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ORIGINAL ARTICLE

Sucrose Synthase, ADP-glucose Pyrophosphorylase and Aldolase Levels in Relation to Grain Development of Wheat

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ABSTRACT

Relative levels of sucrose synthase (SS), ADP-glucose pyrophosphorylase (AGPase) and aldolase were studied at different types of grain (bold and small) growing in the same spikelet of wheat (*Triticum aestivum* L. var. *PBW-343*). Ten labeled spikes were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28th DAA, and at maturity. The sucrose synthase activity increased rapidly in both the types of grains in around 14th DAA. In comparison to bolder grains the smaller grains possessed significantly low levels of this enzyme. The disparity was sustainable throughout the ontogeny of grains' development with a maximum gap at 7th DAA. The AGPase and aldolase activities were increased steadily and reached to maximum values at 21st DAA and were followed by gradual declensions in both the types of grains upto maturity. However, at maturity the absolute levels of aldolase were definitely more than the initial period of anthesis in both the types of grains. AGPase and aldolase activities were also detected in comparatively higher amounts in bolder grains than smaller grains at all stages of grains' development with maximum gaps at 7th DAA. The results bring forth, in no uncertain terms, the findings that the ear of wheat is a developing place for a definite number of grains which intern are separate biological entities endowed with their inherent potentials to grow.

Key words: Anabolic enzymes; spikelet; *Triticum aestivum* L.

Introduction

A casual look into the present global food supply reveals that the cereals constitute 2/3 component of its resource. An appraisal of parameters regulating cereals productivity divulges that their full potential to yield is still unrealized. One of the grey areas, which has remained untapped is the host of physiological and genetical barriers of developing kernels to grow to an optima and their manipulation by desirable traits and methodologies. The potential up gradation of components constituting the total yield in wheat (number of productive tillers m^{-2}, grains per spike and 1000-grain weight), would help to raise the production substantially. Though, significant milestones have been achieved in the first two parameters the last component, the individual grain weight has eluded scientific investigations and rather paradoxically has declined with the advent of high yielding varieties. A study into the physiology of grain yield shows the existence of variation among different varieties or genotypes or even the grains developing in the same ear [3,21,17,23,11]. It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks [25,19,20,7]. Various sugar responsive genes in plants

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potentially affect the partitioning [8] and have been stressed to be key determinant of plant productivity [9]. Dry matter partitioning also plays a paramount role in growth rate of sink organs [12]. Working on the grain growth in wheat and buckwheat variation among varieties was traceable to endogenous hormone production in variety vis-à-vis that in the ear [6,5]. A few biochemical components as advocated by Abrol *et al.* [2], Hakaka [11] and Hasan and Kamal [12], might be of significance in determining sink efficiency and/or the grain yield. In the present study, it is proposed to analyse the relative levels of a few enzymes belonging to the anabolic enzymes namely, sucrose synthase (SS), ADP-glucose pyrophosphorylase (AGPase) and aldolase in different grains growing in the same spikelet and to assess whether they are variable amongst the differentially participating grains or not.

Materials and methods

Crop Management and Sampling:

The investigation was conducted with a common bread wheat (*Triticum aestivum* L. var. *PBW-343*), which was sown in circular earthenware pots (50x30x30 cm) containing 35 kg of soil mixed with farmyard manure (4:1). Eight seeds per pot were sown and after 15 days, seedlings were thinned to two. Hoagland's nutrient solution [14] was supplied to the pots. The plants were grown in a screen covered hall under otherwise natural conditions. Ten labeled main spikes were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28^{th} DAA, and at maturity. Grains were usually taken from three different segments in the ear. The labeled samples of grains were brought to laboratory and separated to two types of grains (small and bold) and the following biochemical analysis was carried out in the above aged grains.

Sucrose Synthase Analysis:

Sucrose synthase was studied according to the method of Morell and Copeland [16] with minor modifications as follows:

Extraction of the enzyme - 500 mg of grain sample was homogenised with 10 mM potassium phosphate buffer (pH of 7.2) containing 1 mM EDTA and 5 mM 2-mercaptoethanol. It was filtered through eight layers of cheese cloth and centrifuged at 30,000 g for 15 minutes. All steps were carried out at 4°C and the supernatant was used as an enzyme source for estimation.

Estimation of the enzyme - The estimation of enzyme was performed by taking 0.2 ml of above supernatant to which 0.2 ml HEPES- KOH buffer 0.1 M (pH of 7.5), 0.2 ml sucrose 0.5 M, 0.2 ml UDP 0.01 M, and 0.1 ml UDP-glucose dehydrogenase were added. The spectrophotometer was set to zero absorbance at 340 nm. Subsequently 0.1 mL NAD^+ 0.015 M was added quickly to the test solution and the initial absorbance was recorded with a timer. The reaction mixture without NAD^+ was used as a blank. The decrease in A_{340} was recorded every minute until no further reaction was observed. The change in absorbance at 340 nm is 12.0 for each micromole of glucose per milliliter. The enzyme activity was expressed as micromole glucose formed per mg of protein in a particular time.

Aldolase and AGPase analysis:

These two enzymes activity were estimated from the same enzyme extract obtained as follows:

Extraction of enzymes - Samples of 1 g plant tissue were grounded in a chilled glass homogenizer with 5 ml of chilled buffer containing 100 mM tricine – NaOH (pH of 8.0), 8 mM $MgCl_2$, 2mM EDTA, 50mM 2-mercaptoethanol and 12.5 percent (V/V) glycerol. The homogenate was transferred to Ependorf tubes and centrifuged at 14,000 g for 5 minutes. The supernatant was collected, stored in ice and used as an enzyme source.

Estimation of aldolase - Aldolase enzyme activity was measured according to the method of Beisenherz *et al*. [4] as follows:

Preparation of solutions:

I. Buffer-iodoacetate-FDP solution; The solution had the following composition i.e., 0.679 g collidine, 6.2 mg Na iodoacetate and 100 mg FDP-Na_3H in 90 ml distilled water all adjusted to pH of 7.4 with 5 N HCl and diluted to 100 ml with distilled water.

II. Reduced diphosphopyridine nucleotide; 25 mg DPNH-Na_2 in 2 ml of 1 percent $NaHCO_3$ solution.

III. Glyceral-1-phosphate dehydrogenase-triose phosphate isomerase, GDH-TIM.

The commercially available crystalline suspensions with 2.4 M ammonium sulphate solution was diluted and mixed. All solutions were stored at 0-4°C for further use. The spectrophotometer was set at 340 nm and 2.74 ml of buffer-iodoacetate-FDP (solution I), 0.05 ml DPNH (solution II) was placed in the cuvette and equilibrated for 5-10 minutes and then mixed in 0.01 ml GDH-TIM (suspension III). Finally 0.2 ml of enzyme extract was added to the cuvette. The aldolase reaction was started after 1-2 minutes. The optical density of enzyme was measured at 340 nm and at the same time, started the stop watch. The optical densities was read at 2

minutes intervals for 20 minutes the result of this reading was used for the calculations according to the method described by Nakamura *et al*. [18]. The aldolase activity was expressed as nanomol per minute per gram fresh weight of sample.

Estimation of AGPase- The AGPase activity was determined at 30°C by measuring the rate of ATP formation in the following reaction system of the pyrophosphate (PPi)-dependent degradation of ADP-glucose. The reaction mixture contained 100 mM HEPES-NaOH (PH of 7.6), 5 mM $MgCl_2$, 5 mM DTT, 3mM PPi and 1.5 mM ADP-glucose. The reaction was initiated by adding an aliquot of enzyme preparation and terminated after 30 minutes of incubation by transferring to boiling water for 1 minute. The formed ATP was monitored according to the procedures described by Wang *et al.* [22]. AGPase activity was expressed as nanogram ATP per mg of protein per minute in sample.

Result and discussion

In order to establish whether the bold and small grains were separate biological entities produced as a consequence of the specific biochemical process or were simple chance manifestations of development, the relative levels of a few enzymes belonging to the anabolic enzymes namely, sucrose synthase, ADP-glucose pyrophosphorylase and aldolase were studied in them at different stages of growth and development. The salient features of results are given below.

Sucrose synthase:

As apparent from the data in Figure 1 sucrose synthase activity increased rapidly in two types of grains around 14th DAA. Analysis of data revealed that its levels increased to the tune of 39.0 and 25.6 percents at 14th and 21st DAA stages respectively followed by a decrease of about 4.0 and 28.4 percents at 28th DAA as well as at maturity in bold grains respectively. Similarly, the increments in its levels were also observed in small grains to the tune of 129.6, 85.2 and 43.2 percents at 14th, 21st and 28th DAA stages respectively followed by an abatement of 3.7 percent at maturity. Looking at the overall deduction recorded from 7th DAA to maturity, the smaller grains showed an overall decrease by 3.7 percent whereas, the bolder grains showed an overall abatement in the level of this enzyme by 28.4 percent.

As evident from the data in Figure 4, the levels of sucrose synthase in bold and small grains, correspondingly also showed a significant disparity with respect to its distribution in the two types of grains. In comparison to bolder grains the smaller grains possessed significantly low levels of this enzyme. The disparity was sustainable throughout the ontogeny of grains' development with maximum gap at 7th DAA stage i.e., 54.0 percent lesser than bold grains with a further recorded gap of 24.1, 32.1 and 31.4 percents at 14th, 21st and 28th DAA stages and ending up with a final disparity of 38.1 percent at maturity respectively.

ADP-glucose Pyrophosphorylase:

AGPase activity was detected in comparatively higher amounts in bolder grains than smaller grains at all stages of grains' development (Figure 2). The disparity between the two types of grains was maximum at 7th DAA (57.9 percent lesser in small grains than bold grains) stage and it was 49.5, 37.1 and 34.8 percents at 14th, 21st and 28th DAA which finally ended up with a disparity of 47.6 percent at maturity respectively. AGPase activity increased steadily and reached its maximum value at 21st DAA and was followed by gradual declensions in both the types of grains upto maturity. The overall depreciation in the absolute levels of AGPase was of a magnitude approximately 26.3 percent in bolder grains as compared to 8.3 percent in smaller grains.

Aldolase:

The scrutiny of the data of another key enzyme, aldolase likewise also offered some interesting disclosures (Figures 3 and 4). As evident from the figures, the levels of this enzyme also showed a significant disparity with respect to its distribution in the two types of grains. The gap was sustainable throughout the ontogeny of grains' development with a maximum gap at 7th DAA (34.8 percent lesser in small grains than bold grains) and 32.8, 30.3 and 28.9 percents gaps at 14th, 21st and 28th DAA stages respectively. At maturity smaller grains possessed 25.6 percent lesser aldolase than bolder grains. Enzyme activity in both the types of grains was highest at 21st DAA and declined later towards maturity. However, at maturity the absolute levels of this enzyme were definitely more (approximately 3 folds) than the initial period of anthesis in both the types of grains.

The results bring forth, in no uncertain terms, the findings that the ear of wheat is a developing place for a definite number of grains which intern are separate biological entities endowed with their inherent potentials to grow and accumulate dry matter. This axiom was advocated by Abolina [1] and is in line with the observations of innumerable workers [21,15,23,17,24].

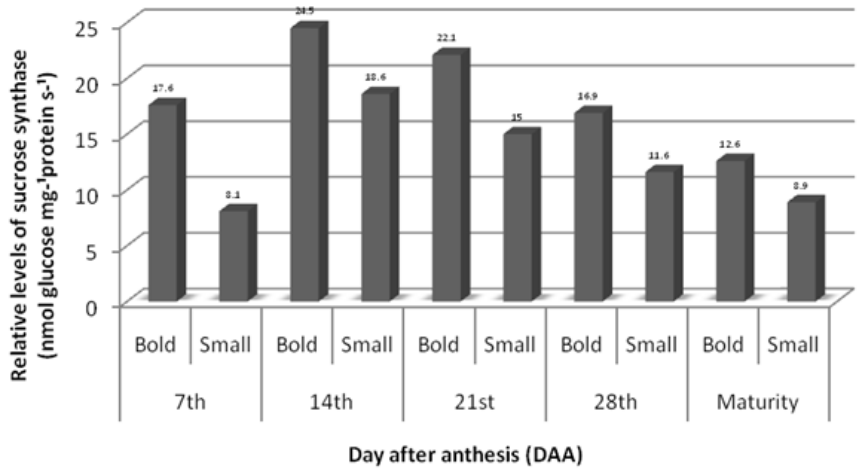

Fig. 1: Relative levels of sucrose synthase (nmol glucose mg^{-1} protein s^{-1}) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*)

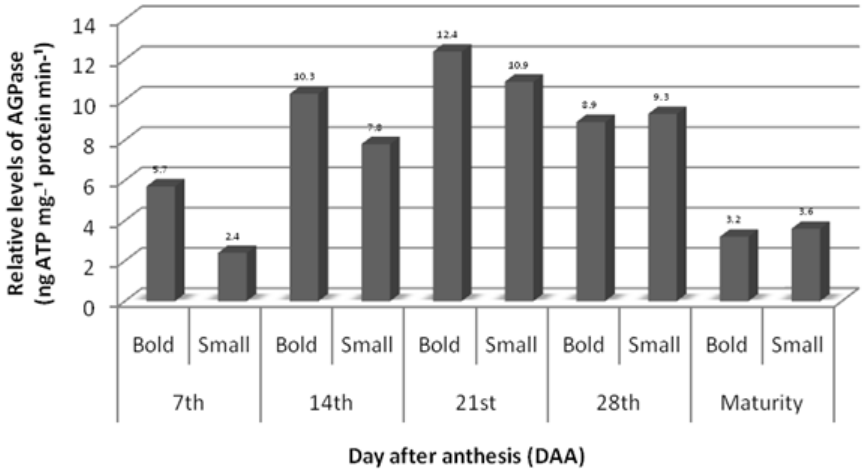

Fig. 2: Relative levels of ADP-glucose pyrophosphorylase (AGPase) (ng ATP mg^{-1} protein min^{-1}) within developing grains (bold and small) of wheat (Triticum aestivum var. PBW-343)

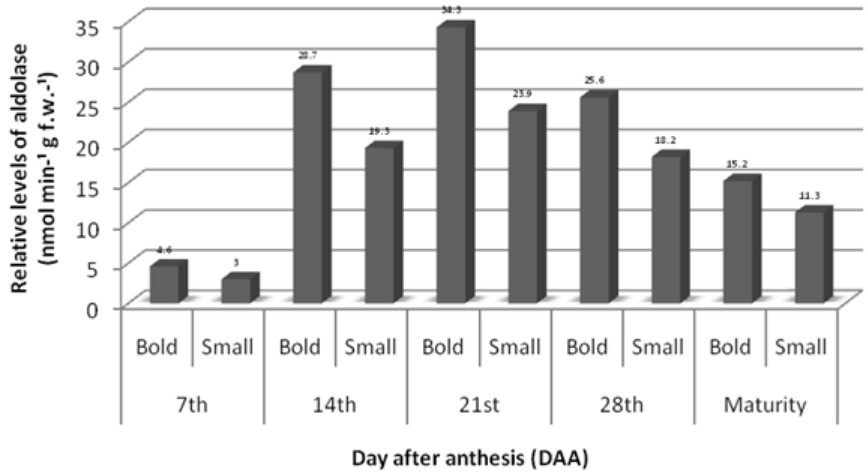

Fig. 3: Relative levels of aldolase (ng mol min^{-1} g fresh weight) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*)

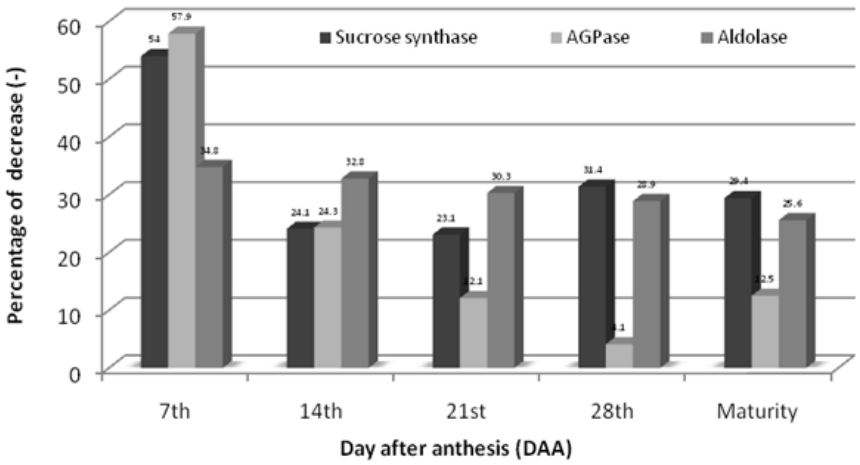

Fig. 4: Percentage decrease (-) in relative levels of sucrose synthase, AGPase and aldolase in small grains over their counterparts bold grains

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