78bcd	8.61s
P_5	S_5	9.25jk	64.75w	3.87h-k	2.65d-h	8.74o
P_5	S_6	10.25hi	71.5t	3.9g-j	2.52f-k	8.57tu
P_5	S_7	8.75kl	67v	2.6y	2.54f-j	8.95ij
P_5	S_8	8.25lm	78.25jklm	2.28z	2.3mno	8.84lm
P_5	S_9	9jk	63.75w	3.39o-t	2.4i-n	8.68q
P_5	S_{10}	7.75mno	68.5u	3.31r-w	2.61d-h	8.62s

RL: root length, PH: plant height, SDW: spike dry weight, GNP: grain N percentage, GMP: grain moisture percentage; values in each column followed by the same letter are not statistically different at $P<0.05$ using Duncan's multiple range test. The P treatments (P_1-P_5) are P_1 (spring planting without priming) to P_5 (the stratification (wet chilling) of the seeds), and the S treatments (S_1-S_{10}) are S_1 (control without spraying) to S_{10} (GA_4 and GA_7 at 100 mg/l and kinetin at 200 mg/l).

in the highest PH (93.13 cm), and the combination of spring planting and moisturized stratification (75.05 cm) resulted in the least PH (Table 2). However, for the spraying treatments the maximum and minimum plant heights were related to treatments with GA_{3+7} (79.85 cm) and GA_{3+7}+kinetin (72.15 cm), respectively (Table 2). According to the interaction of priming and spraying, the combination of autumn planting and spraying with GA_{3+7} (97 cm) and autumn planting with kinetin (97 cm) resulted in the highest, and the combination of spring planting without spraying and spraying with kinetin + GA_{3+7} (58.25 cm) resulted in the lowest pH (Table 3, Fig. I).

Spike dry weight

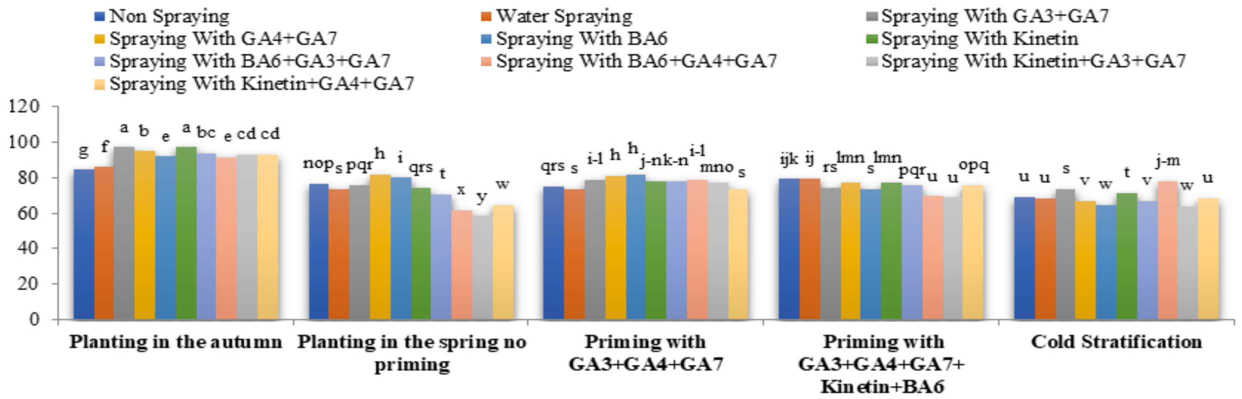

Fig. I. Effects of priming and spraying on plant height at physiological maturity of autumn wheat

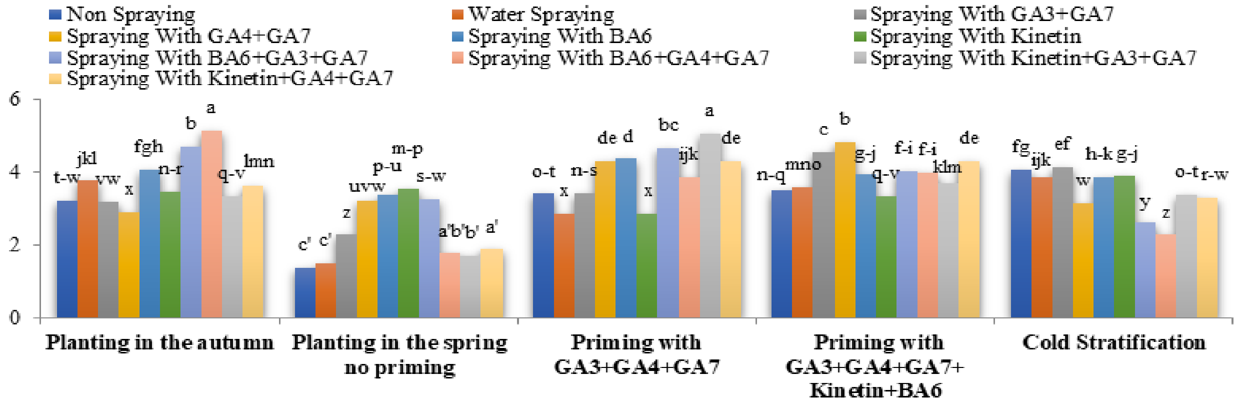

Fig. II. Effects of priming and spraying on spike dry weight at physiological maturity of autumn wheat

The single and the interaction effects of priming and spraying significantly affected spike dry weight (SDW) at the physiological maturity (P< 0.01) (Table 1). According to the priming treatments, the highest SDW was related to the spring planting with GA_{3+4+7}+kinetin+BA6 (3.97 g), and the lowest weight was related to the spring planting without priming (2.39 g) (Table 2). However, in the spraying treatments, the highest and the lowest SDW values were related to BA6 (3.91 g) and Ga_{3+7}+BA6 (3.85 g), and in the control treatment, the lowest weight levels were related to spraying with water (3.11 g) and without spraying (3.12 g) (Table 2). According to the interaction of priming and spraying, the highest SDW resulted from autumn planting + spraying with GA_{4+7}+BA6 (5.15 g) and spring planting + priming with GA_{3+4+7} + spraying with GA_{3+7} + kinetin (5.06 g), and the minimum weights were a result of spring planting without priming and spraying (1.38 g) and spring planting + spraying with water (1.51 g) (Table 3, Fig. II).

Grain N%

The single and the interaction effects of priming and spraying significantly affected grain N percentage (GNP) at the physiological maturity (P<0.01) (Table 1). The treatment of spring planting + priming with GA_{3+4+7} + Kinetin + BA6 resulted in the highest (2.7%), and the treatment of autumn planting resulted in the least GNP (2.14%) (Table 2). The spraying treatments indicated that the water treatment (2.37%) and the control treatment (without spraying) (2.44%) resulted in the least GNP (Table 2). The interaction of priming and spraying resulted in the highest GNP by the following treatments: spring planting without priming + spraying with GA_{3+7}+kinetin (3%), spring planting with moisturized cold stratification + without priming (3%), spring planting + priming with GA_{3+4+7}+kinetin+BA6 + spraying with GA_{3+7} + kinetin (2.9%), spring planting + priming with GA_{3+4+7}+kinetin+BA6 + spraying with kinetin (2.86%), and spring planting + without priming + spraying with GA_{4+7}+BA6

(2.85%). However, the least GNP values were related to the following treatments: autumn planting + spraying with GA_{3+7} +BA6 (1.94%), autumn planting + without spraying (1.96%), autumn planting + spraying with water (1.99%), and autumn planting + spraying with GA_{3+7} (2.12%) (Table 3).

Grain moisture percentage

Significant effects of the single factors and the interaction effects of priming and spraying grain moisture percentage (GMP) were indicated by the analysis of variance at physiological maturity ($P<0.01$) (Table 1). Among the priming treatments, spring planting without priming (8.9%) resulted in the highest GMP, and spring planting + cold stratification (8.68%), and spring planting with GA_{3+4+7}+kinetin+BA6 (8.7%) resulted in the least GMP values (Table 2). According to the spraying treatments, the highest and the lowest GMP values resulted from spraying with kinetin (8.92%), and control (without spraying), respectively (Table 2). The interaction effects of the factors under study indicated that the highest GMP was related to spring planting + priming with GA_{3+4+7} + kinetin +BA6 + spraying with kinetin (9.52%) while the lowest effects were related to spring planting + priming with GA3+4+7 + Kinetin and spraying with GA_{3+7} + BA6 (8.23%) (Table 3).

Discussion

Findings of the present study further confirms the earlier studies on the effect of PGR on wheat growth and seed components and subsequent vernalization, under drought stress(Askarnejad et al., 2021; Shourbalal et al., 2019). The obtained results on different wheat morphological indices (root length and plant height), and seed components (spike dry weight, grain N percentage, and grain moisture percentage) are discussed as follows.

RL

Vernalization (transition from the vegetative to the reproductive stage) is the important and essential process for the growth and yield production of winter wheat. However, if such process is not completed properly, for example under drought stress conditions (due to decreased water and nutrient uptake), yield reduction is resulted. It is a process which is under hormonal control, and if the level of hormones is controlled in plant, it may be possible to regulate vernalization (Dziurka et al., 2016). Accordingly, in the present research it was hypothesized that it is possible to regulate or shorten vernalization by affecting wheat morphology (wheat root growth and height) and seed components (SDW, GNP, and GMP) using PGR and seed cold stratification under drought stress conditions.

The highest RL was related to spraying with GA_{4+7}. Gibberellins, which are produced in plant roots and regulate different plant physiological mechanisms including cellular activities, are essential for root growth (Dziurka et al., 2016). However, the mechanism of effects of gibberellins on regulation of root growth have yet to be indicated. The interaction of priming and spraying indicated that the highest RL was resulted from autumn plantin + spraying with GA_{4+7} + BA6.

Gibberellins can regulate different plant activities including the elongation of different plant tissues (Miransari and Smith, 2014). Effects of BA6 on plant growth are by influencing the cytokinin pathway and by increasing cellular division and growth. The absorbed BA6 is catalyzed into 6-benzylamino-9-glucopyranosylribosyl-purine and 6-benzylamino-9-glucopyranosylribosyl-purine, which are able to reduce plant cytokinin (Zhang et al., 2010).

The results of the presented research indicated the positive effects of cytokinin in combination with gibberellins on plant growth, which is a function of rate, time of use, and growth conditions. Small and Degenhardt (Small and Degenhardt, 2018) in their review suggested the use of PGR for the growth of native plants. PGRs, including the natural and synthetic plant hormones, are able to improve the ecological functions of the ecosystem and increase the rate of land reclamation. The reason for the enhanced effects of combined PGRs tested in this experiment can be the cross-talk between gibberellins, cytokinin, and brassinosteroids (Martínez et al., 2016).

Different biological activities including cellular division and differentiation are resulted from PGRs including gibberellins, cytokinin, and brassinosteroids, which are used to treat different parts of the plant under different conditions including stress. A wide range of positive effects of PGRs on plant growth and yield production has also been indicated by research including seed related processes, root and aerial part growth, stress tolerance, improved water and N use efficiency, and enhanced photosynthesis. However, more has yet to be investigated on the use of PGRs for different purposes including the reclamation of land and improved plant tolerance under stress (Ali et al., 2017; Martínez et al., 2016; Small and Degenhardt, 2018).

According to our results, the single and combined use of PGRs increased SDW. Geng et al. (Geng et al., 2017)found that the allelic variation of *TaGW2-6A* increases the size of wheat grains, probably due to the alteration of endosperm cells and dry matter, by regulation of cytokinin and starch genes. They, accordingly, found that in such an allelic region, the genes responsible for cytokinin synthesis and starch biosynthesizing enzyme are upregulated, and the cytokinin degrading genes and the negative regulators of starch biosynthesis are down regulated.

Nitrogen metabolism is among the most important physiological processes, which is influenced under drought stress, and PGRs are able to affect N metabolism under different conditions including stress. According to the results, seed priming affected wheat morphology more than spraying, however, spraying was more effective on wheat seed components. The significant effects of PGR on plant growth and yield production are mainly through the regulation of gene expression and activity. Accordingly, PGRs are able to trigger plant response under stress by activating different signaling pathways, which are able to alter plant physiology in a way so that plant tolerance increases under stress (Gupta and Thind, 2019; Miransari et al., 2008).

Shourbalal et al. (2019) found that if PGRs can alter plant physiology so that the plant can allocate photosynthates to the grains, without the process of vernalization (which is done under cold conditions), it would be possible to plant winter wheat as spring wheat. The present research also confirms that the PGRs under investigation were able to increase plant morphology and seed components, both in the autumn and spring planting.

PH

The genetics of wheat PH is complicated and is determined by the Rht gene, which reduces PH. The plant hormone, gibberellin, regulates different plant growth mechanisms, especially in the stem growth. Results indicated that spraying the plants with GA_{3+7} and GA_{4+7} resulted in the highest PH. Gibberellins increase cellular division and growth in plant stem. The interaction of priming and spraying indicated that the highest PH was related to autumn planting and spraying with GA_{3+7} and kinetin. Gibberellins can importantly increase wheat stem. Zhang et al. (Zhang et al., 2007) investigated two types of gibberellins, GA_3 and GA_4 on two different hybrids of wheat, compared with their parents, and found that plant stem was significantly increased by GA_4. Priming with GA_{3+4+7} and spraying with GA_{4+7} resulted in the highest plant height in spring wheat. This is similar to the results of the experiments by Zhang et al. (2007), who found that priming wheat seeds with 250-1000 mg/l gibberellins increased PH in the genotypes planted with a 2- to 4-week delay from the suitable time of planting. Ullah et al. (Ullah et al., 2019) also reviewed the effects of seed priming with mineral elements and PGRs on plant growth under stress.

SDW

The results indicated that the effects of priming with GA_{3+4+7}+kinetin +BA6 led to the highest SDW in spring wheat. A high level of gibberellins was found in the endosperm of the seeds, which are expanding, used for cellular division (Miransari and Smith, 2014)Increased levels of gibberellins during the initial growth phase of the seeds indicates that the gibberellins can regulate the transfer of metabolites to the expanding seeds. Accumulation of cytokinins in seeds is also an important component of cellular division in the endosperm (Zhu et al., 2019). Accordingly, the combination of gibberellins and cytokinins in the present research favorably affected wheat growth and yield production without the need for the vernalization process. Cytokinins can enhance the process of seed filling (Farouk and Sanusi, 2019); however, in this study the positive effects of cytokinins along with gibberellins were observed on seed filling, which, to our knowledge, has not been reported in the literature.

GNP

Positive effects of cytokinins and gibberellins were found on GNP. These plant hormones may increase seed demand for N during the process of seed filling, and as a result, wheat plants may be more willing to absorb higher N. Activation of the genes which are essential for N metabolism may also be enhanced in the presence of such PGRs and subsequently direct more N to the seeds, which can increase wheat yield and improve seed quality by increasing the protein content of the seeds (Kong et al., 2016).

GMP

The effects of PGRs on yield production (Abbaszadeh et al., 2020)are exerted by affecting seed size, flour quality, flowering, and growth and germination of wheat seeds. The present research indicated the effects of PGRs on GMP especially by gibberellins during the spring. The PGRs under study can affect plant growth through hormonal pathways, which can affect different plant activities including the uptake of water and the leaf stomata. Accordingly, the plant can manage its water behavior more efficiently affecting its growth, yield production, and grain moisture under different conditions including stress (Abid et al., 2017).

Conclusion

Effects of different PGRs, including gibberellins, kinetin, benzyl adenine 6, and cold stratification, as priming and spraying treatments, on the morphology (root growth and plant height) and seed components (spike dry weight, grain N percentage and grain moisture percentage) of winter wheat were examined. Results indicated that the effects of PGRs were more evident on plant morphology during the autumn and on seed components during the spring. The PGRs under study were more effective than cold stratification on plant morphology and seed components. In fact, the most efficient treatment was the combined use of the PGRs, which can be a function of the cross-talk among these PGRs. These growth regulators are able to strengthen wheat in different conditions including stress by triggering the activity of different genes, which results in the activation of different signaling pathways including hormonal ones. The obtained results confirmed previous research indicating that it is possible to shorten or remove vernalization process in winter wheat, which is required for wheat transition from the vegetative to the reproductive stage, using PGR, under drought stress conditions in Isfahan, Iran, and the similar areas, worldwide.

References

Abbas, K., S. K. Khalil and A. S. M. Hussin. 2010. Modified starches and their usages in selected food products: A review study. *Journal of Agricultural Science,* 2, (2) 90.

Abbaszadeh, B., M. Layeghhaghighi, R. Azimi and N. Hadi. 2020. Improving water use efficiency through drought stress and using salicylic acid for proper production of Rosmarinus officinalis L. *Industrial Crops and Products,* 144, 111893.

Abid, M., Y. Shao, S. Liu, F. Wang, J. Gao, D. Jiang, Z. Tian and T. Dai. 2017. Pre-drought priming sustains grain development under post-anthesis drought stress by regulating the growth hormones in winter wheat (Triticum aestivum L.). *Planta,* 246, 509-524.

Ali, S., T. C. Charles and B. R. Glick. 2017. Endophytic phytohormones and their role in plant growth promotion. *Functional importance of the plant microbiome:*

implications for agriculture, forestry and bioenergy, 89-105.

Askarnejad, M. R., A. Soleymani and H. R. Javanmard. 2021. Barley (Hordeum vulgare L.) physiology including nutrient uptake affected by plant growth regulators under field drought conditions. *Journal of Plant Nutrition,* 44, (15) 2201-2217.

Chandra, D., R. Srivastava, V. V. Gupta, C. M. Franco, N. Paasricha, S. K. Saifi, N. Tuteja and A. K. Sharma. 2019. Field performance of bacterial inoculants to alleviate water stress effects in wheat (Triticum aestivum L.). *Plant and soil,* 441, 261-281.

Daei, G., M. Ardekani, F. Rejali, S. Teimuri and M. Miransari. 2009. Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. *Journal of plant physiology,* 166, (6) 617-625.

Dwivedi, S., A. Arora, V. Singh and G. Singh. 2018. Induction of water deficit tolerance in wheat due to exogenous application of plant growth regulators: membrane stability, water relations and photosynthesis. *Photosynthetica,* 56, 478-486.

Dwivedi, S., V. Singh, G. Singh and A. Arora. 2012. Combined effect of cytokinin, paclobutrazol and ascorbic acid on nitrogen metabolism and yield of wheat (Triticum aestivum L.) under water deficit stress condition. *Indian Journal of Plant Physiology,* 17, (3&4) 259-267.

Dziurka, M., K. Dziurka, B. Jurczyk, A. Ostrowska, F. Dubert, A. Janeczko and J. Biesaga-Koœcielniak. 2016. Changes in gibberellin levels during winter wheat vernalization. *ACTA BIOLOGICA CRACOVIENSIA,*

Farouk, S. and A. B. a.-J. Sanusi. 2019. Potent induction of wheat flowering and its related to yield components. *Journal of Applied Sciences,* 19, (4) 270-278.

Geng, J., L. Li, Q. Lv, Y. Zhao, Y. Liu, L. Zhang and X. Li. 2017. TaGW2-6A allelic variation contributes to grain size possibly by regulating the expression of cytokinins and starch-related genes in wheat. *Planta,* 246, 1153-1163.

Gupta, N. and S. Thind. 2019. Foliar application of glycine betaine alters sugar metabolism of wheat leaves under prolonged field drought stress. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences,* 89, 877-884.

Gupta, N., S. K. Thind and N. S. Bains. 2014. Glycine betaine application modifies biochemical attributes of osmotic adjustment in drought stressed wheat. *Plant Growth Regulation,* 72, 221-228.

Kamali, N., M. K. Pour and A. Soleymani. 2020. Light absorption and light extinction in barley (Hordeum vulgare L.) as affected by planting dates and plant genotypes. *Theoretical and Applied Climatology,* 142, 589-597.

Kong, L., Y. Xie, L. Hu, B. Feng and S. Li. 2016. Remobilization of vegetative nitrogen to developing grain in wheat (Triticum aestivum L.). *Field Crops Research,* 196, 134-144.

Li, N., S. Zhang, Y. Liang, Y. Qi, J. Chen, W. Zhu and L. Zhang. 2018. Label-free quantitative proteomic analysis of drought stress-responsive late embryogenesis abundant proteins in the seedling leaves of two wheat (Triticum aestivum L.) genotypes. *Journal of Proteomics,* 172, 122-142.

Lv, S., K. Feng, S. Peng, J. Wang, Y. Zhang, J. Bian and X. Nie. 2018. Comparative analysis of the transcriptional response of tolerant and sensitive wheat genotypes to drought stress in field conditions. *Agronomy,* 8, (11) 247.

Martínez, C., A. Espinosa-Ruiz and S. Prat. 2016. Gibberellins and plant vegetative growth. *Annual Plant Reviews, Volume 49: Gibberellins, The,* 285-322.

Miransari, M., H. Bahrami, F. Rejali and M. Malakouti. 2008. Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (Triticum aestivum L.) growth. *Soil biology and biochemistry,* 40, (5) 1197-1206.

Miransari, M. and D. Smith. 2014. Plant hormones and seed germination. *Environmental and experimental botany,* 99, 110-121.

Shourbalal, S. K. S., A. Soleymani and H. R. Javanmard. 2019. Shortening vernalization in winter wheat (Triticum aestivum L.) using plant growth regulators and cold stratification. *Journal of Cleaner Production,* 219, 443-450.

Small, C. C. and D. Degenhardt. 2018. Plant growth regulators for enhancing revegetation success in reclamation: A review. *Ecological engineering,* 118, 43-51.

Tahaei, A., A. Soleymani and M. Shams. 2016. Seed germination of medicinal plant, fennel (Foeniculum vulgare Mill), as affected by different priming techniques. *Applied biochemistry and biotechnology,* 180, 26-40.

Ullah, A., B. Shahzad, M. Tanveer, F. Nadeem, A. Sharma, D. J. Lee and A. Rehman. 2019. Abiotic stress tolerance in plants through pre-sowing seed treatments with mineral elements and growth regulators. *Priming and pretreatment of seeds and seedlings: implication in plant stress tolerance and enhancing productivity in crop plants,* 427-445.

Zafari, M., A. Ebadi, S. Jahanbakhsh and M. Sedghi. 2020a. Safflower (Carthamus tinctorius) biochemical properties, yield, and oil content affected by 24-epibrassinosteroid and genotype under drought stress. *Journal of agricultural and food chemistry,* 68, (22) 6040-6047.

Zafari, M., A. Ebadi, M. Sedghi and S. Jahanbakhsh. 2020b. Alleviating effect of 24-epibrassinolide on seed oil content and fatty acid composition under drought stress in safflower. *Journal of Food Composition and Analysis,* 92, 103544.

Zamani, S., M. R. Naderi, A. Soleymani and B. M. Nasiri. 2020. Sunflower (Helianthus annuus L.) biochemical properties and seed components affected by potassium fertilization under drought conditions. *Ecotoxicology and environmental safety,* 190, 110017.

Zhang, H., K. J. Horgan, P. H. Reynolds and P. E. Jameson. 2010. 6-Benzyladenine metabolism during reinvigoration of mature Pinus radiata buds in vitro. *Tree physiology,* 30, (4) 514-526.

Zhang, Y., Z. Ni, Y. Yao, X. Nie and Q. Sun. 2007. Gibberellins and heterosis of plant height in wheat (Triticum aestivum L.). *BMC genetics,* 8, (1) 1-10.

Zhu, Y., J. Chu, X. Dai and M. He. 2019. Delayed sowing increases grain number by enhancing spike competition capacity for assimilates in winter wheat. *European Journal of Agronomy,* 104, 49-62.