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ORIGINAL ARTICLES

Effect of Salicylhydroxamic Acid on Sucrose Synthase, ADP- Glucose Pyrophosphorylase and Aldolase Activities in Relation to Grain Development of Wheat

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ABSTRACT

The effects of salicylhydroxamic acid (SHAM) on 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*). The plants were grown in a screen covered hall under otherwise natural conditions. A concentration of 10 ppm salicylhydroxamic acid was applied at anthesis stage in five replications with the help of cotton plugs, which remained on ears of mother shoots for 48 hours. Labeled spikes were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28th DAA, and at maturity. The spikelets were divided into two grain types included basal (bold) and apical (small). The salient points emerging through the use of salicylhydroxamic acid were that (i) both bold and small grains showed a significant increase in relative levels of sucrose synthase, AGPase and aldolase from about 28th DAA stage, and (ii) in spite of aforementioned increment, they continued to exhibit the disparity between them.

Key words: Anabolic enzymes; SHAM; inhibitor; CN-resistant respiration; *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 (Asana 1968, Stoy 1969; Nautiyal *et al.*, 1999; Yang *et al.*, 2003; Gutam *et al.*, 2008). It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks (Yin *et al.*, 1998; Ravi *et al.*, 2001; Sharma-Natu and Ghildiyal 2005; Foulkes *et al.*, 2010). Various sugar responsive genes in plants potentially affect the partitioning (Geiger et al., 1996) and have been stressed to be key determinant of plant productivity (Gifford *et al.*, 1984). Dry matter partitioning also plays a paramount role in growth rate of sink organs (Heuvelink and Bertin 1994). 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 (Dua and Sehgal 1981; Dua *et al.*, 1990). A few biochemical components as advocated by Abrol *et al.*, (1984), Hakaka (1998) and Hasan and Kamal (1998), might be of significance in determining sink efficiency and/or the grain yield. In the present study, it is proposed to analyze the relative levels of a few enzymes belonging to the anabolic enzymes namely, sucrose synthase, ADP-glucose pyrophosphorylase and aldolase as affected by specific inhibitor of salicylhydroxamic acid in different grains growing in the same spikelet of wheat.

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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 (Hoagland and Arnon, 1939) 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 28th 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 (1958) 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.,* (1953) as follows:

Preparation of Solutions:

1. 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.
2. Reduced diphosphopyridine nucleotide; 25 mg DPNH-Na_2 in 2 ml of 1 percent $NaHCO_3$ solution.
3. 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.,* (1989). 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.,* (1999). AGPase activity was expressed as nanogram ATP per mg of protein per minute in sample.

Results and discussion

The salient points emerging through the use of salicylhydroxamic acid were that (i) both bold and small grains showed a significant increase in relative levels of sucrose synthase, AGPase and aldolase from about 28th DAA stage (Figures 1, 2 and 3), and (ii) in spite of aforementioned increment, they continued to exhibit the disparity between them (Figure 4).

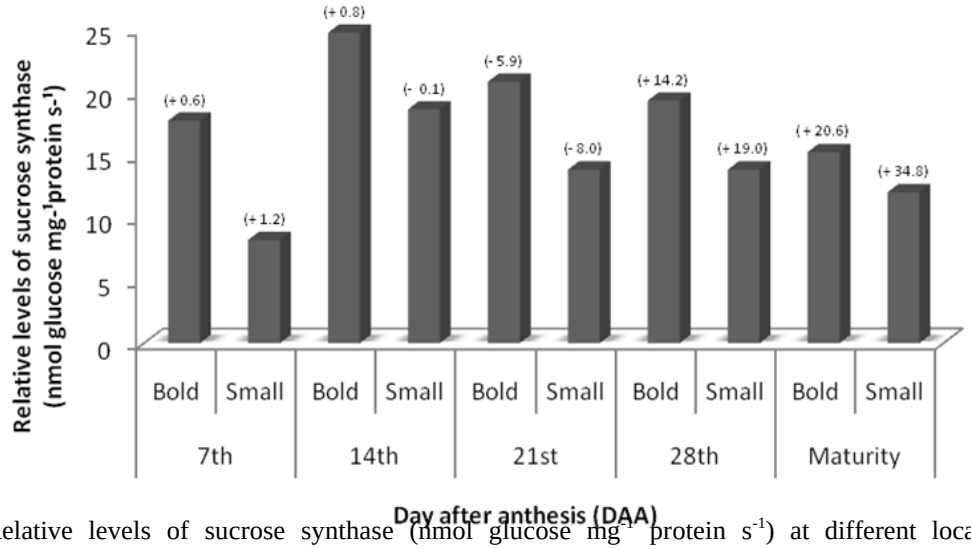

Fig. 1: Relative levels of sucrose synthase (nmol glucose mg^{-1} protein s^{-1}) at different location within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*) as influenced by salicylhydroxamic acid; Values within parenthesis indicate percentage of decrease (-) or increase (+) in level of sucrose synthase over control.

As apparent from the data in Figure 1, the relative levels of sucrose synthase as affected by the inhibitor (SHAM) significantly increased in two types of grains from 28th DAA. Analysis of data revealed that its levels increased to the tune of 14.2 and 20.6 percents at 28th DAA and at maturity in bold grains respectively. Similarly, the increments in its levels were also observed in small grains to the tune of 19.0 and 34.8 percents at 28th DAA and at maturity respectively.

As evident from the data in Figure 4, the effect of salicylhydroxamic acid on the relative levels of sucrose synthase in bold and small grains 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., 53.7 percent lesser than bold grains with a further recorded gap of 24.7, 33.6 and 28.5 percents at 14th, 21st and 28th DAA stages and ending up with a final disparity of 21.0 percent at maturity respectively.

AGPase activity was detected in comparatively higher amounts in bolder grains than smaller grains at all stages of grains' development (Figure 2). As affected by SHAM, the disparity between the two types of grains was still 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 23.1 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 representation of the data in Figures 3 and 4 indicates the level of aldolase as influenced by salicylhydroxamic acid in two different types of grains. 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 (38.3 percent

lesser in small grains than bold grains) and 34.2, 29.1 and 25.9 percents gaps at 14th, 21st and 28th DAA stages respectively. At maturity smaller grains possessed 16.8 percent lesser aldolase than bolder grains. The 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 than the initial period of anthesis in both the types of grains.

We have investigated the relative levels of sucrose synthase, ADP-glucose pyrophosphorylase and aldolase as affected by salicylhydroxamic acid in bold and small grains growing in the same spikelet of wheat(Houshmandfar A and Asli, 2011). 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. This axiom was advocated by Abolina (1959) and is in line with the observations of innumerable workers (Cook and Evans 1978; Larsson and Hensen 1992; Wang *et al.*, 1998; Yang *et al.*, 2003). Nevertheless, the sequence of events, piloting the yielding ability, is the metabolic profile and if augmented through the use of plant growth regulators (Yang *et al*., 2000; Houshmandfar and Eradatmand-Asli 2011) or by imposing a shift in metabolic events (Dua *et al*., 1990) promotery effects are achievable (Hayashi 1961; Michael and Beringer 1980). In present context, the central point which came to light in the present endeavor is that an unusual path of aerobic respiratory chain (CN-resistant respiration) plausibly switches-on during the grain filling stage and if checked, through the immaculate use of salicylhydroxamic acid, can increase the relative levels of sucrose synthase, ADP-glucose pyrophosphorylase and aldolase in the grains. Of course, SHAM or regulator of alternate oxidase pathway was not successful in eliminating the disparities between the two types of grains.

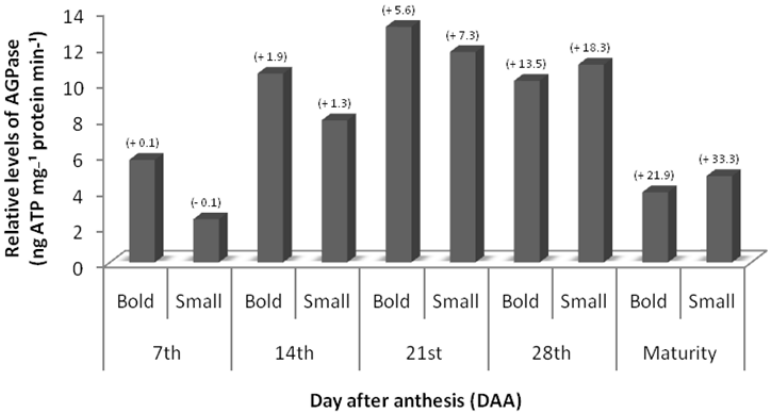

Fig. 2: Relative levels of ADP-glucose pyrophosphorylase (AGPase) (ng ATP mg^{-1} protein min^{-1}) at different location within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*) as influenced by salicylhydroxamic acid; Values within parenthesis indicate percentage of increase (+) or decrease (-) in level of AGPase over control.

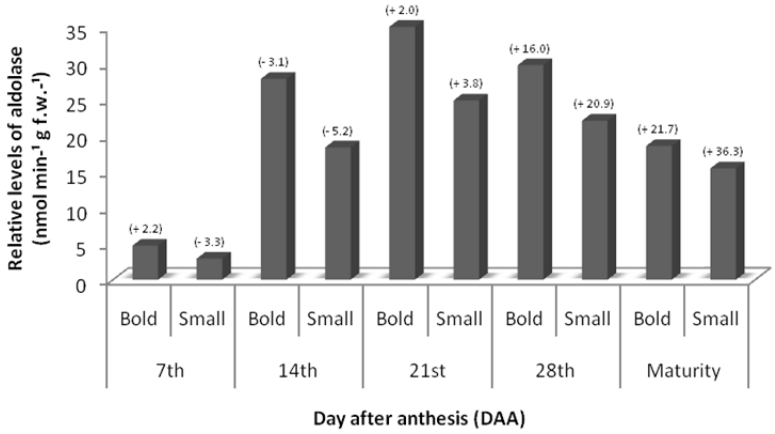

Fig. 3: Relative levels of aldolase (ng mol min^{-1} g fresh weight) at different location within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*) as influenced by salicylhydroxamic acid; Values within parenthesis indicate percentage of increase (+) or decrease (-) in level of aldolase over control.

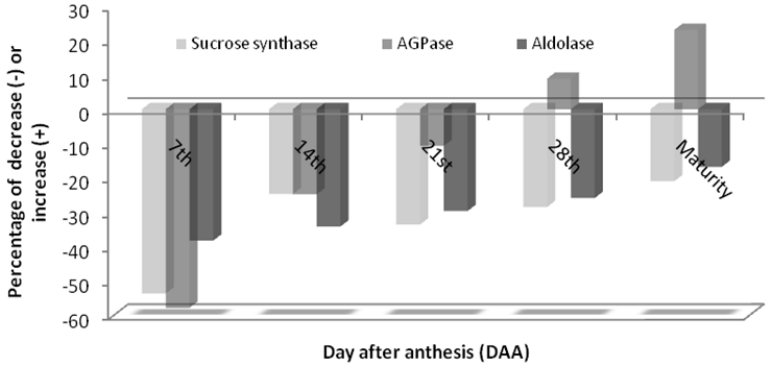

Fig. 4: Percentage increase (+) or decrease (-) in relative levels of sucrose synthase, AGPase and aldolase in small grains over their counterparts bold grains.

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