MEASUREMENT OF INDOLYL-3-ACETIC ACID AND GIBBERELLIN LEVELS AT VARIOUS GRAIN TYPE AND POSITION WITHIN DEVELOPING GRAINS OF WHEAT

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Abstract

Grain growth rate (GGR), gibberellin and indolyl-3-acetic acid (IAA) levels were studied at different grain type and position within developing grains of wheat (Triticum aestivum L. var. Bahar). Main spikes were divided into three grain positions included proximal, middle, and distal regions, and further into two grain types included basal and apical grains. Grain dry matter accumulation, gibberellins including GA_1, GA_3 and GA_4,and IAA levels were determined in ten labeled spikes which sampled five times, seven days interval started from seventh day after anthesis (DAA) up to 30^{th} DAA, and also in maturity. Gibberellins and IAA contents increased until 16^{th} and 23^{st} DAA, respectively. The maximum level of grain growth rate (GGR) was observed at 16^{th} DAA. Furthermore, the differences in both gibberellins and IAA contents, among spikelets in different regions of the spike, and also among grains within a spikelet were correlated with the differences in dry matter accumulation. The results suggest that both gibberellins and IAA levels play an important role in regulating grain filling pattern.

Key words: Gibberellins; IAA; spike; grain development; wheat

Wheat (*triticum aestivum* L.) is the most important cereal crop in the food culture of both developed and developing countries in the world. World wheat production increases by approximately 1.5% annually to meet the growing demand for food that results from population growth and economic development [5]. A Substantial increase in grain yield potential, together with good use of water and fertilizer is required to ensure food security in the future. For improvements in photosynthetic capacity to result in additional wheat yield, extra assimilates must be partitioned to develop grains and/or potential grain weight be increased to accommodate the extra assimilates [8,11,12]. The position of grain within a spike to some extent determines its final grain weight which can range from 20 to 60 mg. the grains from spikelet's in the middle region of the spike and from the basal region within each spikelet are more towards the upper level of this rand [7,13].

Various factors such as assimilate availability and/or transport capacity [2] or the possible role of plant growth regulators [4] are offered to explain these differences. Gibberellins play important role in regulating plant growth and development such as intensity and direction of assimilate flow, cell elongation, and cell division rat [7,9] In cereals, high levels of gibberellins are generally found in the endosperm of developing seeds, which may be required for active cell division during the early phase of grain setting [14,16]. The position of grain within a spike to some extend determines its final grain weight which can range from 20 to 60 mg.

The grains from spikelet's in the middle region of the spike and from the basal region within each spikelet are more towards the upper level of this range [4]. Various explanations such as assimilate availability and/or transport capacity [25] or the possible role of plant growth regulators [30] are offered to explain these differences. Gibberellins (GAs) play an important role in regulating plant growth and development such as stem

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elongation, germination, dormancy, synthesis of α-amylase, flowering, sex expression, enzyme induction and leaf and fruit senescence [7,17,15,6]. Asthir et al. [2] reported that gibberellins act as positive modulators of grain sink activity, whereas, IAA acts as a negative modulator. It was confirmed by Zhang et al. [30] by exogenous application of gibberellins that resulted in improvement of sink activity due to the GAs role as modulators of sugar metabolism. On the other hand, indolyl-3-acetic acid (IAA) is another plant growth regulator which plays an important role in some of the physiological responses such as stimulation of the closure of stomata, inhibition of shoot growth, synthesize storage proteins of seed, inhibition of the affect of gibberellins on stimulating de novo synthesis of α-amylase, and maintenance of dormancy [7,22,24]. Ahmadi and Baker [1] observed a reduction in sucrose transportation into the grains with lowered the ability of starch synthesis in intact grains. Ober [20] demonstrated that IAA could be trans located from leaf tissue to grains and acts as a sensory link between developing reproductive structures and maternal tissues deprived of water. Furthermore, IAA also may influence early establishment of sink size through regulation of cell number [20]. Both field and pot trials of Goldbach [11] indicated that IAA content in the grain increased up to the start of grain ripening and then decreased gradually with the cessation of dry matter accumulation and rapidly later as the moisture content of the grain decreased. Wang et al. [27] suggested that the poor grain filling of rice was associated with low grain doses of both IAA and IAA. Evaluation the relation of dry matter accumulation, gibberellins and IAA levels at different grain type and position could be important to identifying the role of plant growth regulators on differences in dry matter accumulation of grains in a spike, which could be the key in developing wheat with higher grain yield potential. Hence, the objective of this study was to evaluate the GAs and IAA levels along with dry matter accumulation at different grain type and position within a spike of Bahar wheat.

MATERIAL AND METHOD

Experimental Setup and Plant Sampling

Single plants of the wheat (*Triticum aestivum* L. var. Bahar-138) were grown in plastic containers with a diameter of 4.5 cm and depth of 20 cm. The pots were filled with a pasteurized soil which classified as a clay loam with 30.1% sand, 25.7% clay and 46.2% silt, an electrical conductivity (EC_e) of 1.2 dS m^{-1} , a pH of 7.1 (saturated paste), and organic C of 0.62%. The plants were grown in a screen covered hall under otherwise natural conditions. The pots were watered as described by Houshmandfar et al. [14], and fertilized once a week with half strength Peter's solution (NPK = 10:10:10) [5]. The secondary tillers were removed as they appeared. Ten labeled spikes were sampled five times, seven days interval started from seventh day after anthesis (DAA) up to 30^{th} DAA, and also in maturity. Spikes were divided into three grain positions included proximal (spikelet number 1 to 5), middle (spikelet number 6 to 15), and distal (spikelet number 16 to 20) regions, and further into two grain types included basal (bold) (grain No. 1 and 2) and apical (small) (grain No. 3 upward). All samples were divided into two parts, one was dried in an oven at 70 °C for 72 h, and then weighed for dry matter accumulation, and another was frozen in liquid N_2 for one min and kept in a freezer at -70 °C for gibberellins included GA_1 and GA_4 , and also IAA analysis. Grain growth rate (GGR) was calculated using the following equation [10]:

$$GGR\ (mgd^{-1}) = \frac{W_2 - W_1}{T_2 - T_1}$$

Where,
W_1 = Total dry matter of grain at time t_1
W_2 = Total dry matter of grain at time t_2
T_1 = Time of first observation
T_2 = Time of second observation

Gibberellins and IAA contents were expressed on fresh weight. Linear regression was used to evaluate the relationships between traits. The data were analyzed statistically using analysis of variance and critical differences (CD) at 5 percent level were computed.

Gibberellins and IAA analysis

The methods for analysis of GAs and IAA were modified from those described by Sundberg [26] and Yang et al [28]. Samples consisting of 3.0 g dehulled and frozen grains were ground in an ice-cold mortar in 10 ml 80% (v/v) methanol extraction medium containing 1 m mol L^{-1} butylated hydroxytoluence (BHT) as an antioxidant. The homogenate was filtered and the solid residue was further extracted twice with the same solvent. The metabolic extracts were kept for continuous stirring at 4 °C in the dark for about 4 h and centrifuged at 10,000 rpm for 10 min at the same temperature. The supernatants were combined and

concentrated to a water residue in vacuum at 40 °C by rotatory evaporation. The volume was adjusted to 10 ml with 0.05 M Na phosphate buffer, pH of 7.5, and neutral compounds were removed by partitioning with 2 x 5 ml fresh diethyl ether in a 20 ml glass vial. The ether was layered on to the aqueous phase and the two-phase system was gently stirred for 3 min on a multipoint magnetic stirrer. After discarding the ether phase, the aqueous phase was adjusted to pH of 2.7 with 1 M HCl and partitioned as described above with 3 x 5 ml fresh diethyl ether. Saving the aqueous phase for further purification of gibberellins, the combined ether phases were reduced to dryness (anhydrous Na_2SO_4 was added to remove water from the ether phase) and used for the estimation of IAA. The aqueous phase after partitioning against diethyl ether was partitioned two times against 10 ml of ethyl acetate and the aqueous phase was discarded. The ethyl acetate layer was partitioned two times against 10 ml of 0.2 M K_2HPO_4 and the organic layer was discarded.

The aqueous phase was adjusted to pH of 2.5 with H_3PO_4 . The acidified phase was partitioned two times against 10 ml of ethyl acetate. The ethyl acetate layer was dried over anhydrous Na_2SO_4 overnight and used for the estimation of gibberellins. The dried extracted samples were reconstituted in 5 ml HPLC grade methanol and were analyzed by a modular HPLC system consisting of a Spectra physics Spectra System P2000 pump, an AS 3000 auto sampler (Thermo Separation Products, San Jose, CA, USA). The retention time of the peaks in authentic hormones and in the samples obtained by the chromatograph was compared and the peak area was measured with single channel computing integrator and was used to quantify the amount of hormone present in the sample. The endogenous Gibberellins and IAA levels in grains were determined by ELISA as described by He et al. [13].

RESULTS AND DISCUSSIONS

Figure 1 demonstrates the GGR levels at different grain type and position within developing grains of wheat. Generally, grain growth rate (GGR) was high during 9th to 30th DAA. GGR improved from 9th DAA to 16th - 23st DAA then decreased from 16th- 23st DAA to maturity. The lowest amount of GGR was observed during 30th DAA to maturity. Furthermore, grain growth rate was diversely affected due to different grain positions. The GGR level of middle region of spike as compare with proximal and distal regions, and GGR level of proximal region as compared distal region, improved at all sampled DAA. According to the grain types, the maximum levels of GGR were obtained in basal grains in the direction of all sampled DAA. The only exception was at maturity, middle position basal grains, which the GGR was slightly lower as compared with middle position apical grains. Table 1 indicates the gibberellins content of different grain type and position at various DAA. The gibberellins level slightly increased from 9th DAA until 16th DAA, and then decreased from 16th DAA until maturity. The maximum levels of grain gibberellins for all different grain type and position were observed at 16th DAA. The differences in gibberellins concentration of grains positively correlated with the differences in related GGR levels at various DAA (r^2=0.9382). The correlation hold true both for comparisons between spikelet's in various regions of the spike, and also between florets within spikelets. Hence, the maximum gibberellins levels of grain were also obtained in middle region of spike and in basal grains at all sampled DAA. The maximum disparities between two types of grains in the contents of GAs was observed at 9th DAA, which were to the tune of 36.2, 31.0 and 50.7 percent in proximal, middle and distal segments of spike, respectively.

At maturity, the differences suppressed to 26.9, 22.0 and 42.8 percent lower in apical grain as compared to basal grains at the same position respectively. Table 2 presents the IAA content at different grain type and position within developing grains of wheat. The IAA levels increased with grain development from 9th to 23st DAA, and then decreased from 23st DAA until maturity in all the three segments of spike. The highest and lowest levels of grain IAA for all different grain types and positions were observed at 9th and 23st DAA, respectively. The IAA levels were diversely affected due to different grain positions. The IAA levels of distal region of spike as compare with proximal and middle regions, and IAA level of proximal region as compared middle region, reduced at all sampled DAA. According to grain types, the maximum levels of grain IAA were obtained in apical grains for all determined growth stages. The quantum of disparities between basal and apical grains was maximum at 9th DAA. However, there were no significant differences in the levels of IAA between the two types of grains at 9th DAA in all the three segments of spike. Observations similar to those at 9th DAA were recordable at maturity. The differences in IAA concentration of grains negatively correlated with the differences in GGR levels at different DAA.

Grain filling period is an influential stage of crop life cycle which strongly effected grain yield. Plant growth regulators play an important role in regulating plant growth and development. The differences in dry weight per grain are highly flexible [4]. Middle region of spike as compare with proximal and distal regions produces the

maximum level of grain dry matter accumulation [3]. We have investigated the relation between gibberellins and IAA content, along with GGR levels at different grain type and position within developing grains of wheat. Both gibberellins and IAA levels were possessed during middle phase of grain setting while the GGR was high. The differences in gibberellins levels, among spikelets in different regions of the spike, and also among grains within a spikelet were positively correlated with the differences in GGR levels. On the contrary, the differences in the indolyl-3-acetic acid contents, among all aforementioned segments were negatively correlated with the differences in GGR levels. Furthermore, the basal grains were conspicuous in having relatively higher level of gibberellins at subtle stages while the level of indolyl-3-acetic acid was invariably higher in apical grains.

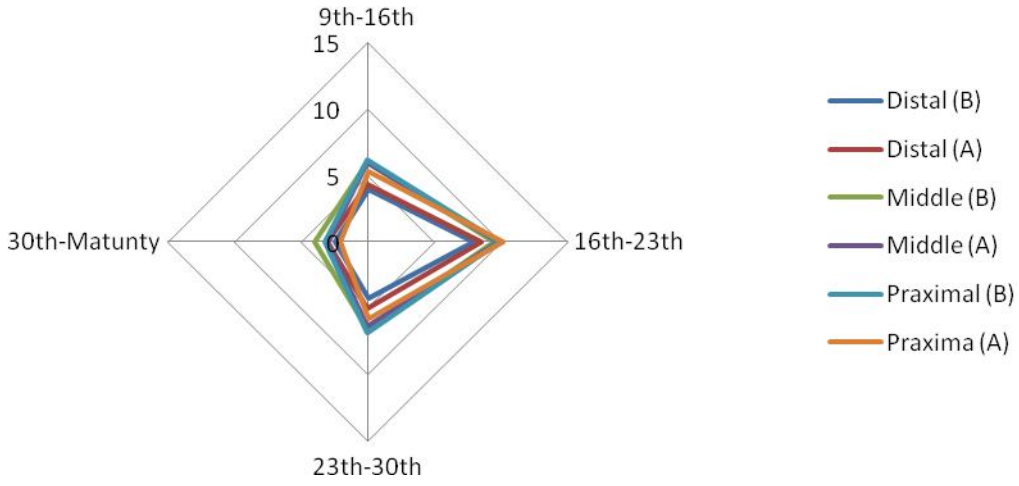

Figure 1: **Grain growth rate (GGR) at different grain type and position within developing grains of wheat. B and A are basal and apical grains, respectively.**

Table 1

Gibberellins (GA_1 + GA_3 +GA_4) content (ng g^{-1} fresh weight) at different grain type and position within developing grains of Wheat

Days after anthesis (DAA)	Proximal			Middle			Distal		
	Basal		Apical	Basal		Apical	Basal		Apical
9th	801.4	(– 36.2)	499.5	841.5	(– 30.0)	519.0	669.4	(– 50.9)	329.0
16th	999.8	(– 33.0)	689.9	1139.1	(– 29.0)	959.1	948.9	(– 45.0)	521.9
23st	635.5	(– 30.8)	320.8	663.1	(– 26.6)	413.3	520.3	(– 40.4)	310.2
30th	182.1	(– 23.0)	240.2	183.9	(– 24.0)	149.2	194.4	(– 46.0)	94.2
Maturity	50.5	(– 26.9)	29.6	42.2	(– 22.0)	32.9		33.6	(– 42.8)	19.2

Values within parenthesis indicate percentage of decrease (-) in small grains over bold grains growing in the same spikelets; CD at 5% level; Age: 41.3; Position: 18.6; Age × Position: 53.5

Table 2

Indolyl-3-acetic acid (IAA) content (ng g^{-1} fresh weight) at different grain type and position within developing grains of wheat

Days after anthesis (DAA)	Proximal			Middle			Distal		
	Basal		Apical	Basal		Apical	Basal		Apical
9th	48.2	(+ 29.0)	59.4	43.6	(+ 26.6)	55.2	46.9	(+ 39.1)	64.3
16th	225.6	(+ 19.9)	290.9	218.0	(+ 19.4)	260.4	228.3	(+ 30.0)	296.8
23st	591.9	(+ 15.5)	660.9	564.9	(+ 9.0)	604.4	599.2	(+ 24.3)	920.2
30th	153.2	(+ 14.2)	195.0	148.9	(+ 9.4)	162.9	159.1	(+ 18.4)	186.1
Maturity	3.9	(+ 9.9)	4.2	3.4	(+ 11.8)	3.8	4.2	(+ 14.3)	4.8

Values within parenthesis indicate percentage of decrease (+) in small grains over bold grains growing in the same spikelets; CD at 5% level; Age: 25.2; Position: 17.6; Age × Position: 32.1

According to previous studies, the increase in gibberellins content at early embryonic stage where a rapid enlargement of embryo [21] takes place implies that gibberellins had signaled the translocation of metabolites to the active sink such as the developing grain [19]. The characteristic decrease in gibberellins content at 23st DAA can be explained by

the hypothesis put forth by Krishnamoorthy[16] that at early stage, conjugation might have taken place and it existed in the storage from in the matured grain to be used during germination. There are also reports by earlier workers that the IAA is elevated in the grains during maturation for induction of dormancy. The higher levels of IAA in hard dough stage, along with relatively lower level of gibberellins at approximately middle stages of grain development [12] suggests that at this stage, maintenance of embryo dormancy appears to be an active process involving IAA [18]. In conclusion, the result suggest that both gibberellins and IAA levels of grains during the middle phase of grain development play an important role in regulating grain filling pattern and grain growth rate of Bahar-138 wheat. Furthermore, it could be possible to improve grain weight by manipulating gibberellins and IAA levels in grain, especially during the filling stage either through breeding or crop management.

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