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ORIGINAL ARTICLE

Relative Levels of Protein and Free Amino Acids Within Developing Grains of Wheat

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ABSTRACT

Relative levels of protein and free amino acids were studied at different grain locations 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. To summarize the data, it is apparent that the grains differing in their growth potential also differed in the endogenous levels of their proteins as well as total free amino acids. While the levels of proteins were significantly higher in bolder grains than smaller grains, the reverse was true with regard to the levels of free amino acids. A unique revelation here was that the diminutions in the levels of proteins as well as total free amino acids were more or less of the same order and there were no significant differences in the behavior of bold or small grains in losing the quantum of their proteins or free amino acids over a period of time.

Key words: Spike; spikelet; bold grain; small grain; *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 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 [2,18,14,20,7]. It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks [21,16,17,5]. Various sugar responsive genes in plants potentially affect the partitioning and have been stressed to be key determinant of plant productivity [6]. 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 [3,4]. A few biochemical components as advocated by Abrol *et al.* [1], Hakaka [8] and Hasan and Kamal [9], 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 protein and free amino acids in different grains growing in the same spikelet and to

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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 [11] 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.

Protein analysis:

The total proteins content were determined by method described by Lowry *et al.* [13].

(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 regents were used for estimation of total proteins:

Solution A: 2 percent Na_2CO_3 in 0.1 N NaOH; Solution B: 0.5 percent $CuSO_4.5H_2O$ 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 regent (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 [12] 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.

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 protein and free amino acids were studied in them at different stages of growth and development. The salient features of results are given below.

The dynamics in the levels of proteins are summed up in the Figure 1. The data reveal that the total proteins decreased in their absolute levels as the grains headed towards ripening except in the second week stage where there was a significant increase in their levels in all grains, irrespective of their precipitation potential. The enhancement was to the tune of 43.4, 43.7 and 46.7 percents in bold grains in proximal, middle and distal segments of spike respectively, while the increment in smaller grains was 46.8, 40.6 and 50.0 percents at the same positions. Subsequent to this stage i.e., third week onwards there was a gradual decrease in their levels ultimately settling at a level which was approximately half than its values from the first stage of sampling (7th DAA).

A relative look in the levels of proteins revealed that bolder grains significantly possessed a higher level of proteins as compared to its next neighbor i.e., smaller grains. The disparities were in the range of 10.9 percent to 36.4 percent and were more prominent in the third and fourth weeks after anthesis as well as at maturity (Figure 3). The disparities

were uniformly detectable amongst grains from proximal, middle and distal spikelets.

A simultaneous look into the Figure 2 shows the levels of free amino acids in the two types of grains. Their quantities on per unit basis showed that the bold grains possessed relatively lesser free amino acids as compared to smaller grains at all the stages of grains' development and hence this behavior by and large, reflected a similar pattern as that of total proteins. Further like proteins their levels also increased in first two weeks after ripening followed by a gradual decline irrespective of their positions in the spike or spikelet. However, a unique contrast was apparent when their relative distribution and levels were analyzed in bolder and smaller grains. Herein smaller grains, at any juncture, relatively possessed significantly higher levels of free amino acids than the bolder grains. The differences were almost in the same range of 16 to 28 percents approximately (more in smaller grains than bolder grains) and were independent of the positions of grains whether they were growing in proximal, middle or distal spikelets (Figure 3).

To summarize the data of the present study, it is apparent that the grains differing in their growth potential also differed in the endogenous levels of their proteins as well as total free amino acids. While the levels of proteins were significantly higher in bolder grains than smaller grains, the reverse was true with regard to the levels of free amino acids. A unique revelation here was that the diminutions in the levels of proteins as well as total free amino acids were more or less of the same order and there were no significant differences in the behavior of bold or small grains in losing the quantum of their proteins or free amino acids over a period of time. In addition to the data on proteins, a higher quantity of free amino acids in smaller grains could possibly be linked to a higher rate of hydrolysis of proteins (owing to higher hydrolytic enzymes) and/or the inability of the sinks to utilize these free amino acids. These findings also deduce the support from the contributions of Tribol *et al.* [19] and Panozzo *et al.* [15].

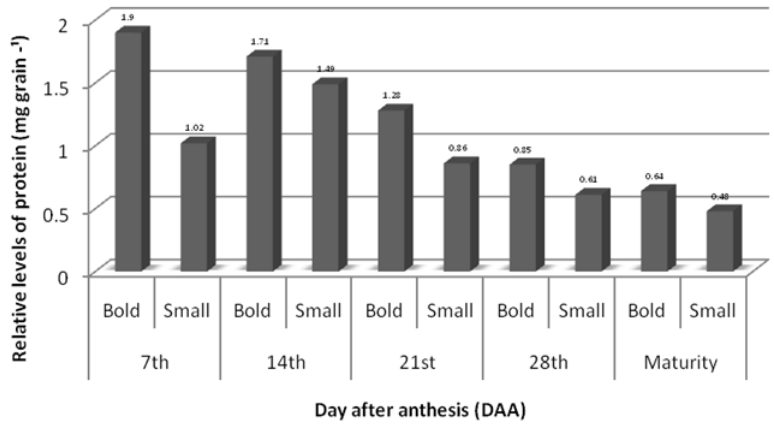

Fig. 1: Relative levels of protein (mg grain^{-1}) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*)

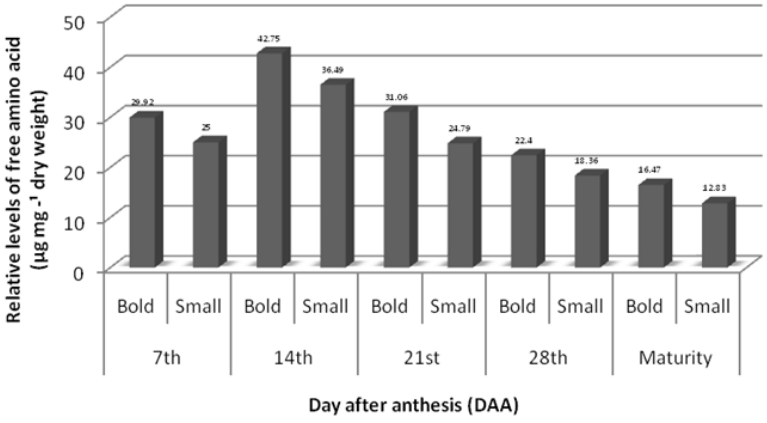

Fig. 2: Relative levels of free amino acids (µg mg^{-1} DW) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*)

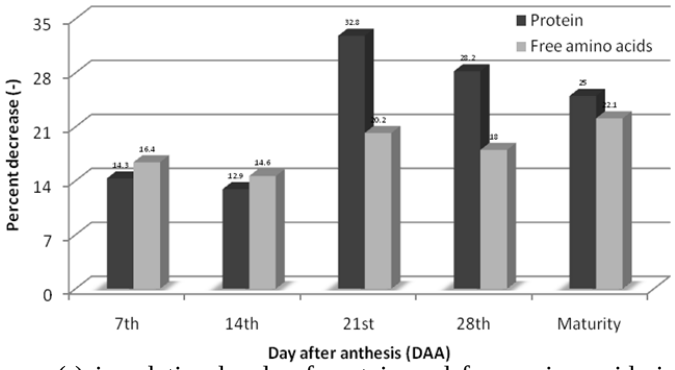

Fig. 3: Percentage decrease (-) in relative levels of protein and free amino acids in small grains over their counterparts bold grains

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