Salicylhydroxamic Acid Effects on Relative Levels of Ascorbic Acid Oxidase and Amylases in Different Grains Growing in the Same Spikelet of Wheat

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ABSTRACT

The salicylhydroxamic acid effects on relative levels of ascorbic acid oxidase, a- and b- amylases were studied within developing grains 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 decrease in relative levels of ascorbic acid oxidase, a-amylase and b-amylase from about 14th DAA stage and (ii) in spite of the aforementioned decline, they continued to exhibit the disparity between them and at maturity the smaller grains still showed higher ascorbic acid oxidase, a- and b- amylases than the bolder grains.

Key words: 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 their productivity divulges that their full potential to yield is still unrealized. One of the grey areas, which have remained untapped, is the host of physiological and genetrical 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 [3,26,21,30,12, Nawaz et al., 2009]. It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks [31,23,24,9]. 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 [11]. Dry matter partitioning also plays a paramount role in growth rate of sink organs [16]. 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 [8,7]. A few biochemical components as advocated by Abrol et al. [2], Hakaka [13] and Hasan and Kamal [14], 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 without performing the target aim of creating procticy and may downgrade the overall impetus of meristems to grow by 10 to 25 percent [25]. 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 [27]. 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 a few enzymes belonging to the hydrolytic class namely, ascorbic acid oxidase, a- and b- amylases 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 [17] 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.

Ascorbic Acid Oxidase Analysis:

The method used for estimation of ascorbic acid oxidase was according to Oberbacher and Vines [22] and it’s briefly described as follows:

(i) Extraction of the enzyme - 100 mg of grain tissue was homogenated in a clean and pre-cooled mortar and pestle with 5ml of 0.1 M phosphate buffer (pH of 6.5). The homogenate was centrifuged at 15,000g for 10 minutes at 4°C. All procedures were carried out at 0-4°C and the supernatant was used as a source for enzyme estimation.

(ii) Estimation of the enzyme - In order to quantify ascorbic acid oxidase, 3 ml of substrates solution (prepared by making 8.8 mg ascorbic acid dissolved in 300 ml phosphate buffer at pH of 5.6) was added to reference cuvette of a spectrophotometer. 0.1 ml of enzyme extract was added to the cuvette (enzyme was added so as to get a positive increase in absorbance values). The absorbance change was measured at 265 nm in 30 sec intervals time for 5 minutes. For calculation of the enzyme from the linear phase of reaction, the change in optical density per minute was computed. Enzyme activity was expressed as micromole oxygen per minute according to above method.

a-amylase analysis:

The method of Fuma (1954) with certain modifications was used. The method is outlined below:

(i) Extraction of the enzyme - 1 g of grain was homogenized in 7 ml of phosphate buffer (pH of 7.5) containing 0.001 M cysteine and 0.8 M KCl. The extract was centrifuged at 15,000 g for 30 minutes. The residue was re-extracted with 3 ml phosphate buffer and supernatant was pooled together and kept at 0-4°C for estimation.

(ii) Estimation of the enzyme - The following reagents were used for estimation of this enzyme.

1) KI solution, 25.4 mg of I₂ and 0.4 g KI were dissolved in 100 ml of distilled water.
2) 1 percent starch solution, a fresh solution by dissolving 1 g starch in 100 ml acetate buffer was prepared.
3) Phosphate buffer (0.05 M pH of 7.5) A - Monobasic sodium phosphate (1.39 g/200 ml of distilled water); B - Dibasic sodium phosphate (1.78 g/200 ml of distilled water); For desired pH of 7.5, 16 ml of A and 84 ml of B were pooled together and diluted to 200 ml with distilled water.
4) Cysteine 0.242 g/200 ml buffer.
5) KCl 11.92 g/200 ml buffer.

4 ml of the 1 percent starch solution and 1 ml acetate buffer at pH of 5.2 were added to 0.5 ml of enzyme extract and the reaction mixture was incubated at 30°C for 2 hours. The reaction was stopped by adding 1.0 ml of 1 N HCl and the amount of starch left unhydrolysed was determined by adding 3 ml of KI solution. The resultant solution was determined at OD 520 nm. The enzyme activity was expressed as mg glucose released per unit gram.
fresh weight of tissue and was calculated from the standard curve prepared by using different glucose concentrations.

\textit{b-amylase analysis}

The \textit{b-amylase} was estimated according to the method of Bernfield [4] with slight modifications given as below:

(i) Extraction of the enzyme – 1 g of acetone defatted grains was homogenized in 66 mM phosphate buffer (pH of 7.0) containing 0.5 M NaCl. The extract was centrifuged at 20,000 g for 15 minutes. The residue re-extracted with 3 ml phosphate buffer and supernatant was pooled together and kept in refrigerator at 4°C for estimation. All operations were carried out at 4°C.

(ii) Estimation of the enzyme - 1 ml of starch solution mixed with 1 ml of properly diluted enzyme extract was incubated at 27°C for 15 minutes. After 15 minutes 2 ml of dinitro salicylic acid was added to stop the reaction. The solution was then heated in boiling water bath for 5 minutes and allowed to cool. When the tubes were warm, 1 ml of potassium sodium tartrate solution was added. The tubes were cooled in running tap water and the volume was made upto 10 ml by addition of 6 ml water. The OD of blue coloured solution was measured with spectrophotometer at 560 nm. The enzyme activity was expressed as mg of maltose produced during 5 minutes incubation with 1 percent starch and was calculated from the standard curve prepared by using different maltose (0-100 mg) concentrations.

\textbf{Results and discussion}

The salient points emerging through the use of salicylhydroxamic acid were that (i) both bold and small grains showed a decrease in relative levels of ascorbic acid oxidase, \textit{a-amylase} and \textit{L98}\textit{\textsuperscript{a}}-\textit{amylase} from about 14\textsuperscript{th} DAA stage (Figures 1, 2 and 3) and (ii) in spite of the aforementioned decline, they continued to exhibit the disparity between them and at maturity the smaller grains still showed higher ascorbic acid oxidase, \textit{a-} and \textit{b-} amylases than the bolder grains (Figure 4).

The representation of the data in Figure 1 indicates the level of ascorbic acid oxidase as influenced by salicylhydroxamic acid in two different types of grains. As apparent its levels increased in first 14 days post-anthesis stages followed by a gradual decrease which continued until maturity. The fall was to the tune of 36.6 percent at 21\textsuperscript{st} DAA which further fell by another 13.8 percent at 28\textsuperscript{th} DAA followed by another decline of 28.0 percent at maturity, thereby showing an overall declension to the tune of 78.4 percent in bold grains. A more or less similar pattern was apparent in smaller grains, which despite the fact being endowed with higher levels of ascorbic acid oxidase showed a declension right from 14\textsuperscript{th} DAA onwards, reaching to a minimum level at maturity. The distribution of the enzyme was unequivocally higher in smaller grains than bolder grains at all the stages of investigations e.g., at 7\textsuperscript{th} DAA its level was higher by 106.9 percent and by 84.3, 63.3 and 50.0 percents more at 14\textsuperscript{th}, 21\textsuperscript{st} and 28\textsuperscript{th} DAA stages respectively with a final figure of 40.5 percent more in smaller grains at harvest (Figure 4).

The data on the levels of amylases reveal that as the grains progressed towards maturity the levels of these hydrolytic enzymes increased correspondingly (Figures 2 and 3). A comparative look into the levels of \textit{a-amylase} and its distribution in bold and small grains, disclosed that smaller grains were endowed with its relatively higher levels at all stages of investigations. The analysis of data revealed that the higher quantum of distribution in smaller grains was maximum at 7\textsuperscript{th} DAA (108.0 percent more than bolder grains) and subsequent to that the differences were to the tune of 20.0, 11.3 and 36.4 percents more in smaller grains at 14\textsuperscript{th}, 21\textsuperscript{st} and 28\textsuperscript{th} DAA (Figure 4). At maturity smaller grains possessed relatively more (80.0 percent) of \textit{a-amylase} than their counterpart bolder grains. With regard to the levels of \textit{b-amylase} a similar pattern as that of \textit{a-amylase} was recordable. As evident it showed a significant disparity with respect to its distribution in the two types of grains. The disparity was sustainable throughout the ontogeny of grains’ development with a maximum gap at 7 days after anthesis (114.3 percent higher in smaller grains) and the values were 35.7, 10.7, 23.7 and 68.9 percents more at 14\textsuperscript{th}, 21\textsuperscript{st} and 28\textsuperscript{th} DAA and at maturity respectively (Figure 4).

\textbf{Discussion:}

We have investigated the relative levels of ascorbic acid oxidase, \textit{a-} and \textit{b-} amylases as affected by salicylhydroxamic acid in bold and small grains growing in the same spikelet of wheat. 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 [6,19,28,30]. Nevertheless, the sequence of events, piloting the yielding ability, is the metabolic profile and if augmented through the use of plant growth regulators [29,18] or by imposing a shift in metabolic events [7] promotory effects are achievable [15,20]. 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)
Fig. 1: Relative levels of ascorbic acid oxidase (µ mol oxygen 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 decrease (-) in level of ascorbic acid oxidase over control.

Fig. 2: Relative levels of α-amylases (µg glucose g⁻¹ fresh weight 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 α-amylases over control.

Fig. 3: Relative levels of β-amylases (µg maltose g⁻¹ 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 β-amylases over control.
plausibly switches-on during the grain filling stage and if checked, through the immaculate use of salicylhydroxamic acid, can decrease the relative levels of ascorbic acid oxidase, a- and b- amylases 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.

References


