

## WHEAT GRAIN QUALITY AND ITS RELATIONSHIP WITH PLANT GROWTH REGULATORS

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Because of its widespread consumption, bread wheat is an important cereal grain worldwide that is valued for its nutritional and economic properties. However, the quality of wheat is affected by various stresses and environmental conditions. This study investigated the effects of the exogenous application, in the early grain-filling stage, of different plant hormones (3-indoleacetic acid [IAA], gibberellic acid [GA<sub>3</sub>], and 6-benzylaminopurine [6-BAP]) and distilled water as the control on two wheat cultivars (Rijaw and Azar-2). The results showed that interaction effects of plant hormones × genotypes were significant on gluten index, total gluten and falling number. As well, grain-filling duration was affected by plant hormone and genotype. However, hectoliter weight was affected only by genotype. There was a positive relationship between gluten index and grain-filling duration in both years of the experiment. Overall, the application of plant hormones can increase wheat grain-filling duration under dry-land conditions, leading to increases in total gluten and gluten index.

**Keywords:** Falling number, gluten index, grain-filling rate, rainfed, plant hormone, wheat.

### INTRODUCTION

Wheat is consumed mainly in the form of bread. In the bread-making process, the proteins derived from wheat flour play a major role in determining viscoelastic properties. The amount of protein and its composition are two important factors affecting the baking quality of wheat flour (Khan *et al.*, 2013). In addition to protein content, traits such as total gluten, gluten index, hectoliter weight (which represents the conversion efficiency of grain to flour), falling number (which describes alpha-amylase activity), and starch content play a vital role in determining the quality of the final product prepared from the wheat flour (Souza *et al.*, 2002; Shewry, 2009). The measured wheat gluten in a sample indicates the quantity of gluten in the flour, and gluten quality is shown by the gluten index (which describes the ratio of strong gluten to total gluten). Bread quality can be affected by grain genetics and by environmental factors such as soil conditions, irrigation, weather, seed storage conditions, and seed content (Noorka *et al.*, 2009).

Water deficits, high temperatures, and nutritional requirements during the post-anthesis and grain-filling stages have significant effects on many biochemical properties of wheat grains (Ashraf, 2010) and can directly affect leaf photosynthesis and the availability of assimilates (Kuanar *et al.*, 2010; Zhang *et al.*, 2010). Indeed, these factors can change the grain-filling rate and duration. In addition, genotypes with tolerance to drought stress have higher values

for traits related to baking quality (Balla *et al.*, 2011; Ashraf, 2014).

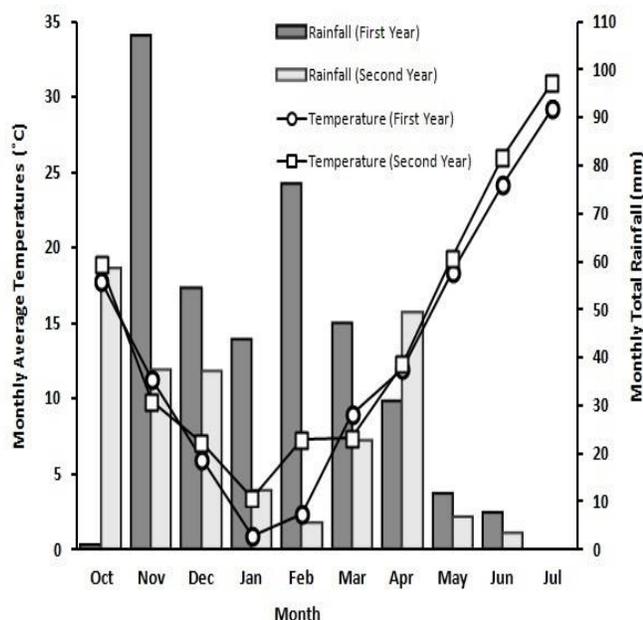
This experiment was conducted to investigate the effects of the application of different plant hormones on certain characteristics related to grain and baking quality in two bread wheat genotypes under dry-land conditions.

### MATERIALS AND METHODS

**Experimental site:** A two-year study was conducted in the experimental field at the Campus of Agriculture and Natural Resources of Razi University in Kermanshah, Iran (34°21'N; 47°9'E; 319 m AMSL). This location is in a semi-arid zone.

**Experimental design:** The experiment was carried out as a factorial based on a randomized complete block design with three replications in the 2013–2014 and 2014–2015 cropping seasons. Weather characteristics, including monthly average temperatures (°C) and total rainfall (mm), for both years of the experiment are shown in Figure 1. The factors were: (i) exogenous application of different plant growth regulators, namely, the auxin 3-indole acetic acid (IAA), gibberellic acid (GA<sub>3</sub>), and 6-benzylaminopurine (6-BAP), and distilled water as the control in the early grain-filling stage; and (ii) two dry-land wheat genotypes (cv. Rijaw and cv. Azar-2), which have optimum and low grain yield potential respectively in Kermanshah province. The cultivar Rijaw is a newly released rainfed cultivar. The concentration applied for each hormone was 50 µM. Each plot contained six planting rows. The

distance between planting rows was 25 cm, and the planting density was 300 seeds/m<sup>2</sup>. The distance between plots was 50 cm. Sowing was performed manually. For the hormone application step, to ensure that the plant growth regulators were absorbed, foliar application was performed on three consecutive days after sunset (to prevent the plant growth regulators from being degraded by sunlight).



**Figure 1. Monthly average temperatures (°C) and total rainfall (mm) in the two years (2013–2014 and 2014–2015) of the experiment.**

**Measured traits:** After harvest, the following baking quality traits were measured in the Seed Technology Laboratory of the Department of Agronomy and Plant Breeding, Campus of Agricultural and Natural Resources, Razi University, Kermanshah, Iran.

Falling number was measured by the standard method based on alpha-amylase activity. The falling number value was determined with a Perten FN 1500 instrument (method adapted from Method 65-81B; AACC, 2000b).

Gluten index was determined using a Glutomatic Gluten Washer and a Gluten Index Centrifuge and measured by a Glutomatic System (method adapted from Method 38-12A; AACC, 2000a).

Total gluten (g) was determined by adding together the amounts of weak gluten (g) and strong gluten (g).

The grain-filling duration was considered to be the duration between the 50% anthesis stage and physiological maturity, and the grain-filling rate was obtained by dividing the final grain weight by the grain-filling duration (Egli, 1999).

Hectoliter weight was determined by a hectoliter instrument and expressed in kilograms per hectoliter.

Statistical analyses were performed using SAS 9.1 software. Differences between means were determined by the least significant difference (LSD) test at a 5% probability level.

## RESULTS

**Grain-filling duration and rate:** The effect of the exogenous application of different plant hormones was significant on grain-filling duration. There was also a significant difference between the genotypes for this trait, but their interaction effect was not significant (Tables 1 and 2). In both years of the experiment, the plants treated with 6-BAP had the longest grain-filling duration, and there was no significant difference between the GA<sub>3</sub>, IAA, and control plants (Table 4). Of the two genotypes, Azar-2 had a longer grain-filling duration than Rijaw did (Table 3). Nevertheless, the effects of hormone, genotype, and their interaction on grain-filling rate were not significant in either year of the experiment (Tables 1 and 2).

**Falling number:** As Tables 1 and 2 show, only the effects of genotype and the interaction between genotype and plant hormone were significant on falling number. Average falling number was higher for the Azar-2 genotype than for Rijaw, even with the application of various plant hormones. Of the plant hormones, the application of GA<sub>3</sub> resulted in the lowest number in Rijaw, whereas Azar-2 did not show such a response (Table 5).

**Gluten index and total gluten:** As Tables 1 and 2 show, the effects of plant hormone, genotype, and the interaction between them were significant on gluten index and total gluten in both years of the experiment. Of the hormones, 6-BAP resulted in the highest gluten index (89.7% and 75.5% in the first and second years respectively) and total gluten (4.9 g and 5.0 g in the first and second years, respectively) in the Rijaw genotype (Table 5). In addition, there was a positive and significant relationship between gluten index and grain-filling duration in both years of the experiment ( $R^2 = 0.83$  and  $R^2 = 0.96$  for the first and second years, respectively) (Fig. 2).

**Hectoliter weight:** Hectoliter weight was different between the genotypes, and the application of different plant hormones did not have a significant effect on this trait (Tables 1 and 2). Hectoliter weight was higher in the Rijaw genotype than in Azar-2 in both years of the experiment (Table 3).

**Table 1. Analysis of variance table for the grain quality traits of two wheat genotypes (Rijaw and Azar-2) treated with different plant hormones (3-indoleacetic acid, gibberellic acid, and 6-benzylaminopurine) in the early grain-filling stage in the first year of the experiment (MS).**

Source of variation	df	Grain-filling rate	Grain-filling duration	Falling number	Gluten index	Total gluten	Hectoliter weight
Replication	2	0.40 <sup>ns</sup>	88.6 <sup>**</sup>	3,248.1 <sup>ns</sup>	14.9 <sup>ns</sup>	0.47 <sup>ns</sup>	72.6 <sup>ns</sup>
Genotype (G)	1	0.01 <sup>ns</sup>	243.3 <sup>**</sup>	84,847.0 <sup>**</sup>	1,708.5 <sup>**</sup>	3.01 <sup>**</sup>	450.6 <sup>*</sup>
Hormone (Hr)	3	0.02 <sup>ns</sup>	49.4 <sup>*</sup>	10,065.4 <sup>ns</sup>	358.4 <sup>**</sup>	0.95 <sup>**</sup>	42.0 <sup>ns</sup>
G × Hr	3	0.01 <sup>ns</sup>	11.2 <sup>ns</sup>	10,180.7 <sup>*</sup>	283.3 <sup>**</sup>	0.62 <sup>*</sup>	144.3 <sup>ns</sup>
CV%	-	17.91	12.45	12.27	10.57	10.97	16.88

df, degrees of freedom; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; ns, not significant; CV%, coefficient of variance.

**Table 2. Analysis of variance table for the grain quality traits of two wheat genotypes (Rijaw and Azar-2) treated with different plant hormones (3-indoleacetic acid, gibberellic acid, and 6-benzylaminopurine) in the early grain-filling stage in the second year of the experiment (MS).**

Sources of variation	df	Grain-filling rate	Grain-filling duration	Falling number	Gluten index	Total gluten	Hectoliter weight
Replication	2	0.04 <sup>ns</sup>	16.5 <sup>ns</sup>	3,248.1 <sup>ns</sup>	14.9 <sup>ns</sup>	0.04 <sup>ns</sup>	73.5 <sup>ns</sup>
Genotype (G)	1	0.11 <sup>ns</sup>	155.0 <sup>**</sup>	85,920.0 <sup>**</sup>	1,965.6 <sup>**</sup>	6.72 <sup>**</sup>	636.5 <sup>**</sup>
Hormone (Hr)	3	0.01 <sup>ns</sup>	58.3 <sup>*</sup>	6,912.7 <sup>ns</sup>	158.2 <sup>**</sup>	0.05 <sup>ns</sup>	130.6 <sup>ns</sup>
G × Hr	3	0.009 <sup>ns</sup>	4.3 <sup>ns</sup>	12,557.0 <sup>**</sup>	98.3 <sup>*</sup>	1.87 <sup>**</sup>	39.1 <sup>ns</sup>
CV%	-	19.82	13.66	11.91	9.07	13.91	15.16

df, degrees of freedom; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; ns, not significant; CV%, coefficient of variance.

**Table 3. Mean differences in grain-filling duration and hectoliter weight between two wheat genotypes (Rijaw and Azar-2) in each year of the experiment.**

Genotype	Grain-filling duration (d)		Hectoliter weight (kg hL <sup>-1</sup> )	
	First year	Second year	First year	Second year
Rijaw	25.6	24.6	56.6	60.3
Azar-2	31.9	29.7	47.9	50.0
LSD5%	3.13	3.25	7.73	7.32

**Table 4. Mean comparison of effects of different plant hormones on grain-filling duration under dry-land conditions in each year of the experiment.**

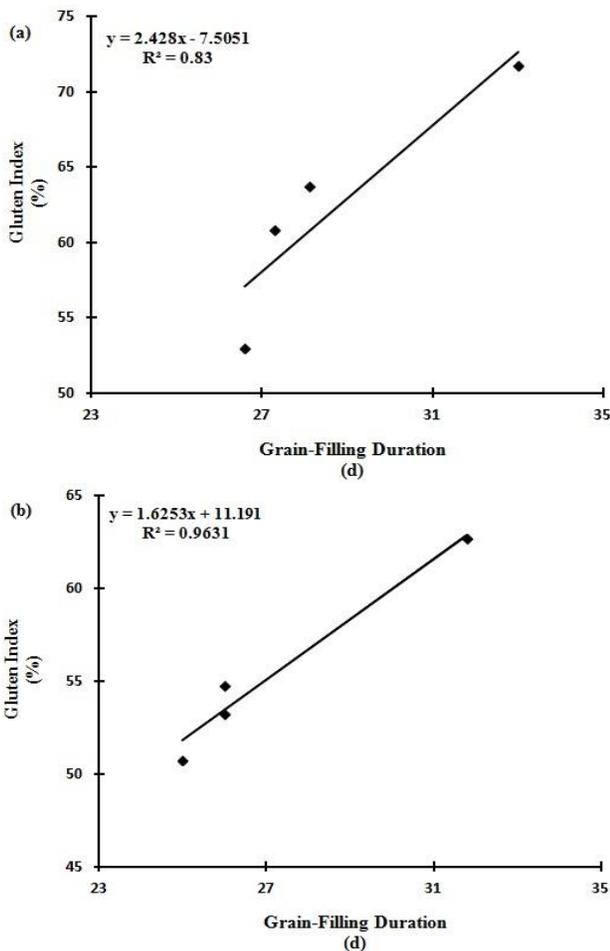
Hormone	Grain-filling duration (d)	
	First year	Second year
Control	26.6 <sup>b</sup>	25.0 <sup>b</sup>
IAA	27.3 <sup>b</sup>	26.0 <sup>b</sup>
GA <sub>3</sub>	28.1 <sup>b</sup>	26.0 <sup>b</sup>
6-BAP	33.0 <sup>a</sup>	31.8 <sup>a</sup>
LSD5%	4.44	4.60

Means with the same letter are not significantly different. IAA, 3-indoleacetic acid; GA<sub>3</sub>, gibberellic acid; 6-BAP, 6-benzylaminopurine.

**Table 5. Interaction effects between exogenous applications of different plant hormones and two wheat genotypes on grain quality traits in the early grain-filling stage in each year of the experiment.**

Genotype	Hormone	Falling number (s)		Gluten index (%)		Total gluten (g)	
		First year	Second year	First year	Second year	First year	Second year
Rijaw	Control	448.0 <sup>a</sup>	403.0 <sup>c</sup>	57.5 <sup>cde</sup>	54.4 <sup>cd</sup>	4.0 <sup>b</sup>	3.9 <sup>bc</sup>
	IAA	425.6 <sup>b</sup>	405.6 <sup>c</sup>	69.5 <sup>b</sup>	62.0 <sup>bc</sup>	4.0 <sup>b</sup>	4.5 <sup>ab</sup>
	GA <sub>3</sub>	271.3 <sup>c</sup>	301.3 <sup>d</sup>	66.3 <sup>bc</sup>	66.1 <sup>b</sup>	3.9 <sup>b</sup>	3.9 <sup>bc</sup>
	6-BAP	348.0 <sup>bc</sup>	434.0 <sup>bc</sup>	89.7 <sup>a</sup>	75.5 <sup>a</sup>	4.9 <sup>a</sup>	5.0 <sup>a</sup>
Azar-2	Control	507.6 <sup>a</sup>	572.6 <sup>a</sup>	48.5 <sup>e</sup>	47.3 <sup>de</sup>	2.7 <sup>c</sup>	2.7 <sup>d</sup>
	IAA	469.0 <sup>a</sup>	443.0 <sup>bc</sup>	52.1 <sup>de</sup>	44.6 <sup>e</sup>	3.8 <sup>b</sup>	3.4 <sup>c</sup>
	GA <sub>3</sub>	489.6 <sup>a</sup>	524.6 <sup>ab</sup>	61.2 <sup>bcd</sup>	43.6 <sup>e</sup>	3.8 <sup>b</sup>	3.7 <sup>bc</sup>
	6-BAP	502.3 <sup>a</sup>	482.3 <sup>abc</sup>	53.7 <sup>de</sup>	50.0 <sup>de</sup>	3.7 <sup>b</sup>	3.6 <sup>bc</sup>
LSD5%	-	93.0	93.0	11.54	8.80	0.70	0.93

Means with the same letter are not significantly different. IAA, 3-indoleacetic acid; GA<sub>3</sub>, gibberellic acid; 6-BAP, 6-benzylaminopurine.



**Figure 2. Relationship between gluten index and grain-filling duration in the first (a) and second (b) years of the experiment.**

## DISCUSSION

Grain-filling duration has a major impact on wheat bread quality. An increase in grain-filling duration causes an increase in the transport of assimilates to grains. Grain quality is influenced by the supply of assimilates during anthesis and later stages (Rotundo *et al.*, 2009; Seebauer *et al.*, 2010). The availability of assimilates is directly correlated with photosynthesis activity (Kuanar *et al.*, 2010), and any factor that affects the grain-filling duration can have an impact on grain quality. Guedira *et al.* (2002) and Guttieri *et al.* (2001) reported that water stress played a key role in reducing the moisture percentage and also increased protein and gluten content. Moeinian *et al.* (2011) observed a significant difference between irrigation treatments for seed gluten content: the highest percentage of grain gluten, 9.47%, was obtained in the severe stress treatment, and the lowest percentage, 7.4%, was obtained in the normal irrigation treatment. Noorka *et al.* (2009) reported that although drought has negative effects on yield and yield components, a low amount of aggregation, especially during reproductive stages, increases the gluten percentage. Guttieri *et al.* (2005) observed that genotype, nitrogen fertilizer, and irrigation affected grain protein concentration, which differed significantly among their optimum nitrogen levels for grain yield. This relationship is shown in our results, in that the application of 6-BAP increased grain-filling duration. As well, the application of this hormone enhanced many photosynthesis characteristics in the treated wheat plants (data not shown). Therefore, by increasing the rate of photosynthesis and the grain-filling period, this hormone increased the quantity (total gluten) and quality (gluten index) of gluten. However, the effect of genotypes should not be ignored, given that a response was observed in the Rijaw genotype only. The Rijaw plants treated with GA<sub>3</sub> showed a

significant reduction in falling number. This hormone plays an important role in alpha-amylase synthesis and the stimulation of alpha-amylase activity in grain and flour (Kılıç Apar and Özbek, 2004; Kondhare *et al.*, 2012). However, alpha-amylase activity has a negative relationship with falling number. Therefore, increasing the alpha-amylase activity by the application of GA<sub>3</sub> led to the decrease in falling number.

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## REFERENCES

- AACC. 2000a. Method 38-12A. In: Approved methods of the American Association of Cereal Chemists. 10th ed. AACC, St. Paul, MN.
- AACC. 2000b. Method 65-81B. In: Approved methods of the American Association of Cereal Chemists. 10th ed. AACC, St. Paul, MN.
- Ashraf, M. 2010. Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv.* 28:169-183.
- Ashraf, M. 2014. Stress-induced changes in wheat grain composition and quality. *Crit. Rev. Food Sci. Nutr.* 54:1576-1583.
- Balla, K., M. Rakszegi, Z. Li, F. Békés, S. Bencze and O. Veisz. 2011. Quality of winter wheat in relation to heat and drought shock after anthesis. *Czech J. Food Sci.* 29:117-128.
- Egli, D.B. 1999. Seed biology and the yield of grain crops. CAB International, Wallingford, UK.
- Guedira, M., P.J. McCluskey, F. MacRitchie and G.M. Paulsen. 2002. Composition and quality of wheat grown under different shoot and root temperatures during maturation. *Cereal Chem.* 79:397-403.
- Guttieri, M.J., R. McLean, J.C. Stark and E. Souza. 2005. Managing irrigation and nitrogen fertility of hard spring wheats for optimum bread and noodle quality. *Crop Sci.* 45:2049-2059.
- Guttieri, M.J., J.C. Stark, K. O'Brien and E. Souza. 2001. Relative sensitivity of spring wheat grain yield and quality parameters to moisture deficit. *Crop Sci.* 41:327-335.
- Khan, S., A.B. Ghanghro, A.N. Memon, I. Tahir, A.M. Shah, M.A. Sahito, F.N. Talpur and S. Qureshi. 2013. Quantitative analysis of wheat proteins in different varieties grown in Sindh, Pakistan. *Int. J. Agri. Crop Sci.* 5:1836-1839.
- Kılıç Apar, D. and B. Özbek. 2004. Corn, rice and wheat starch hydrolysis by using various alpha-amylase enzymes at temperature 40°C. *J. Eng. Nat. Sci.* 2:55-67.
- Kondhare, K.R., P.S. Kettlewell, A.D. Farrell, P. Hedden and J.M. Monaghan. 2012. Effects of exogenous abscisic acid and gibberellic acid on pre-maturity  $\alpha$ -amylase formation in wheat grains. *Euphytica.* 188:51-60.
- Kuanar, S.R., R. Panigrahi, E. Kariali and P.K. Mohapatra. 2010. Apoplasmic assimilates and grain growth of contrasting rice cultivars differing in grain dry mass and size. *Plant Growth Regul.* 61:135-151.
- Moeinian, M.R., K. Zargari and J. Hasanpour. 2011. Effect of boron foliar spraying application on quality characteristics and growth parameters of wheat grain under drought stress. *Am. Eur. J. Agric. Environ. Sci.* 10:593-599.
- Noorka, I.R., S. Rehman, J.R. Haidry, I. Khaliq, S. Tabasum and G. Mueen-ud-Din. 2009. Effect of water stress on physico-chemical properties of wheat (*Triticum aestivum* L.). *Pak. J. Bot.* 41:2917-2924.
- Rotundo, J.L., L. Borrás, M.E. Westgate and J.H. Orf. 2009. Relationship between assimilate supply per seed during seed filling and soybean seed composition. *Field Crops Res.* 112:90-96.
- Seebauer, J.R., G.W. Singletary, P.M. Krumpelman, M.L. Ruffo and F.E. Below. 2010. Relationship of source and sink in determining kernel composition of maize. *J. Exp. Bot.* 61:511-519.
- Shewry, P.R. 2009. Wheat. *J. Exp. Bot.* 60:1537-1553.
- Souza, E.J., R.A. Graybosch and M.J. Guttieri. 2002. Breeding wheat for improved milling and baking quality. *J. Crop Prod.* 5:39-74.
- Zhang, T., Z. Wang, Y. Yin, R. Cai, S. Yan and W. Li. 2010. Starch content and granule size distribution in grains of wheat in relation to post-anthesis water deficits. *J. Agron. Crop Sci.* 196:1-8.