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ORIGINAL ARTICLE

Grain Development of Wheat in Relation to Levels of Catalase, Peroxidase and Ascorbic Acid Peroxidase

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ABSTRACT

Relative levels of catalase, peroxidase and ascorbic acid peroxidase were studied at different grain types (bold and small) within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*). The plants were grown in a screen covered hall under otherwise natural conditions. 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 data uncover that as the grains progressed towards maturity, the levels of catalase and peroxidase increased in both the types of grains while, the levels of ascorbic acid peroxidase decreased. A further look into the levels of aforementioned enzymes, with regard to their distribution in bold and small grains, disclosed that smaller grains possessed a relatively higher levels of catalase, peroxidase and ascorbic acid peroxidase at all the stages of investigations. The disparity was more at 7th DAA as compared to other stages of grain development.

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

Introduction

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 [2,15,10,20]. It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks [21,16,18,7]. Various sugar responsive genes in plants 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 [13]. 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.* [1], 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 hydrolytic class namely, catalase, peroxidase and ascorbic acid peroxidase in different grains growing in the same spikelet and to assess whether they are

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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 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.

Extraction of Enzymes:

The activities of catalase, peroxidase and ascorbic acid peroxidase enzymes in grain samples were estimated from the same enzyme extract obtained as follows:

1 g of fresh plant tissue was homogenized with 3ml of 0.1 M phosphate buffer pH of 7.0 in a chilled pestle and mortar. The homogenate was centrifuged at 18,000 g at 5°C for 15 minutes. The homogenate was used for determination of enzyme activity. The whole extraction procedure was carried out at 4°C.

Estimation of Catalase:

The catalase activity of the crude extract was assayed by the titrimetric method of Chance and Maehly [3]. The extract and H_2O_2 in 0.1 M phosphate buffer (pH of 7.0) was incubated at 30°C for 1 minute. The reaction was stopped by adding an excess of 5 percent H_2SO_4, and the residual H_2O_2 was titrated with 0.05 N $KMnO_4$ solution. The catalase activity was expressed as micromole oxygen released per gram fresh weight of sample.

Estimation of Peroxidase:

The incubation mixture for peroxidase assay consisted of pyrogallol, 0.03 ml hydrogen peroxidase solution and 0.1 ml enzyme extract in 0.1 M phosphate buffer (pH of 6.0) maintained at 30^{o}C for 3 to 5 minutes [17]. 2N H_2SO_4 was added to stop the reaction and extracted the purporgallin that formed with 1-butanol. The spectrophotometer was set on 420 nm and the concentration of purpurogallin in 1-butanol was measured colorimetrically. The peroxidase content was calculated from a standard curve prepared with purpurogallin and expressed as micromol purpurogallin per gram fresh weight of tissue.

Estimation of Ascorbic Acid Peroxidase:

For estimation of this enzyme, 0.1 ml of above mentioned aliquot, 2.6 ml of 50 mM phosphate buffer (pH of 7.0), 0.1 ml of 0.5 mM ascorbic acid and 0.1 ml of 0.1 mM ethylene dinamine tetra acetic acid (EDTA) were added into reference cuvette of a spectrophotometer. Just before reading the absorbance OD at 290 nm in spectrophotometer, 0.1 ml of 0.1 mM hydrogen peroxide was added to the cuvette. The absorbance change was measured at 290 in 30 sec intervals time for 5 minutes. Ascorbic Acid peroxidase activity was determined by procedure of Chang and Kao [4] and it was expressed as micromol per gram fresh weight of test 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 hydrolytic class namely, catalase, peroxidase and ascorbic acid peroxidase were studied in them at different stages of growth and development. The salient features of results are given below.

Catalase:

The data uncover that as the grains progressed towards maturity, the levels of catalase increased in both the types of grains (Figure 1). The pattern showed that there was an increase in the level of catalase in the bolder grains to the tune of 241.7, 15.8 and 85.3 percents from 7th to 14th, 14th to 21st and 21st to 28th DAA respectively, whereas from 28th DAA to maturity the increase was to the tune of 37.5 percent. Similarly, the increase in small grains was 166.7, 12.5 and 58.8 percents at the aforementioned stages with a final increase by 17.7 percent at maturity from 28th DAA. A further look into the levels of catalase, with regard to their distribution in bold and small grains, disclosed that smaller grains possessed a relatively higher levels of catalase at all

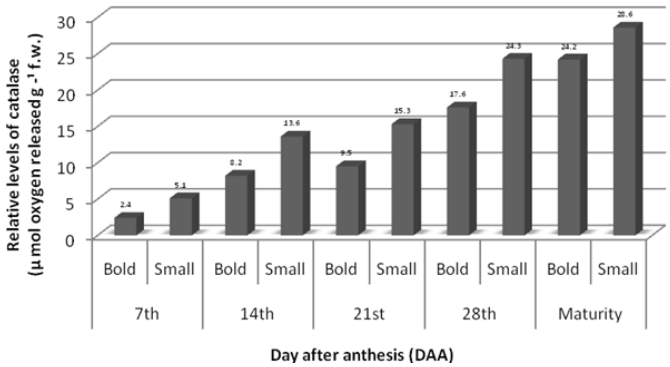

Fig. 1: Relative levels of catalase (µ mol oxygen released g^{-1} fresh weight) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*).

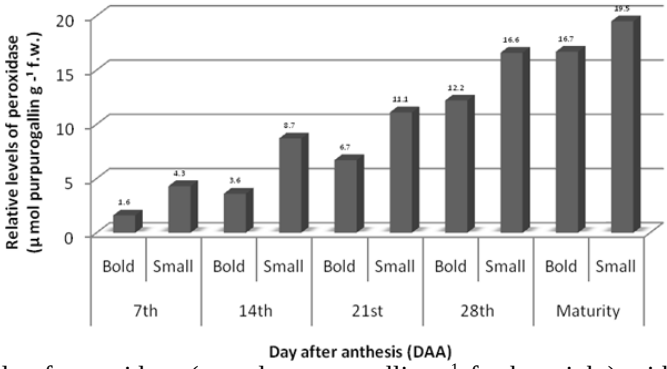

Fig. 2: Relative levels of peroxidase (µ mol purpurogallin g^{-1} fresh weight) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*).

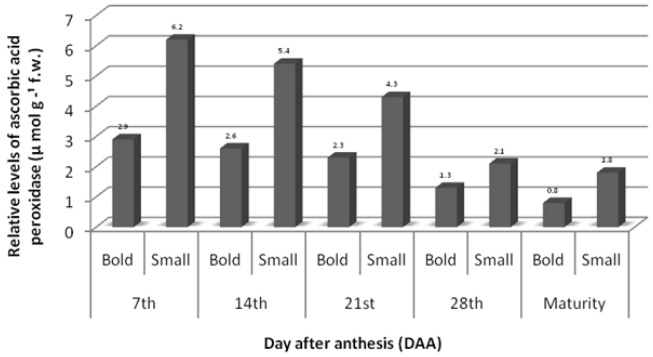

Fig. 3: Relative levels of ascorbic acid peroxidase (µ mol g^{-1} fresh weight) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*).

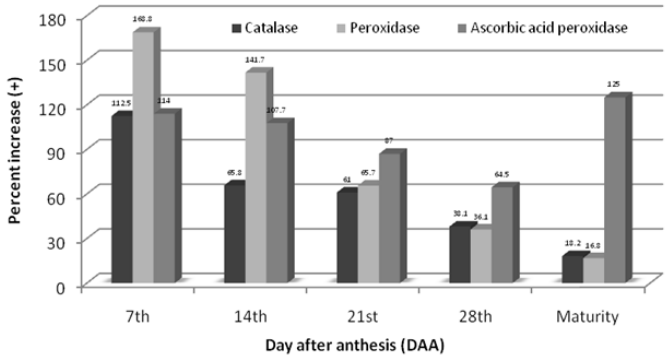

Fig. 4: Percentage increase (+) in relative levels of catalase, peroxidase and ascorbic acid peroxidase in small grains over their counterparts bold grains

stages of investigations. The analysis of data revealed that the higher quantum of distribution in small grains was maximum at 7th DAA (112.5 percent higher) and subsequent to that the difference was to the tune of 65.8, 61.0 and 38.1 percents more in small grains at 14th, 21st and 28th DAA stages. At harvest the smaller grains possessed relatively more (18.2 percent) of catalase than their co-developer bolder grains (Figure 4).

Peroxidase:

The scrutiny of the data of second hydrolytic enzyme (peroxidase) also offered some interesting lineaments (Figure 2). As apparent from the data the levels of peroxidase activity was comparatively higher in smaller grains than bolder grains at different stages of grain development. This disparity was more at 7th DAA as compared to other stages of grain development (168.8 percent). Smaller grains exhibited 141.7, 65.7, 36.1 and 16.8 percents higher peroxidase activity than bolder grains at 14th, 21st and 28th DAA and at maturity respectively. The net increase in enzyme activity from 7-day stage to maturity was about 10 and 4.5 times for bolder and smaller grains respectively (Figure 4).

Ascorbic Acid Peroxidase:

Studies on the behaviour of grains with regard to levels of ascorbic acid peroxidase unfolded certain interesting revelations. As apparent from data in Figures 1 and 4, the levels of ascorbic acid peroxidase were comparatively higher in smaller grains than bolder types at all the stages of grain development. Enzyme activity in both the types of grains was highest at 7th DAA which narrowed down towards maturity.

Smaller grains registered 114.0, 107.7, 87.0, 61.5 and 125.0 percents higher ascorbic acid peroxidase activity as compared to bolder grains at 7th, 14th, 21st and 28th DAA and at maturity respectively. A look into the data revealed that the rate of deduction in enzyme activity in initial days after anthesis was more pronounced in smaller grains as compared to bold grains. However, an exception was at the time of maturity where there was more reduction in ascorbic acid peroxidase in bolder than smaller grains (38.5 and 14.3 percents in bold and small grains respectively).

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