Grain Growth Rate and Iaa Levels Within Developing Grains of Different Wheat Genotypes (*Triticum Aestivum* L.)

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Grain growth rate (GGR) and indolyl-3-acetic acid (IAA) levels were studied within developing grains of different wheat genotypes (*Triticum aestivum* L.) included DL 1266-2, DL 1266-5 and PBW 343. Grain dry matter accumulation and IAA levels were determined in ten labelled spikes which sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 35th DAA. Grain yield, number of grain per spike, 1000-grain weight, and harvest index (HI) were evaluated at maturity. Grain growth rate level was high during 7th to 28th DAA, while IAA increased rapidly from 7th to 14th-21st DAA. The DL 1266-5 genotype with maximum amount of IAA produced a maximum level of GGR, grain yield, number of grain per spike, 1000-grain weight, and HI. The results suggest that IAA level is an important factor in the regulation of grain dry matter accumulation in different genotypes. Furthermore, it might be possible to improve grain weight by increasing IAA level in grain, especially at the early to middle grain filling stage.

**Key words:** Grain growth rate; IAA; spike; grain development; wheat.

**Introduction**

Wheat is the most important cereal crop which ubiquitous in the food culture of both developed and developing countries in the world. World wheat production must increase by approximately 1.5% annually to meet the growing demand for food that will result from population growth and economic development [1]. A substantial increase in grain yield potential, along with better use of water and fertilizer is required to ensure food security in future decades. For improvements in photosynthetic capacity to result in additional wheat yield, extra assimilates must be partitioned to developing grains and/or potential grain weight increased to accommodate the extra assimilates [2]. Although, the efficient assimilate partitioning has being considered as an important factor in the regulation of plant productivity, the basis of its control has not been fully exploited. The genotypic variation in grain weight of wheat drives from the interaction between potential storage capacity or volume and realization of this potential [3]. The variation in grain filling is also the result of interaction between the availability of assimilates to the grain, metabolism of intermediates and synthesizing complex [4]. Various explanations such as a role of plant growth regulators are offered to explain these differences [5,6,7]. Auxin play an important role in regulating plant growth and development such as shoot growth, root branching, fruit ripening, tropisms and flowering [8]. IAA is a major auxin involved in regulating grain development [9,10]. Awan and Alizai [11] observed that the application of IAA significantly reduced spikelet sterility in rice. Wang *et al.*, [12] suggested that the poor grain filling of rice was associated with low grain doses of both IAA and ABA (abscisic acid). Furthermore, Darroch and Baker [13] have reported

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about the significant genotypic differences in rate and duration of grain filling in spring wheat. Sayed and Gadallah [14] reported that grain yield in wheat was more closely related to the rate than the duration of grain filling, whereas Gebeeyehou et al., [15] found that both rate and duration of grain filling were positively associated with final grain weight. Evaluation the relation between grain growth rate, dry matter accumulation, and IAA levels, during grain filling of different wheat genotypes could be important to identifying the role of plant growth regulators on differences in dry matter accumulation of wheat genotypes, which could be the key factor in developing wheat with higher grain yield potential. Hence, the objective of this study was to evaluate the GGR and IAA levels along with grain yield and yield components at different bread wheat genotypes.

Materials and method

Experimental Setup and Plant Sampling:

Single plants of different wheat genotypes (*Triticum aestivum* L.) included DL 1266-2, DL 1266-5 and PBW 343, 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 27.3% Sand, 27.2% Clay and 45.5% Silt, an electrical conductivity (ECe) of 1.1 dS m⁻¹, a pH of 7.3 (saturated paste), and organic C of 0.43%. The plants were grown in a screen covered hall under otherwise natural conditions. The pots were watered as described by Houshmandfar et al. [16], and fertilized once a week with half strength Peter’s solution (NPK = 10:10:10) [17]. The secondary tillers were removed as they appeared. Grain dry matter accumulation, GGR, and also IAA levels were determined in ten labelled spikes which sampled five times, seven day intervals started from seventh day after anthesis (DAA) up to 35th DAA. All samples were divided into two parts; one was dried in an oven at 70 °C for 72 h then weighed for dry matter accumulation, and another was frozen in liquid N2 for one min and kept in a freezer at -70 °C for IAA analysis. Grain yield, number of grain per spike, 1000-grain weight, and harvest index (HI) were evaluated at maturity.

Harvest index (HI), and Grain growth rate (GGR) [15] were calculated using the following equations:

\[
HI (\%) = \frac{\text{Grain dry matter accumulation (g)}}{\text{Plant dry matter accumulation (g)}} \times 100
\]

\[
\text{GGR (mg d}^{-1}) = \frac{W_2 - W_1}{T_2 - T_1}
\]

Where,

\[W_1 = \text{Total dry matter of grain at time } t_1\]
\[W_2 = \text{Total dry matter of grain at time } t_2\]
\[T_1 = \text{Time of first observation}\]
\[T_2 = \text{Time of second observation}\]

Linear regression was used to evaluate the relationship between traits. The data were analysed statistically following randomized complete block design (RCBD) using SAS software version 8 [18], and Duncan's multiple range test (DMRT) [19] at the 0.05 level of probability was used to evaluate the difference among treatment means.

Indolyl-3-acetic acid analysis:

The method for extraction and purification of IAA was modified from those described by Bollmark et al. [20] and Yang et al. [21]. In brief, samples consisting of approximately 50 dehulled and frozen grains were ground in an ice-cold mortar with 10 ml 80% (v/v) methanol extraction medium containing 1 mmol L⁻¹ butylated hydroxytoluence (BHT) as an antioxidant. The methanolic extract was incubated at 4 °C for 4 h and centrifuged at 4,000 rpm for 15 min at the same temperature (4 °C). The supernatant was 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 × 5 ml fresh diethyl ether in a 20 ml glass vial. The ether was layered to the aqueous phase and the two-phase system was gently stirred for 3 minutes on a multi-point magnetic stirrer. The combined ether phases were discarded and reduced to dryness. The dried extracted samples were reconstituted in 5 ml HPLC grade methanol and were analysed by a modular HPLC system consisting of a Spectraphysics Spectra System P2000 pump, an AS 3000 autosampler (Thermo Separation Products, San Jose, CA, USA).

Results:

Table 1 indicates the grain dry matter accumulation within developing grains of different wheat genotypes. The grain dry weight was positively correlated with the age of the plant from 7th to 35th DAA (r²=0.9745). Variation in the set of accessions was not possible to discern grain dry weight of different genotypes at 7th DAA. However, accessions differed significantly at all the later sampled DAA. The DL 1266-5 genotype with maximum levels of percentage increase in 7th to 14th, 14th to 21st, and 21st to 28th DAA, produced a maximum level of grain dry weight at all determined DAA.

The GGR was diversely affected by the age of the plant from 7th to 35th DAA (Figure 1). The maximum level of GGR was observed during 14th to 21st DAA for all investigated genotypes.
Table 1: Grain dry matter accumulation (mg grain⁻¹) within developing grains of different wheat genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>7&lt;sup&gt;th&lt;/sup&gt; DAA</th>
<th>14&lt;sup&gt;th&lt;/sup&gt; DAA</th>
<th>21&lt;sup&gt;st&lt;/sup&gt; DAA</th>
<th>28&lt;sup&gt;th&lt;/sup&gt; DAA</th>
<th>35&lt;sup&gt;th&lt;/sup&gt; DAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL 1266-2</td>
<td>5.19±</td>
<td>18.07±</td>
<td>31.43±</td>
<td>43.36±</td>
<td>48.22±</td>
</tr>
<tr>
<td></td>
<td>(+248.16)</td>
<td>(+73.93)</td>
<td>(+37.95)</td>
<td>(+11.20)</td>
<td></td>
</tr>
<tr>
<td>DL 1266-5</td>
<td>5.57±</td>
<td>19.57±</td>
<td>34.06±</td>
<td>47.08±</td>
<td>51.93±</td>
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<tr>
<td></td>
<td>(+251.34)</td>
<td>(+74.04)</td>
<td>(+38.22)</td>
<td>(+10.30)</td>
<td></td>
</tr>
<tr>
<td>PBW 343</td>
<td>5.10±</td>
<td>17.60±</td>
<td>30.35±</td>
<td>41.50±</td>
<td>44.90±</td>
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<tr>
<td></td>
<td>(+245.09)</td>
<td>(+72.44)</td>
<td>(+36.73)</td>
<td>(+8.19)</td>
<td></td>
</tr>
</tbody>
</table>

Within a column, means followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's multiple range test; Values within parenthesis indicate percentage of increase (+) between two sampled DAA.

Fig 1: Grain growth rate (GGR) within developing grains of different wheat genotypes.

The overall mean levels of GGR in different genotypes during 21<sup>st</sup> to 28<sup>th</sup>, 28<sup>th</sup> to 35<sup>th</sup> DAA reduced with a decrease of 11.40 and 67.88% as compare with GGR level of 14<sup>th</sup> to 21<sup>st</sup>, respectively. During the first three sampled stage included 7<sup>th</sup> to 14<sup>th</sup>, 14<sup>th</sup> to 21<sup>st</sup>, and 21<sup>st</sup> to 28<sup>th</sup> DAA, the highest level of GGR was observed in DL 1266-5 genotype. However, during the last determined growth stage (28<sup>th</sup> to 35<sup>th</sup> DAA), GGR was slightly higher in DL 1266-2 genotype as compared DL 1266-5 (Figure 1). PBW 343 genotype produced the minimum level of GGR in all sampled stages.

Table 2 demonstrates the IAA content of different wheat genotypes at various DAA. The IAA level increased from 7<sup>th</sup> DAA until 14<sup>th</sup> DAA, and then decreased from 14<sup>th</sup> DAA until 35<sup>th</sup> DAA. Therefore, the maximum level of grain IAA for all studied genotypes was observed at 14<sup>th</sup> DAA. Variation in the set of accessions was not possible to discern IAA levels of different genotypes at 35<sup>th</sup> DAA. However, accessions differed significantly at all the earlier sampled DAA. The differences in doses of IAA in various genotypes at 7<sup>th</sup> DAA closely correlated with the differences in related GGR levels at 7<sup>th</sup> to 14<sup>th</sup>, 14<sup>th</sup> to 21<sup>st</sup>, and 21<sup>st</sup> to 28<sup>th</sup> DAA, with a correlation of 0.9384, 0.9468, and 0.9482, respectively. Furthermore, the differences in the doses of IAA in investigated genotypes at 14<sup>th</sup> and 21<sup>st</sup> DAA also closely correlated with the differences in their GGR levels at aforementioned DAA with a correlation of 0.9339, 0.9443, and 0.9495 for 14<sup>th</sup> DAA, and 0.8416, 0.8716, and 0.9160 for 21<sup>st</sup> DAA, respectively. According to genotype IAA differences, the maximum level was observed in DL 1266-5 at all sampled grain growth stages.

Table 3 presents the various traits of yield and yield component in different wheat genotypes. The DL 1266-5 produced the highest quantum of grain yield per spike with an increase of 12.29 and 29.24%, number of grain per spike with an increase of 3.85 and 13.32%, 1000-grain weight with an increase of 8.02 and 15.12%, and HI with an increase of 9.63 and 18.25%, as compare with DL 1266-2 and PBW 343, respectively.

Discussion:

Plant growth regulators play an important role in regulating plant growth and development. Grain filling period is an important stage of cereal life cycle which strongly influenced final grain yield. Differences in grain dry weight within genotypes are
highly flexible. We have investigated the relation between GGR and IAA levels, along with grain yield, number of grain per spike, 1000-grain weight, and HI, in different bread wheat genotypes. The IAA level increased remarkably in the early phase of grain setting while the GGR was max. The correlation between IAA content at different DAA and the GGR among different genotypes indicated a significant correlation between the GGR and IAA levels during the early to middle filling stage. Furthermore, the new genotypes with a maximum amount of IAA produced maximum levels of GGR, grain yield, number of grain per spike, 1000-grain weight, and HI. Bhardwaj and Verma [22] reported that the developing grains which are capable of producing higher levels of auxins and also could mobilize greater proportions of assimilates from flag leaf leading to their higher accumulation in grains and resulting in bold size grains at maturity. Moulla et al. [23] observed a maximum auxin content of barley grains between 22rd and 29th DAA. The level of IAA was estimated in the developing wheat grains collected at 10th, 20th, 30th and 40th DAA using GLC by Singh et al. [24] and found that IAA content was highest (4.3 μg grain⁻¹) at 30th DAA. Gutam et al. [3] reported a maximum IAA level of wheat grains in 15th DAA. You et al. [25] showed that the content of IAA was less in young wheat panicles of male sterile lines, WAV41A and KV41A compared to V41B during the period when the young panicle developed. Furthermore, it was suggested that the level of male sterility is related to the loss of endogenous hormone especially, IAA. Higher IAA content in the grain at the early grain filling stage may promote the division of endosperm cells [26], thus constitute a powerful sink [22], and enhance assimilate transport and its accumulation in the developing grains [27,28,29].

**Conclusion:**

In conclusion, the result suggest that IAA level of grains during the early to middle phase of grain development play an important role in regulating grain filling pattern and dry matter accumulation. The rapid grain growth phase is crucial for any genotype and the new genotypes with a maximum amount of IAA produced a highest level of GGR, grain yield, number of grain per spike, 1000-grain weight, and HI. Hence, it might be possible to improve grain weight by increasing IAA level of grains, especially at the early to middle filling stage either through breeding or crop management.

**Table 2:** IAA content (ng g⁻¹ fresh weight) within developing grains of different wheat genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Day after anthesis (DAA)</th>
<th>7*</th>
<th>14*</th>
<th>21*</th>
<th>28*</th>
<th>35*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL 1266-2</td>
<td>DL 1266-5</td>
<td>278.89b</td>
<td>818.75b</td>
<td>779.29b</td>
<td>87.20b</td>
<td>20.78a</td>
</tr>
<tr>
<td></td>
<td>(193.57)</td>
<td>(-4.82)</td>
<td>(-88.82)</td>
<td>(-76.18)</td>
<td>21.06a</td>
<td>19.03*</td>
</tr>
<tr>
<td>PBW 343</td>
<td>DL 1266-5</td>
<td>338.31a</td>
<td>925.82a</td>
<td>866.07a</td>
<td>107.33a</td>
<td>21.06*</td>
</tr>
<tr>
<td></td>
<td>(173.66)</td>
<td>(-6.47)</td>
<td>(-87.61)</td>
<td>(-80.38)</td>
<td>21.06*</td>
<td>19.03*</td>
</tr>
<tr>
<td></td>
<td>PBW 343</td>
<td>243.93b</td>
<td>751.06b</td>
<td>665.01c</td>
<td>86.13b</td>
<td>19.03*</td>
</tr>
</tbody>
</table>

Within a column, means followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's multiple range test; Values within parenthesis indicate percentage of decrease (-) and increase (+) between two sampled DAA.

**Table 3:** Overall mean values of yield and yield components of different wheat genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Grain yield per spike (g)</th>
<th>No. of grain per spike</th>
<th>1000-grain weight (g)</th>
<th>HI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL 1266-2</td>
<td>3.66*</td>
<td>69.85*</td>
<td>48.84*</td>
<td>42.89*</td>
</tr>
<tr>
<td>DL 1266-5</td>
<td>4.11*</td>
<td>72.54*</td>
<td>52.76*</td>
<td>42.89*</td>
</tr>
<tr>
<td>PBW 343</td>
<td>3.18*</td>
<td>64.01*</td>
<td>45.83*</td>
<td>36.27*</td>
</tr>
</tbody>
</table>

Within a column, means followed by the same letter are not significantly different at the 0.05 level of probability by Duncan's multiple range test.

**References**