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Effects of Nitrogen and ABA Application on Basal and Distal Kernel Weight of Wheat

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ABSTRACT

Individual grain weight of wheat kernels differs with their positions on a spike. Cultivation practices (such as fertilizer nitrogen and plant growth regulators application) can be used to improve weight of basal and distal kernels. For this purpose, two experiments based on randomized complete block design were carried out with three replications. The aim of this research was to study the mechanisms related to sink strength as well as the regulatory factors affecting sink activity. One cultivar of a facultative wheat, *cv* Yangmai15, was used. Treatments of the experiments included application of nitrogen (two levels) and plant growth regulators [abscisic acid (ABA) and Fluridone (inhibitor of ABA synthesis)] in basal and distal kernels, respectively. Results showed that nitrogen application increased grain yield and its components. Grain filling in basal kernels started earlier and its rate was higher than that of the distal kernels. Nitrogen fertilizer increased the individual kernel weight both in basal and distal kernels, and the rate of increment was higher than the control, even in distal kernels. The application of ABA resulted in increase in grain weight, whereas a considerable decrease in grain weight was observed in response to Fluridone compared to the control. Nitrogen application together with ABA application enhanced the activity of SuSase, AGPase, SSS, and SBE in basal and distal kernels and the increment in the activity was higher in distal kernels. It is concluded that simultaneous application of nitrogen and ABA enhanced grain weight by regulating the activity of key enzymes involved in starch synthesis.

Keywords: Basal and distal kernels, Grain growth, Plant growth regulators, Sink activity, Wheat (*Triticum aestivum* L.).

INTRODUCTION

It is well known that the process of assimilate uptake by the grains is limited, and that individual grains do not attain their potential weights (Hay and Walker, 1989). However, within an ear there is considerable variation in grain weight at maturity, depending on the position of the grain within the spikelet and of the spikelet on the ear. Assuming that there is adequate assimilate supply, the final weight of an individual grain will reflect its inherent capacity to accumulate dry matter and its resistance to assimilate movement (Hay and Walker, 1989). Studies have shown that differences in grain weight between and within spikelets is related to the grain's capacity for growth, which, in turn, may depend on e.g. endosperm cell number (Gleadow *et al*., 1982) and the activity of starch synthesizing enzymes (Jiang *et al*., 2003).

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Individual grain weight is a key component of grain yield in cereals like wheat and rice, and is considered as an essential factor in grain yield improvement (Calderini *et al*., 1999). Starch comprises 65-74% of the wheat grain weight, therefore, starch biosynthesis is important for determination of the final grain weight (Keeling *et al*., 1988). Dry matter partitioning, the final destination for assimilate flux from source organs, happens via accessing the transfer route of sink organs, and sink strength is considered as the determinant of dry matter distribution throughout the whole plant (Marcelis, 1996). Four enzymes are considered to play a key role in converting sucrose to starch in the grains. These are: sucrose synthase (SuSase; EC 2.4.1.13), ADP-glucose pyrophosphorylase (AGPase; 2.7.7.27), starch synthase (StSase; 2.4.1.21), and starch branching enzyme (SBE; 2.4.1.18) (Ahmadi and Baker, 2001; Hurkman *et al*., 2003; Yang *et al*., 2004). Low individual grain weights in the various positions within an ear have been shown to be due to low rate and duration of grain filling, and the low activity of SuSase (Kato, 1995), AGPase, and StSase (Jiang *et al*., 2003).

Abscisic acid (ABA) is a stress hormone and is generally regarded as a very sensitive signal produced during water stress (Davies and Zhang, 1991). ABA is also believed to be involved in senescence and remobilization of assimilates to the grain (Yang *et al*., 2003b). Drought stress increases ABA accumulation in the grain, and its increase is significantly associated with maximal increase in the grain-filling rate (Yang *et al*., 2004). External application of ABA can increase chlorophyll loss as well as remobilization of pre-stored reserves from the stems to endosperm, thereby increasing the grain weight (Yang *et al*., 2003a; Zhang *et al*., 2005; Guóth *et al*., 2009). The activity of some of the key enzymes involved in converting sucrose to starch significantly increases during the controlled drought stress, which is significantly related to the ABA content (Yang *et al*., 2004).

Considering the aforesaid, the objective of this experiment was to determine the activity of key enzymes in sucrose-to-starch conversion in wheat grains in different positions within spike in response to nitrogen treatment, abscisic acid, and fluridone application at grain-filling stage.

MATERIALS AND METHODS

The experiment was conducted at the Yangzhou University research farm, Yangzhou, China, from 30 October 2009 to June 2010. Two separate experiments were carried out based on randomized complete block design with three replications. One highly lodging-tolerant cultivar of facultative wheat (*Triticum aestivum*), *cv*. Yangmai15, currently used in local production, was used. Treatments included two nitrogen levels: Low-nitrogen (LN= Half the HN) and High-nitrogen (HN= 25 g N m^{-2})]. The sowing date was 30 October. The soil was a sandy loam [Typic fluvaquents, Entisols (US taxonomy)] with 24.5 g kg^{-1} organic matter, 106.2 mg kg^{-1} alkali hydrolysable N, 28.5 mg kg^{-1} Olsen-P, and 93.6 mg kg^{-1} exchangeable K. Alkali hydrolysable N (NaOH) was analyzed using the method described by Cornfield (1960), and Olsen-P (0.5 M $NaHCO_3$) and exchangeable K (NH_4OAc) were analyzed using the method of Sparks *et al*. (1996). On the day of sowing, 15 g N m^{-2}, as urea, and 4 g phosphorus m^{-2}, as single superphosphate, were applied to the soil. Thirty two days after sowing (DAS) and 115 DAS, 6 g and 5 g N m^{-2}, in the form of urea, were top-dressed, respectively. In China, N rate of wheat is usually 30~36 g N m^{-2}. The high rate of N is used in this country partly because the wheat varieties are very lodging-tolerant. The rainfall during the wheat growing season was 98.5, 67.7, 14.1, 113.2, 100, 137.6, and 47.5 mm, respectively, in Nov and Dec of 2009, and in Jan, Feb, Mar, Apr, and May 2010. Plot dimension was 4×5m, and the plots were separated by a ridge (20 cm in width) wrapped with plastic film.

Plant Sampling

A total of 100 spikes that headed on the same day were selected and tagged from each plot. Fifteen tagged spikes from each treatment were sampled at 7-day intervals from anthesis to maturity. From basal five to 10 spikelets on the spikes, the first and second basal grains on each spikelet were detached as basal kernels, whereas the most distal grain on the same spikelet was detached as distal kernel. Half of the sampled grains were frozen in liquid nitrogen for 2 minutes and then stored at -80˚C for enzyme measurements. The other half of grains were oven-dried at 70˚C for weighing. In each treatment, plants were harvested at maturity from one square meter, and were oven dried at 70˚C for determining grain yield (GY), biological yield (BY), and harvest index (HI).

The process of grain-filling was fitted by Richards' growth equations (Richards, 1959) as described by Yang *et al*. (2004).

$$R = \frac{AkBe^{-kt}}{N(1+Be^{-kt})^{\frac{(N+1)}{N}}} \qquad (1)$$

$$W = \frac{A}{(1+Be^{-kt})^{\frac{1}{N}}} \qquad (2)$$

Where, R is the grain-filling rate, W is the grain weight (mg), A is the final grain weight (mg), t is the time after anthesis (d), and B, K, and N are coefficients determined by regression.

Enzyme Extraction and Assays

All chemicals and enzymes used for enzymatic measurements were obtained from Sigma Chemical Company. The method for preparation of enzyme extracts was modified from Nakamura *et al*. (1989). Briefly, 30 to 40 dehulled grains were homogenized with a pestle in a pre-cooled mortar that contained 8 ml frozen extraction solution: 100 mM HEPES-NaOH (pH 7.6), 8 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 2 mM EDTA, 12.5% (v/v) glycerol, and 5% (w/v) insoluble polyvinylpyrrolidone 40. After being filtered through 4 layers of cheesecloth, the homogenate was centrifuged at 12,000×g for 10 minutes, and the supernatant was used for the enzyme assay.

The assay of SuSase activity was carried out following the procedure of Smyth and Prescott (1989). The reaction mixture contained 100 mM Hepes-NaOH (pH= 7.5), 50 mM sucrose, 5 mM uridine diphosphate (UDP), 5 mM magnesium acetate, and 5 mM DTT and was made up to a volume of 0.2 ml of enzyme crude extract. After incubation at 30˚C for 30 minutes, the reaction was stopped by heating in boiling water for 1 minute. Subsequently, 0.5 ml of dinitrosalicylic acid (DNS) was added to the solution and was heated for 5 minutes in boiling water. Finally, the formation of fructose catalyzed by SuSase was measured with a spectrophotometer (Beckman, USA) at 540 nm.

The AGPase, SSS, and SBE were assayed by the method of Nakamura *et al*. (1989). The assay of AGPase was conducted in 100 mM HEPES-NaOH (pH 7.4), 1.2 mM ADPglucose, 3 mM pyrophosphate, 5 mM $MgCl_2$, 4 mM DTT, and enzyme preparation in a reaction mixture of 700 µl. After 20 minutes, the reaction was terminated by heating the mixture in boiling water for 30 seconds. The resulting solution was transferred to a micro tube and centrifuged at 15,000×g for 10 minutes. A portion (500 µl) of the supernatant was mixed with 15 µl of 10 mM NADP. The activity was assayed by measuring the increase in absorbance at 340 nm after addition of 1 µl each of P-glucomutase (0.4 unit) and glucose-6-phosphate dehydrogenase (0.4 unit).

The assay of SSS was conducted in 50 mM HEPES-NaOH (pH 7.4), 1.6 mM ADPglucose, 0.7 mg amylopectin, 1–5 mM DTT, and enzyme preparation in a reaction mixture of 300 μl. Twenty minutes after the start of the reaction, the enzyme was inactivated by placing the mixture in a boiling-water bath for 40 seconds. Then, 200 μl of a solution of 50 mM HEPES-NaOH (pH 7.4), 4 mM phosphoenolpyruvate, 200 mM KC1, 10 mM $MgCl_2$, and pyruvate kinase (1.2 unit) was added and incubated for 30 minutes at 30°C. The ADP produced by the starch synthase reaction was converted to ATP and the resulting solution was heated in a boiling-water bath for 30 seconds and then subjected to centrifugation at 15,000×g for 5 minutes. The supernatant (400 μl) was mixed with 400 μl solution of 50 mM HEPES-NaOH (pH 7.4), 10 mM glucose, 20 mM $MgCl_2$, and 2 mM NADP. The enzymatic activity was measured as the increase in absorbance of 340 nm after the addition of 1.5 μl each of hexokinase (1.4 unit) and glucose-6-phosphate dehydrogenase (0.35 unit).

The assay of SBE was conducted in 50 mM HEPES-NaOH (pH 7.4), 5 mM glucose-1-phosphate, 1.25 mM AMP, phosphorylase a (60 unit), and enzyme preparation in a reaction mixture of 200 μl. The reaction was terminated by addition of 50 μl of 1M HCl. The solution was mixed with 500 μl of dimethylsulfoxide, and 700 μl of 0.1% I_2, and 1% KI were added. The enzymatic activity was assayed spectrophotometrically at 540 nm. One unit of enzymatic activity was defined as the amount causing an increase in absorbance of one unit at 540 nm in one minute.

Exogenous ABA and Fluridone Application

The sowing date and cultivation of the plants used for chemical application were the same as in the field experiment. A HN treatment was conducted as described above. Exogenous plant growth regulators and their rate of application were used according to Yang's method (Yang *et al*., 2004). Plant growth regulators were obtained from Sigma Chemical Company. Starting 9 days post-anthesis, either 25×10^{-6} M ABA or Fluridone (an inhibitor of ABA synthesis) were applied at the top of plants for 4 days with 0.1% (v/v) ethanol and 0.01(v/v) Tween20 as surfactant. The plants sprayed with the same volume of deionized water containing same concentrations of ethanol and Tween20 were taken as the control.

Enzymatic activities and grain weight in basal and distal kernels were determined at 16 and 23 days post-anthesis in each treatment. Measurement methods were the same as described above. Ten plants from each treatment were harvested at maturity for the final grain weight measurement.

Statistical Analysis

The data were analyzed through an analysis of variance using the Generalized Linear Model (GLM) procedure of SAS (version 9.1). Data from each sampling date were analyzed separately. Additionally, Least Significant Different (LSD) multiple range comparison tests were used to indicate when data values represent treatment differences with 95% certainty. A difference in treatments exists when the difference between values for treatments is equal to or greater than the LSD. Excel was used for auxiliary statistical works and drawings.

RESULTS AND DISCUSSION

Grain yield was higher when nitrogen treatment (HN) was applied (Table 1). It is generally accepted that nitrogen plays a vital role in increasing yield of the crop (Shekoofa and Emam, 2008). This increase was associated with increase in grain number per spike and grain weight (Table 1). In other words, low nitrogen (LN) resulted in decreased sink size (grain number per spike) and sink activity (1,000-grain weight), and, consequently, decreased harvest index.

High N rate treatment increased the individual kernel weight both in basal and

Table 1. Effects of nitrogen treatment on the yield and its components in Yangmai15 wheat cultivar.

Nitrogen treatment	Grain yield ($g\ m^{-2}$)	Biological yield ($g\ m^{-2}$)	Harvest index (%)	1000-Grain weight (g)	Grain number $spike^{-1}$
HN[a]	646	1910	34	47	40
LN	287	938	31	38	34
% changes[b]	56	51	10	21	16
LSD[c]	80	256	3	2	3

[a] On the day of sowing, 15 g N m^{-2} as Urea was applied to the soil, [b] % changes: [(LN treatment- HN treatment)/HN treatment]×100, [c] Least Significant Different (LSD) multiple range comparison tests were used to determine differences between means (P≤ 0.05).

distal kernels, but the rate of increment was greater in distal kernels. Enhancement of grain weight could be attributed to a stimulated capacity of the kernels to utilize available sucrose. Stimulated mature kernel dry weight and starch accumulation with nitrogen supply was also reported in maize (Singletary *et al.,* 1990).

The kernel dry weight increased linearly until 21 days post-anthesis, and then the rate increment decreased and finally reached a steady state at maturity. Mean kernel weight of basal was higher than that of the distal (Figure 1). Results of this experiment also showed that the grain filling in basal kernels started earlier and its filling rate was higher than that of the distal in both conditions (Figure 1). At the early stage of grain development, the rate of filling and dry weight of grain in basal kernels was higher in HN treatment than LN, but this difference was minimized at later stages of grain filling (Figure 1). The production capacity of a crop is affected by physiological processes

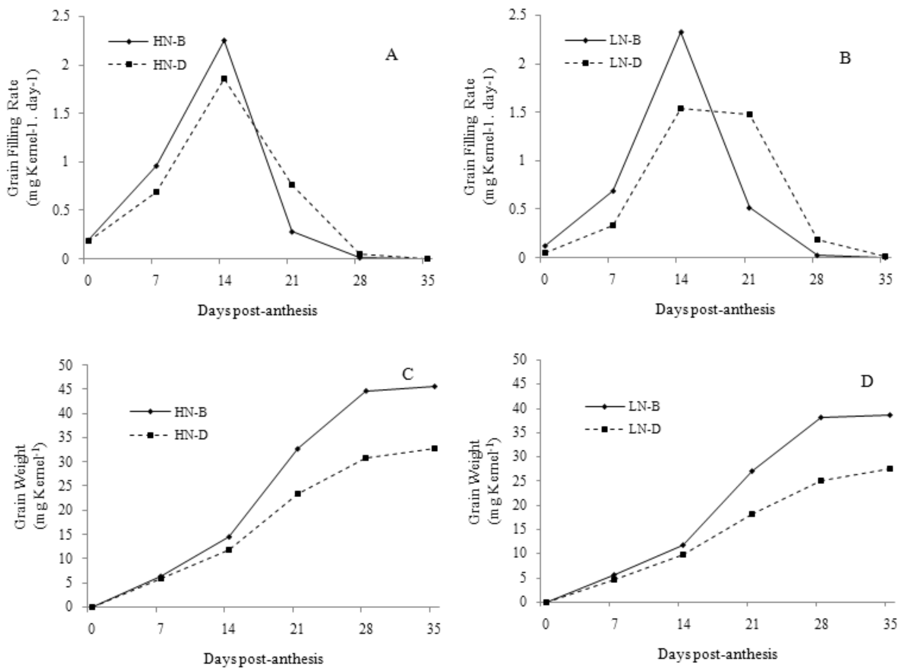

Figure 1. Grain filling rate and grain weight for superior and inferior kernels in treated plants with nitrogen (A,C) and Low-nitrogen (B,D) during grain development wheat. Solid and Dotted line represents superior and inferior kernels, respectively.

related to the synthesis, translocation, and accumulation of photosynthetic products during the post-anthesis phase (Guo *et al*., 1995). The possible factors for large size of basal kernels are differences in starch accumulation rate, the number of endosperm cells (Yang *et al*., 2002), developmental period of kernel, and availability of assimilates (Hay and Walker, 1989). A spike of wheat consists of several spikelets which differ in flowering time and their locations within the spike. The kernels can be classified as basal and distal. In general, basal and distal kernels result from early and late flowering, respectively. The basal kernels filling time are generally prior to distal kernels which lead to high grain weight of kernels (Jiang *et al*., 2003).

Increased grain weight could be due to sink size and sink activity. One of the major components of sink activity is activity of enzymes involved in the sucrose to starch biosynthesis pathway in grain (Wang *et al*., 1993; Riffkin *et al*., 1995). SuSase catalyzes sucrose cleavage into fructose and UDP-glucose (Keeling *et al*. 1988). This enzyme is dominant to accumulate sink carbohydrate reserves, it could be used as a marker for sink strength (Koch, 2004) and is involved in the first step of catalyzing sucrose to starch conversion in endosperm of cereals (Kato, 1995). In our study, SuSase activity exhibited a single peak increase in basal and distal kernels during grain-filling and then reached a maximum 21 days post-anthesis which was higher in HN treatment than in LN (Figure 2). Thereafter, its activity decreased in both treatments. HN treatment slightly enhanced the activity of SuSase in basal and distal kernels and the increment was obvious even in distal kernels. It has been shown that nitrogen supply to sink tissue stimulates sink biosynthesis activity. In the experiment of Singletary *et al*. (1990), where developing maize kernels were cultured *in vitro,* at 4 day after anthesis on a media containing a range of nitrogen concentrations, nitrogen supply stimulated SuSase activity several fold, measured at 20 day after anthesis.

AGPase catalyzes the synthesis of ADP-glucose and pyrophosphate from glucose-1-P and ATP. ADP-glucose, then, serves as the glucose donor for starch biosynthesis (James *et al*., 2003). One key regulatory step that controls the flux of carbon into starch is catalyzed by AGPase (Slattery *et al*., 2000). The activity of this enzyme showed a single peak during grain-filling period (Figure 3). This peak in basal kernels occurred 21 days post-anthesis. AGPase activity in basal kernels was higher than that of distal kernels. HN treatment increased the AGPase activity in distal and basal kernels. Probably, low nitrogen causes a reduction in enzymatic activity which leads to limitations in enzymatic capacity and finally reduction in starch accumulation and grain weight

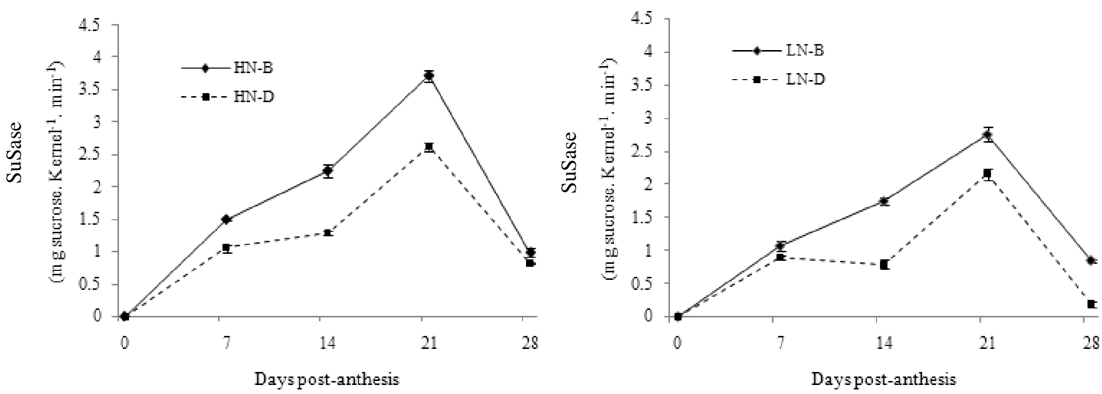

Figure 2. Developmental changes in activity of SuSase for basal (B) (Solid Line) and distal (D) (Dotted Line) kernels in treated plants with high nitrogen (HN) and Low-nitrogen (LN) during grain development in wheat. Vertical bars represent ±SE of means (n= 3).

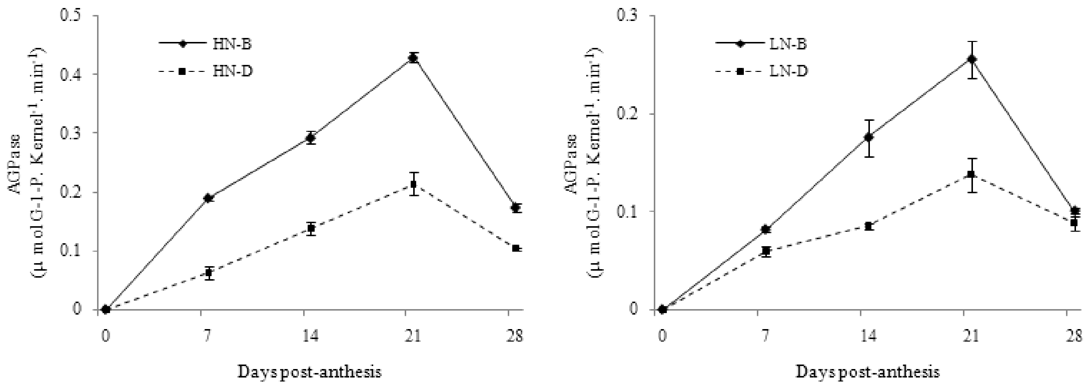

Figure 3. Developmental changes in activity of AGPase for basal (B) (Solid Line) and distal (D) (Dotted Line) kernels in treated plants with nitrogen (HN) and Low-nitrogen (LN) during grain development in wheat. Vertical bar represent ±SE of means (n= 3).

(Jiang *et al*., 2003).

The resulting ADP-glucose is used by starch synthase to transfer the glucose residue onto the non-reducing end of a pre-existing glucan chain *via* a *α*-1, 4 linkage (James *et al*., 2003). It is assumed that this enzyme is responsible for producing the polymers as substrates for SBE to synthesize amylopectin. On the contrary, granular-bound starch synthase (GBSS) has a probable role to synthesize amylose. Starch in wheat grains is mainly formed from amylopectin, thus the role of SSS is more considerable than GBSS (Smith and Denyer, 1992). Results from this research showed that the activity of SSS during grain development increased to a maximum amount 21 days post-anthesis and then decreased again (Figure 4). The activity of this enzyme was higher in basal kernels than in the distal ones. HN treatment increased the activity of SSS in both kernels. In a research on developing maize kernels, it was shown that the enhanced enzyme activity in kernels supplied with high nitrogen concentration in the medium was due to enhanced gene expression. Increase in nitrogen supply almost doubled transcript levels of genes encoding for SuSase, starch synthase, and aldolase (Doehlert, 1993).

Branching *α*-1, 6 linkages between linear chains is catalyzed by SBE (James *et al*., 2003). The activity of SBE was similar to SSS activity (Figure 5). SBE activity in HN

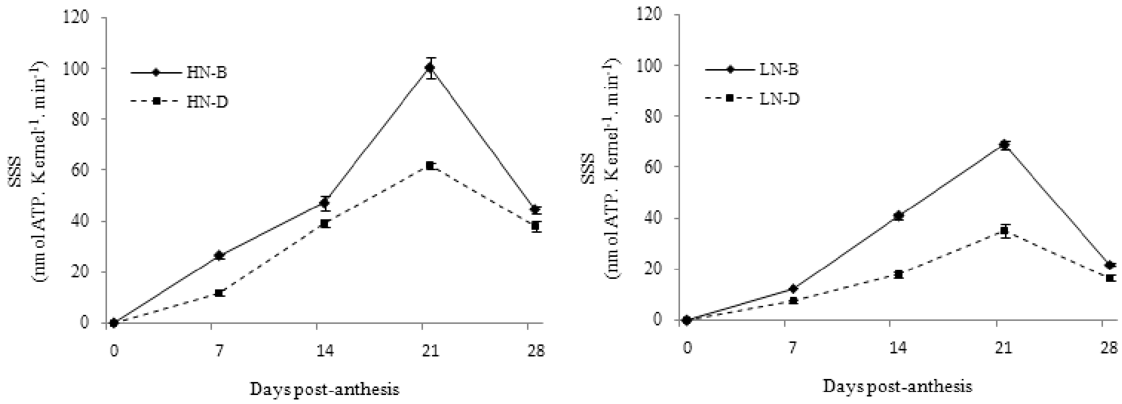

Figure 4. Developmental changes in activity of SSS for basal (B) (Solid Line) and distal (D) (Dotted Line) kernels in treated plants with nitrogen (HN) and Low-nitrogen (LN) during grain development in wheat. Vertical bars represent ±SE of means (n= 3).

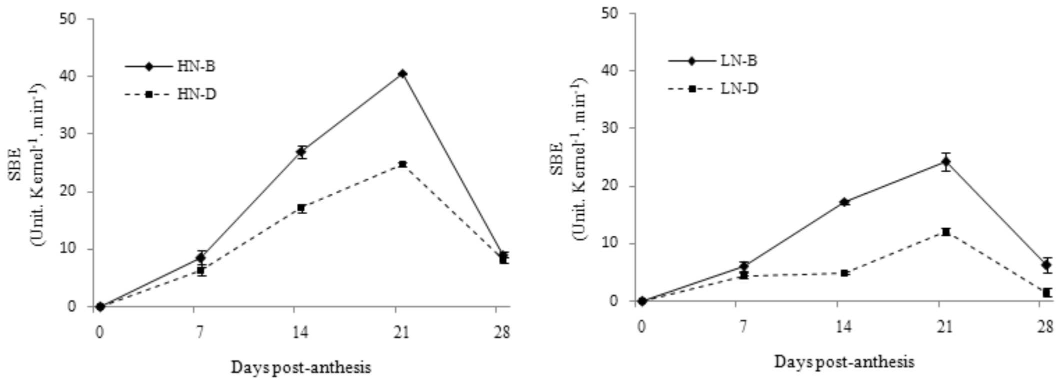

Figure 5. Developmental changes in activity of SBE for basal (B) (Solid Line) and distal (D) (Dotted Line) kernels in treated plants with nitrogen (HN) and Low-nitrogen (LN) during grain development in wheat. Vertical bar represent ±SE of means (n= 3).

treatment was markedly higher than that of LN.

Present results showed that, during wheat kernel development, the activity of SuSase, AGPase, SSS and SBE changed as single peak curve. The application of nitrogen fertilizer could enhance enzyme activity during grain-filling period. Therefore, nitrogen availability to kernels is one of the primary controlling factors for sink strength. In this experiment, we observed that distinct differences in individual grain weight between distal and basal kernels were associated with the strong differences in grain sink strength. These results are consistent with previous studies that reported the key role of SuSase, AGPase, and SSS in starch synthesis regulation in rice, their relevance for amylopectin accumulation in individual grains on wheat spike and SuSase, AGPase, SSS, and GBBS significant roles in amylose accumulation (Jiang *et al*., 2003). In a research on sink strength of wheat, it was shown that the grain sink strength, determined by endosperm cell number and the activity of synthesis-related enzymes, was closely associated with starch accumulation in superior and inferior grains on a wheat spike (Yan *et al*., 2010). Our results also imply that these enzymes have an important role in controlling starch synthesis in grain endosperm and in determining the individual grain weight in wheat, particularly in distal kernels.

Effects of Exogenous Application of ABA on the Sink Strength

To investigate the effects of ABA on the kernel weight, ABA and Fluridone were applied at 9 days post-anthesis. We observed an increase in 1,000-Grain weight with ABA treatment, and a substantial decrease in 1,000-Grain weight with Fluridone compared to the control (Table 2). Exogenous application of ABA increased harvest index (HI) and grain yield (Table 2). Increased HI is possibly caused by increased remobilization of storage compounds from secondary sources, especially stems to developing grains (Yang *et al*., 2003b).

Table 2. Yield and its components of Yangmai15 wheat cultivar in response to plant growth regulators.

Plant growth regulator	Grain yield ($g\ m^{-2}$)	Biological yield ($g\ m^{-2}$)	Harvest index (%)	1000-Grain weight (g)	Grain number $spike^{-1}$
ABA	684 a	1730 ab	40 a	50 a	41 a
Fluridone	516 b	1527 b	34 b	41 c	38 b
Control	646 a	1910 a	34 b	47 b	40 a
LSD[a]	58	261	2	0.37	2

Starting 9 days post-anthesis either 25×10^{-6} M ABA or Fluridone (an inhibitor of ABA synthesis) were applied at the top of plants for 4 days with 0.1% (v/v) ethanol and 0.01(v/v) Tween20 as surfactant. The plants sprayed with the same volume of deionized water containing the same concentrations of ethanol and Tween20 were taken as the control. [a] Least Significant Different (LSD) multiple range comparison tests were used to determine differences between means (P≤ 0.05).

We also observed a significant increase in the activity of key enzyme converting sucrose to starch both in distal and basal kernels (Table 3). The highest increase was found for SSS and AGPase activity in ABA treated plants (Table 3). ABA may play a role in regulation of gene expression (Rock and Quatrano, 1995).

Results indicated that the increase in grain weight of basal and especially of distal kernels was due to increase in the mentioned enzyme activity. In a research on rice, it was shown that superior grains show a higher filling rate because of a higher ratio of ABA to ethylene (Yang *et al.,* 2006). Also, as we know, ABA plays important role in plant growth regulation and plant responses to environmental conditions, including a critical role in the regulation of seed development. High concentration of ABA might enhance the rate of assimilate transport from source to developing grains in wheat (Waters *et al*., 1984). It has also been suggested that ABA might stimulate phloem unloading by decreasing the proton motive force across the sieve tube plasma lemma (Tanner, 1980).

CONCLUSIONS

Low nitrogen caused reduction in individual grain weight, associated with a decrease in the activity of key enzymes involved in sucrose-to-starch conversion. Nitrogen treatment probably increases the strength of developing kernels to utilize available carbohydrate, largely through improving activities of enzymes of carbohydrate metabolism and also changes in kernel metabolic activities. Probably, plants have the ability to sense internal and

Table 3. Effects of ABA and Fluridone application on the activity of enzymes involved in starch synthesis in the grains of Yangmai15 wheat cultivar**.**

Plant growth regulator	Kernel position	SuSase (mg sucrose $kernel^{-1}\ min^{-1}$)	SSS (nmol ATP $kernel^{-1}\ min^{-1}$)	AGPase (µmol G-1-P $kernel^{-1}\ min^{-1}$)	SBE (Unit $kernel^{-1}\ min^{-1}$)
ABA	Basal	4.27 ± 0.05	111.33 ± 5.02	0.46 ± 0.01	43.41 ± 1.06
	Distal	2.81 ± 0.05	66.83 ± 0.78	0.26 ± 0.01	27.95 ± 0.60
Fluridone	Basal	3 ± 0.04	67.91 ± 0.39	0.26 ± 0.01	27.62 ± 0.45
	Distal	2.17 ± 0.05	40.95 ± 1.28	0.14 ± 0.01	12.20 ± 0.29
Control	Basal	3.37 ± 0.07	91.14 ± 1.75	0.36 ± 0.01	33.90 ± 0.65
	Distal	2.27 ± 0.08	49.97 ± 1.22	0.19 ± 0.01	22.60 ± 0.37

Starting 9 days post-anthesis either 25×10^{-6} M ABA or Fluridone (an inhibitor of ABA synthesis) were applied at the top of plants for 4 days with 0.1% (v/v) ethanol and 0.01(v/v) Tween20 as surfactant. The plants sprayed with the same volume of deionized water containing same concentrations of ethanol and Tween20 were taken as a control. Means ± S.E. (n=3).

external nitrogen status, and to adapt to varying nitrogen conditions by modifying gene expression, enzyme activities, and metabolite contents (Sakakibara *et al*., 2006).

When ABA was applied to nitrogen supplied plants at early grain-filling stage (9-13 days post-anthesis), the activity of key enzymes in starch synthesis increased significantly within basal and distal kernels. Thus, ABA levels have a probable key role in enhancing enzyme activity.

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تاثیر کاربرد نیتروژن و ابسزیک اسید روی وزن دانه‌های پایینی و بالایی سنبلچه های گندم

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چکیده

وزن تک‌دانه در دانه‌های گندم بسته به موقعیت‌شان روی یک سنبله متفاوت است. عملیات زراعی (مثل نیتروژن) و کاربرد تنظیم کننده‌های رشد گیاهی می‌توانند به منظور بهبود وزن دانه در دانه‌های قوی و ضعیف مورد استفاده قرار گیرند. بدین منظور دو آزمایش مجزا به صورت طرح بلوک کامل تصادفی در سه تکرار در مزرعه تحقیقاتی دانشگاه یانگجو چین در سال ۲۰۱۰-۲۰۰۹ انجام شد. هدف از انجام آزمایش مطالعهٔ مکانیزم‌های مرتبط با قدرت مخزن و عوامل تاثیر گذار روی فعالیت مخزن بود. یک رقم نیمه‌زمستانه گندم، Yangmai15 استفاده شد. تیمارهای دو آزمایش به ترتیب، کاربرد نیتروژن (دو سطح) و تنظیم کننده‌های رشد گیاهی (ABA و فلوریدون) در دانه‌های قوی و ضعیف بودند. نتایج نشان دادند که تیمار نیتروژن باعث افزایش در عملکرد دانه و اجزاء آن شد. پر شدن دانه در دانه‌های قوی زودتر شروع شده و سرعت پر شدن آن هم بیشتر از دانه‌های ضعیف بود. کود نیتروژن وزن تک‌دانه را هم در قوی و هم در ضعیف افزایش داد، اما میزان افزایش در دانه‌های ضعیف حتی بیشتر بود. کاربرد ABA منجر به افزایش در عملکرد دانه شد در حالیکه کاربرد فلوریدون (بازدارندهٔ سنتز ABA) منجر به کاهش اساسی در عملکرد دانه در مقایسه با کنترل شد. کود نیتروژن به‌همراه کاربرد ABA، فعالیت آنزیم‌های SuSase، SSS، AGPase و SBE را به طور معنی‌داری افزایش داد. بنابراین، کاربرد همزمان نیتروژن و اسید آبسیزیک وزن دانه را از طریق تنظیم فعالیت آنزیم‌های کلیدی دخیل در سنتز نشاسته افزایش می‌دهد.