Endogenous Gibberellins and Abscisic Acid Levels During Grain Development of Durum Wheat Genotypes

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ABSTRACT

Grain growth rate (GGR), gibberellins (GAs), and abscisic acid (ABA) levels were studied within developing grains of different durum wheat genotypes (Triticum durum L.) included HD 4713, HD 4530 and PDW 233. The plants were grown in a screen covered hall under otherwise natural conditions and secondary tillers were removed as they appeared. Grain dry matter accumulation, GAs including GA1, GA3 and GA4, and also ABA 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. The GAs and ABA levels were possessed in a sequence during early to middle phase of grain setting while the GGR was max. The new genotype of HD 4713 which had maximum amounts of both GAs and ABA produced a highest quantum of dry matter accumulation. The results suggest that GAs and ABA contents are important factors governing grain dry matter accumulation in different genotypes. Furthermore, it could be possible to improve grain weight by manipulating GAs and ABA levels in grain, especially during the early to middle stage of grain filling either through breeding or crop management.

Key words: Gibberellins; ABA; grain development; durum wheat

Introduction

A substantial increase in grain yield potential, along with better use of water and fertilizer is required to ensure food security in the future decades [9]. 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 [9]. 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 [12]. 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 [16]. Various explanations such as a role of plant growth regulators are offered to explain these differences [13,20,30]. Gibberellins (GAs) play an important role in regulating plant growth and development such as stem elongation, germination, dormancy, synthesis of α-amylase, flowering, sex expression, enzyme induction and leaf and fruit senescence [6,19,17,5]. Asthir et al. [2] reported that gibberellins act as positive modulators of grain sink activity, whereas, ABA acts as a negative modulator. It was confirmed by Zhang et al. [31] by exogenous application of gibberellins that resulted in improvement of sink

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activity due to the GAs role as modulators of sugar metabolism. On the other hand, abscisic acid (ABA) 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 [6,25,27].

Ahmadi and Baker [1999] observed a reduction in sucrose transportation into the grains with lowered the ability of starch synthesis in intact grains. Ober [23] demonstrated that ABA could be translocated from leaf tissue to grains and acts as a sensory link between developing reproductive structures and maternal tissues deprived of water. Furthermore, ABA also may influence early establishment of sink size through regulation of cell number [23]. Both field and pot trials of Goldbach [11] indicated that ABA 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. [29] suggested that the poor grain filling of rice was associated with low grain doses of both IAA and ABA. Evaluation the relation between grain growth rate, dry matter accumulation, gibberellins and ABA 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 GAs and ABA levels along with dry matter accumulation at different durum wheat genotypes.

Materials and methods

Experimental Setup and Plant Sampling:

Single plants of different durum wheat genotypes (Triticum durum L.) included HD 4713, HD 4530 and PDW 233, 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 (EC,) of 1.1 dS m⁻¹, a pH of 7.3 (saturated paste), and organic C of 0.43% [4]. 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) [3]. The secondary tillers were removed as they appeared. Grain dry matter accumulation, GAs and ABA 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 GAs and ABA analysis. Grain growth rate (GGR) was calculated using the following equation provided by Gebeyehou et al. [10]:

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

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

Linear regression was used to evaluate the relationship between traits. The data were analysed statistically following completely randomized design (CRD) using SAS/STAT software version 8 [28], and Duncan's multiple range test (DMRT) [8] at the 0.05 level of probability was used to evaluate the difference among treatment means.

Gibberellins and ABA Analysis:

Samples consisting of 3.0 g dehulled and frozen grains were grinded into homogenates under ice bath and weak light. Resultant extraction was centrifuged. The supernatant was separated by C-18 solid phase extraction column, transferred into 5 ml plastic centrifuge tube, dried by nitrogen gas, eliminated methanol from extractions and then diluted into a constant volume by sample diluents. The endogenous Gibberellins and ABA levels in grains were determined by ELISA as described by He et al. [14].

Results:

Evaluation of GGR and Grain Dry Weight:

The grain dry weight was closely correlated with the age of the plants from 7th to 35th DAA. 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 (p<0.05). The HD 4713 genotype with maximum levels of percentage increase in 7th to 14th, 14th to 21st, and 21st to 28th DAA, produced maximum levels of grain dry weight at all the determined stages. On the other hand, the HD 4530 genotype which had minimum levels of percentage increase in 14th to 21st, and 21st to 28th DAA, produced lowest amounts of grain dry weight at all the determined stages (Figure 1).
Figure 1 indicates the GGR levels within developing grains of various durum wheat genotypes. The GGR levels increased from 7th-14th DAA until 14th-21st DAA, and then decreased from 14th-21st DAA until 21st-35th DAA. The overall mean values of GGR in different genotypes during 21st to 28th, 28th to 35th DAA reduced with a decrease of 1.25 and 74.54% as compare with GGR level of 14th to 21st, respectively. During the first three sampled stages included 7th to 14th, 14th to 21st, and 21st to 28th DAA, the highest levels of GGR were observed in HD 4713 genotype. However, during the last determined growth stage (28th to 35th DAA), GGR was slightly higher in HD 4530 genotype as compared both HD 4713 and PDW 233. The HD 4530 produced the minimum levels of GGR in 7th to 14th, 14th to 21st, and 21st to 28th DAA.

Evaluation of GAs and ABA:

The GAs levels of grain increased from 7th DAA until 14th DAA, and then decreased from 14th DAA until 35th DAA (Table 1). Variation in the set of accessions was not possible to discern GAs levels of different genotypes at 35th DAA. However, accessions differed significantly at 7th, 14th, 21st and 28th DAA (p<0.05). The differences GAs concentrations of grains in different genotypes at 7th DAA positively correlated with differences in GGR levels at 7th to 14th, 14th to 21st, and 21st to 28th DAA which had r² of 0.9377, 0.9358, and 0.9236, respectively. Furthermore, the differences in grain GAs doses in different genotypes at 14th, 21st and 28th DAA closely correlated to differences in GGR levels at all aforementioned DAA with r² of 0.9398, 0.9241, and 0.9043 for 14th DAA, 0.8243, 0.8859, and 0.9211 for 21st DAA, and 0.8922, 0.9284, and 0.9380 for 28th DAA, respectively. According to genotype GAs differences, the maximum levels were observed in HD 4713 for most of the sampled grain growth stages. The only exception was GAs levels at 35th DAA which PDW 233 produced slightly higher GAs levels as compared HD 4713 (Table 1).

The ABA levels increased with grain development from 7th to 21st DAA, and then decreased from 21st to 35th DAA (Table 2). Variation in the set of accessions was not possible to discern grain ABA levels of different genotypes at 7th and 35th DAA. However, accessions differed significantly at 14th, 21st and 28th DAA (p<0.05). The differences ABA levels of grain in different genotypes at 14th, 21st and 28th DAA positively correlated to differences in GGR levels at 14th to 21st, 21st to 28th and 28th to 35th DAA with r² of 0.9283, 0.9380, and 0.8653 for 14th DAA, 0.9398, 0.9339, and 0.9166 for 21st DAA, and 0.9235, 0.9358 and 0.8543 for 28th DAA, respectively.

Discussion:

Plant growth regulators play an important role in governing plant growth and development. Grain filling stage is an important period of cereal life cycle which strongly influenced final grain weight. Differences in grain dry weight within genotypes are highly flexible. We have investigated the relation between GGR, GAs and ABA levels within developing grains of different durum wheat genotypes. Both gibberellins and ABA levels were possessed during early to middle phase of grain setting while the GGR was high. The maximum amount of GAs was observed at 14th DAA. In a sequence, the maximum level of ABA was obtained at the further stage (21st DAA). The HD 4713 genotype with a maximum amount of both GAs and ABA produced the highest quantum of GGR and dry matter accumulation. The increase in GAs contents at early embryonic stage where a rapid enlargement of embryo [24] takes place implies that GAs had signalled the translocation of metabolites to the active
Table 1: Gibberellins content (GA1 + GA3 + GA4) (ng g\(^{-1}\) fresh weight) within developing grains of various wheat genotypes (Triticum durum L.)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Day after anthesis (DAA)</th>
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<tbody>
<tr>
<td></td>
<td>7(^{th})</td>
</tr>
<tr>
<td>HD 4530</td>
<td>653.5(^{a})</td>
</tr>
<tr>
<td></td>
<td>(+6.67)</td>
</tr>
<tr>
<td>HD 4713</td>
<td>731.2(^{a})</td>
</tr>
<tr>
<td></td>
<td>(+9.57)</td>
</tr>
<tr>
<td>PDW 233</td>
<td>662.3(^{b})</td>
</tr>
<tr>
<td></td>
<td>(+5.99)</td>
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</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 2: Abscisic acid content (ABA) (ng g\(^{-1}\) fresh weight) within developing grains of various durum wheat genotypes (Triticum durum L.)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Day after anthesis (DAA)</th>
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<tbody>
<tr>
<td></td>
<td>7(^{th})</td>
</tr>
<tr>
<td>HD 4530</td>
<td>40.3(^{a})</td>
</tr>
<tr>
<td></td>
<td>(+328.78)</td>
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<tr>
<td>HD 4713</td>
<td>42.0(^{a})</td>
</tr>
<tr>
<td></td>
<td>(+380.71)</td>
</tr>
<tr>
<td>PDW 233</td>
<td>41.2(^{a})</td>
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<tr>
<td></td>
<td>(+339.32)</td>
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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.

Fig. 2: Grain growth rate (GGR) levels within developing grains of various durum wheat genotypes.

sink such as the developing grain [22]. The characteristic decrease in GAs contents at 21\(^{st}\) DAA can be explained by the hypothesis put forth by Krishnamoorthy [18] 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 ABA is elevated in the grains during maturation for induction of dormancy. The higher levels of ABA in hard dough stage, along with relatively lower level of gibberellins at early to middle stage of grain development [12] suggests that at this stage, maintenance of embryo dormancy appears to be an active process involving ABA [21]. In conclusion, the results suggest that both GAs and ABA levels of grain during the early to middle phase of grain development play important roles in regulating grain filling pattern and grain growth rate of different wheat genotypes. Furthermore, it could be possible to improve grain weight by manipulating GAs and ABA levels in grain, especially during the grain filling stage either through breeding or crop management.

References


