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ORIGINAL ARTICLE

Effect of Salicylhydroxamic Acid on Relative Levels of Protein and Free Amino Acids in Bold and Small Grains of Wheat

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ABSTRACT

The effect of exogenous application of salicylhydroxamic acid (SHAM) on relative levels of protein and free amino acids were investigated within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*). 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 (MS) for 48 hours. The labeled spikes were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28th DAA, and at maturity. The main spikes were divided into two grain types within each spikelet 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 an increase in relative levels of protein and free amino acids from 7th and 14th DAA stages respectively and (ii) in spite of the aforementioned increment, they continued to exhibit the disparity between them and at maturity the smaller grains still showed lower protein and free amino acids than the bolder grains.

Key words: CN-resistant respiration; SHAM; inhibitor; spikelet; *Triticum aestivum* L.

Introduction

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 [3,24,20,28,9]. It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks [29,21,22,7]. Various sugar responsive genes in plants potentially

affect the partitioning [8] and have been stressed to be key determinant of plant productivity [8]. Dry matter partitioning also plays a paramount role in growth rate of sink organs [10]. 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 [5,6]. A few biochemical components as advocated by Abrol *et al.* [2], Hakaka [10] and Hasan and Kamal [11], might be of significance in determining sink efficiency and/or the grain yield. Since, the harvest index is the culmination of innumerable events, most of the view points on sink efficiency appears to be speculative and need a holistic approach in isolating obligatory events to produce the net assimilates. The revelation that the electron transport chain, in operation during biological oxidation, might find an alternate route

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without performing the target aim of creating proticity and may downgrade the overall impetus of meristems to grow by 10 to 25 percent [23]. Indeed, it has been reported that higher alternative respiration could be one of the reasons of lower growth of grains at distal position in a spikelet [25]. It is, therefore, advocated that any attempt to interrupt this process may prove beneficial in improving productivity. In the present study, it is proposed to analyse the relative levels of protein and free amino acids as affected by specific inhibitor of salicylhydroxamic acid in different grains growing in the same spikelet of wheat.

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. 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 (MS) for 48 hours. The 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.

Protein Analysis:

The total proteins content were determined by method described by Lowry *et al.* [18].

- (i) Extraction of Total Proteins - 100 mg of dried grains were crushed in 5 ml of 20 percent trichloro acetate (TCA) and centrifuged at 12000 g for 20 minutes. Supernatant was discarded and precipitate was washed twice with 80 percent alcohol and left over night for dissolving in 10 ml 2 M NaOH. The extract thus obtained was used for protein estimation.
- (ii) Estimation of Total Proteins - The following reagents were used for estimation of total proteins:

Solution A: 2 percent Na_2CO_3 in 0.1 N NaOH; Solution B: 0.5 percent $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1 percent sodium potassium tartrate; Solution C: alkaline

copper solution; Solution D: follin-cio-calteau reagent was diluted with distilled water in the ratio of 1:1 mix 50 ml of reagent (A) with 1 ml of reagent (B) at the time of use.

0.3 ml of NaOH treated precipitate extract was taken and to it 3 ml of reagent (C) was added. After 10 minutes 0.3 ml of Folin reagent was further added followed by a vigorous shaking immediately. After 30 minutes the optical density of developed blue colour was read at 560 nm on a Bausch and Lomb Spectronic-20. The proteins contents were quantified using the standard curve prepared with bovine serum albumin (BSA). The results are expressed as mg per grain.

Free Amino Acids Analysis:

Method of Lee and Takahashi [17] was adopted for the estimation of total free amino acids.

- (i) Extraction of total free amino acids - 100 mg of dried material was homogenised in 5 ml of 80 percent ethanol refluxed for 15 minutes on a steam bath and centrifuged at 20,000 g for 20 minutes. The residue was further refluxed twice with 80 percent ethanol. The supernatants were pooled together for free amino acids estimation.
- (ii) Estimation of total free amino acids - Total free amino acids were estimated using the following reagents: Ninhydrin reagent (pH 6.0). This reagent was prepared by mixing the following constituents (A, B and C) in the ratio of 5: 12: 2; (A) 1 per cent ninhydrin in 0.5 M citrate buffer (pH 5.5); (B) Pure glycerol. (C) 0.5 M citrate buffer (pH 5.5).

0.2 ml of extract was added to 3.8 ml of ninhydrin reagent. The contents were heated in boiling water bath for 12 minutes and cooled to room temperature. The purplish blue colour was read at 570 nm. The quantity of total free amino acids was calculated from the standard curve prepared by using glycine (5-50 mg) and expressed as mg amino acids per mg dry weight (DW) of tissue.

Results:

The application of salicylhydroxamic acid presented the unique observations. Ironically, the inhibitor behaved in an enigmatic way and proved to be a promoter when being assessed under the criterion of relative levels of protein and free amino acids.

The salient points emerging through the use of salicylhydroxamic acid were that (i) both bold and small grains showed an increase in relative levels of protein and free amino acids from 7th and 14th DAA stages respectively (Figures 1 and 2) and (ii) in spite of the aforementioned increment, they continued to exhibit the disparity between them and at maturity

the smaller grains still showed lower protein and free amino acids than the bolder grains (Figure 3).

Figure 1 indicates the relative levels of proteins under the influence of salicylhydroxamic acid in bold and small grains of wheat. As apparent from the data, the relative levels of protein increased rapidly in both grain types at around 14th DAA. Analysis of data showed that its levels increased to the tune of 53.0 percent at 14th DAA (as compared 7th DAA) followed by decrease of about 23.2, 50.0 and 26.7 percents at 21st and 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 65.8 percent at 14th DAA (as compared 7th DAA) followed by decrease of about 40.2, 31.9 and 22.8 percents at 21st and 28th DAA as well as at maturity in bold grains, respectively.

Furthermore, the relative levels of proteins in bold and small grains correspondingly 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 protein. The disparity was sustainable throughout the ontogeny of grains development with maximum gap at 21st DAA (25.2 percent lower than bold grains) with a recorded gap of 11.4, 4.0 and

21.8 percent at 7th, 14th and 28th DAA and ending up with a final disparity of 17.6 percent at maturity, respectively (Figure 3).

Figure 2 demonstrates the relative levels of free amino acids under the influence of SHAM in different grains growing in the same spikelet of wheat. As apparent from the data, the relative levels of free amino acids increased rapidly in both grain types at around 14th DAA. Analysis of data showed that its levels increased to the tune of 47.1 percent at 14th DAA (as compared 7th DAA) followed by decrease of about 25.8, 27.1 and 25.9 percents at 21st and 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 48.1 percent at 14th DAA (as compared 7th DAA) followed by decrease of about 29.05, 25.4 and 29.2 percents at 21st and 28th DAA as well as at maturity in bold grains, respectively.

As apparent from the Figure 3, the relative levels of free amino acids in bold and small grains also correspondingly 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 free amino acids. The disparity was sustainable throughout the ontogeny of grains development with maximum gap at maturity (21.3 percent lower than bold grains) with a recorded gap of 16.4, 15.9, 19.5 and 17.6 percent at 7th, 14th, 21st and 28th DAA, respectively.

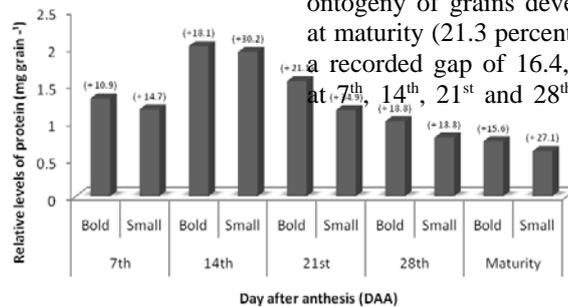


Fig. 1: Relative levels of protein (mg grain⁻¹) at different location within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*) as influenced by salicylhydroxamic acid; Values within parenthesis indicate percentage of increase (+) or decrease (-) in level of protein over control.

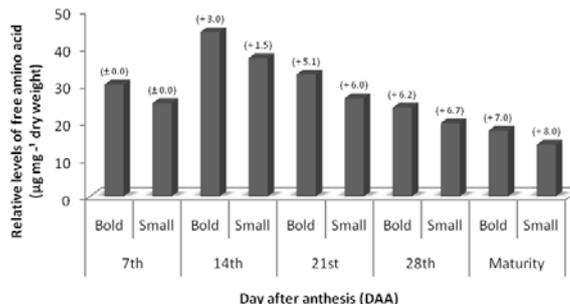


Fig. 2: Relative levels of free amino acids (µg mg⁻¹ DW) at different location within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*) as influenced by salicylhydroxamic acid; Values within parenthesis indicate percentage of increase (+) or decrease (-) in level of free amino acid over control.

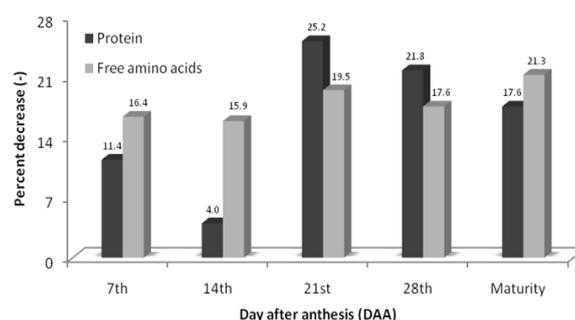


Fig. 3: Percentage decrease (-) in relative levels of protein and free amino acids in small grains over their counterparts bold grains

Discussion:

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 [1] and is in line with the observations of innumerable workers [4,26,28]. Nevertheless, the sequence of events, piloting the yielding ability, is the metabolic profile and if augmented through the use of plant growth regulators [27,15] or by imposing a shift in metabolic events [5] promotory effects are achievable [12,19]. 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 protein and free amino acid 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.

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