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ORIGINAL ARTICLE

An Appraisal of Relative Levels of Starch, Reducing Sugars and Non-reducing Sugars Within Developing Grains of Wheat

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ABSTRACT

Relative levels of starch, total sugars, reducing sugars and non-reducing sugars were studied at different grain locations within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*). The labeled spikes were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28th DAA, and at maturity. All the grains, irrespective of their locations in an ear, revealed a positive correlation between their ages and the levels of starch. The disparity between the bold and small grains in the levels of starch was maximum at 7th DAA. The total sugars increased upto third week after anthesis in small grains in the proximal, middle or distal spikelets, while its values enhanced upto fourth week in the bolder grains in the same segments. The gap amongst the bold and small grains tended to taper with the grains' progression to maturity. When total sugars were studied as two separate components i.e., reducing and non-reducing sugars, it was deciphered that the absolute levels of reducing sugars were significantly more than the non-reducing sugars in all the grains irrespective of their locations in the spike or spikelet. The data sum up that bolder grains contained relatively higher levels of starch with concomitant lower levels of total, reducing and non-reducing sugars as compared to smaller grains which offered a contrasting picture with lower levels of starch and a higher level of total, reducing and non-reducing sugars.

Key words: Carbohydrates; spike; spikelet; *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 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 [2,7,11,22,31,32]. It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks [8,25,27,33]. Various sugar responsive genes in plants potentially affect the partitioning [9] and have been stressed to be key determinant of plant productivity [10]. Dry matter partitioning also plays a paramount role in growth rate of sink organs [17].

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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 [3,4,6]. A few biochemical components as advocated by Abrol *et al.* [1], Hakaka [15] and Hasan and Kamal [16], 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 starch, total sugars, reducing sugars and non-reducing sugars in different grains growing in the same spikelet and to 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 [18] was supplied to the pots. The plants were grown in a screen covered hall under otherwise natural conditions. 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.

Starch Analysis:

Starch contents were estimated by the method described by Hodge and Hofreiter [14]. The brief procedure is as follows:

(i) Extraction of Starch - One gram of powdered dry sample of grains was transferred to 100 ml of volumetric flask. The material was hydrated and gelatinised with 30 ml of distilled water by keeping the flask over boiling water bath for 30 minutes. The flasks were well stoppered so as to prevent any loss of water by evaporation. The flasks were cooled under running cold water for 10 minutes and 60 ml of 60 percent perchloric acid was added slowly with thorough agitation so as to avoid any momentary high concentration of acid. The mixture was allowed to stand, with occasional stirring, for 15 minutes and the volume were made up with distilled water. After shaking, the contents were allowed to settle. The supernatant was used for starch estimation.

(ii) Estimation of Starch - 5 ml of the above supernatant solution was pipetted out into 100 ml volumetric flask. To this, 6 ml of cold distilled water was added. The solution was made incipient alkaline with a few drops of 2N NaOH with the use of phenolphthalein as an indicator. The solution was made just acidic with a drop of 2N acetic acid till the pink colour disappears and to this, 2.5 of 2N acetic acid, 0.5 ml of 10 percent potassium iodide and 5 ml of 0.01 N potassium iodate were added for the colour to develop. The volume was raised to 100 ml and intensity of the colour was measured at 650 nm in a Bausch and Lomb Spectronic 20 using red filter. A single blank containing all the reagents was used to adjust the absorbance at zero. The unknown quantity was estimated from the standard curve prepared with tomato starch and results expressed as mg per grain.

Total Sugars Analysis:

Total sugars were estimated by the method of Dubois *et al.* [5] with some modifications as follows:

(i) Extraction of total sugars - Fresh grains weighing 1 g at different intervals of time after anthesis were cut into small cubes of 1 sq. mm and taken into a vial containing 5 ml of 80 percent ethanol and stored overnight. The samples were extracted with boiling ethanol the next day. The supernatant was decanted into 50 ml beaker. The residue was repeatedly extracted 4 times with 80 percent boiling ethanol. The volume of the combined supernatant was made upto 25 ml with 80 percent ethanol and then centrifuged at 6000 g for 20 minutes. The supernatant so obtained was used for the estimation of total sugars.

(ii) Estimation of total sugars - Total sugars were estimated using the reagents included phenol 5 percent and concentrated H_2SO_4. A suitable amount of the supernatant (0.2 – 0.5 ml) was taken in a test tube and to this 1 ml of phenol reagent and 5 ml of concentrated H_2SO_4 were added and mixed thoroughly. The absorbance was recorded after 20 minutes of colour development at 490 nm. The total sugars content were calculated from a calibration curve prepared with glucose and expressed as mg per grain.

Reducing and non-reducing sugars analysis

(i) Clarification of sugar extracts - A representative sample of the extract obtained as above was evaporated on a water bath taking care not to let the liquid dry out completely. Subsequently, the concentrated extract was heated with 1 ml saturated lead acetate to precipitate the colloidal substances. The mixture was then filtered

(Whatman No. 54) into a beaker containing 1.5 ml of saturated di-sodium hydrogen phosphate (Na_2HPO_4) to precipitate the excess lead as $Pb_3(PO_4)_2$. The contents of the beaker were filtered into a 25 ml volumetric flask and volume was made upto 25 ml with distilled water. An aliquot of this solution was used for determination of reducing sugars.

(ii) Estimation of Reducing Sugars - Reducing sugar was quantified following the modified Somogyi's method [29]. The required reagents were prepared as follows:

1. Somogyi's reagent: (a) prepared by dissolving 24 g of sodium carbonate (Na_2CO_3) and 12 g of Na-K-tartrate in 250 ml of distilled water. 40 ml of 10% solution of $CuSO_4\ 5H_2O$ was then added to it with stirring followed by the addition of 16 g of sodium bicarbonate (Na_2HCO_3). (b) 180 g of sodium sulphate (Na_2SO_4) was dissolved in 500 ml of distilled water, boiled for few minutes to expel air and cooled to room temperature. The two solutions (a) and (b) were mixed thoroughly and the final volume was made upto 1 liter with distilled water.
2. Arseno molybdate reagent: It was prepared by dissolving 25 g of ammonium molybdate in 450 ml of distilled water followed by the addition of 21 ml of concentrated H_2SO_4. Subsequently, 3 g of sodium arsenate ($Na_2HASO_4\ 7H_2O$) dissolved in 25 ml of distilled water was added to the above solution.

An aliquot of 2 ml was mixed with 1 ml of Somogyi's reagent in 30 ml vials. It was heated in a boiling water bath for about 12 minutes followed by its cooling under running water. 1 ml of arseno molybdate reagent was added and the final volume was made upto 10 ml with water. The absorbance was read at 630 nm against a blank prepared with distilled water and reagents. The amount of reducing sugar was calculated from a calibration curve prepared with glucose and the results were expressed in terms of glucose equivalent mg per grain. Non-reducing sugars were calculated by subtracting the amount of reducing sugars from total sugar contents in the two types of grains.

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 starch, total sugars, reducing sugars and non-reducing sugars were studied in them at different stages of growth and development. The salient features of results are given below.

All the grains, irrespective of their size or positions in an ear, revealed a positive correlation between their ages and the levels of starch (Table 1). The first five spikelets, constituting as proximal, the next ten and the last five spikelets as middle and distal segments respectively had their own characteristic variations with a common generalization that the bolder grains possessed a higher levels of starch than the smaller grains at all the stages of grain development. The disparity between the bold and small grains in the levels of starch was maximum at 7^{th} DAA in all the three segments (44.8, 30.0 and 55.6 percents lesser in smaller grains than bolder grains in proximal, middle and distal segments respectively) and further the disparity tended to taper with maturity (Figure 1). Interestingly, during the initial phases of grain growth, there were significant disparities within the smaller grains amongst themselves whether growing at proximal, middle or distal segments, while on the other hand, bolder grains reflected insignificant differences amongst them. As is well known, through the innumerable reports and findings, that starch is a major constituent of wheat grains and also an important factor determining its grain yield [26, 30]. A casual browse into the studies of the levels of this polysaccharide shows that any increase in the dry matter in grains occurred due to a proportionate increase in the levels of starch [28]. Concurrently, a lower dry matter deposition in smaller grains could be directly linked, rationally either with a higher level of starch hydrolyzing enzymes (a and b-amylases), which have also been linked to grains compactness or lower relative levels of enzymes involved in starch synthesis (sucrose synthase, ADP-glucose pyrophosphorylase) [12,19,20,23].

Total sugars along with its two components namely reducing and non-reducing sugars were studied and its data are presented in Table 1. The total sugars increased upto third week after anthesis in small grains in the proximal, middle or distal spikelets, while its values enhanced upto fourth week in the bolder grains in the same segments. Their values were significantly lower in the bolder grains as compared to smaller grains in all the segments by a margin ranging from 8.1 to 46.9 percents. These disparities were maximum around three weeks after anthesis and this generalization was true for all the three segments under (45.6, 46.9 and 46.3 percents higher in smaller than bolder grains in proximal, middle and distal segments respectively). Interestingly the gap amongst the bold and small grains tended to taper with the grains' progression to maturity (Figure 1).

Table 1: Relative levels of starch, total sugars, reducing sugars and non-reducing sugars (mg grain^{-1}) in bold and small grains in (*Triticum aestivum* var. *PBW-343*) wheat

Day after anthesis (DAA)	Grain type	Starch	Total sugars	Reducing sugars	Non-reducing sugars
7th	Bold	1.9	3.42	1.80	1.62
	Small	1.3	4.65	1.88	2.77
14th	Bold	9.2	4.17	2.20	1.97
	Small	6.7	5.86	2.37	3.49
21st	Bold	17.7	4.58	2.58	2.00
	Small	14.1	6.73	3.28	3.45
28th	Bold	25.9	4.81	2.71	2.10
	Small	21.6	6.06	2.95	3.11
Maturity	Bold	27.6	4.56	2.60	1.96
	Small	23.1	4.93	2.28	2.65

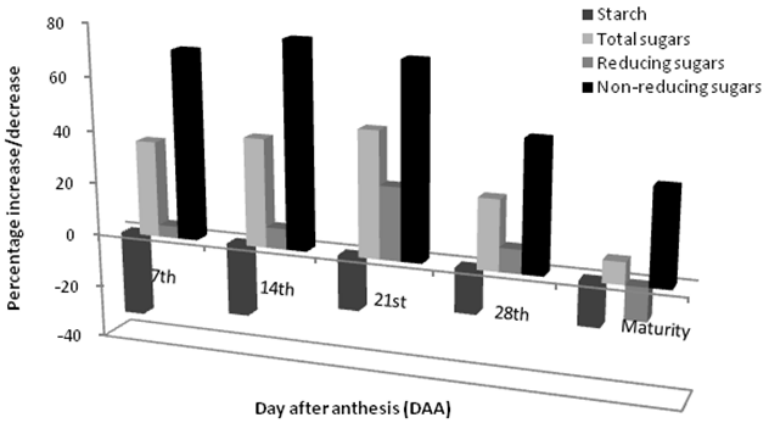

Fig. 1: Percentage increase (+) or decrease (-) in relative levels of starch, total sugars, reducing sugars and non-reducing sugars in small grains over their counterparts bold grains

When total sugars were studied as two separate components i.e., reducing and non-reducing sugars, it was deciphered that like total sugars both the components increased in the first three to four weeks after anthesis followed by a decline. The absolute levels of reducing sugars were significantly more than the non-reducing sugars in all the grains irrespective of their positions in the spike or spikelet. However, in smaller grains the levels of non-reducing sugars surpassed than the reducing sugars in the initial days after anthesis or at maturity. A critical conclusion was that reducing sugars constituted more than 50 percent component of the total sugars in the bolder grains while their counterparts (smaller grains) reflected the same story for non-reducing sugars. This derivation was applicable to all the three segments of the spike and at all the stages of grains' development.

Further a comparison in the levels of non-reducing sugars shows that relatively their lesser quantities were present in bolder grains as compared to smaller grains. The differences were more in the first three weeks as compared to the fourth week after anthesis or at maturity. As apparent from Figure 1 the smaller grains possessed 71.0, 77.2 and 71.0 percents more non-reducing sugars than the bolder grains in the first, second and third weeks after anthesis respectively. In the fourth week and at maturity the gap narrowed and it was to the tune of 40.5 and 32.2 percents respectively. By and large values in this range were also available in the proximal and distal segments.

Similarly, reducing sugars were also more in their quantity in smaller grains in comparison to bolder grains in the first four weeks after anthesis. Surprisingly the bolder grains possessed more reducing sugars in all the segments of the ear at maturity. Another striking observation was that reducing sugars did not offer huge imbalances as was observable in the case of non-reducing sugar. Here the observations of Jeng *et al.* [13], Liang *et al.* [21] and Paul and Foyer [24] gather the relevance who advocated that the relative levels of reducing, non-reducing sugars and sucrose along with starch in totality would steer the destiny of grains in terms of their final dry weights. The present findings, by going a step forward, reveal that the sugars in smaller grains were not limiting but what lacked in them was their inability to convert them into polysaccharides like starch.

The data sum up that bolder grains contained relatively higher levels of starch with concomitant lower levels of total, reducing and non-reducing sugars as compared to smaller grains which offered a contrasting picture with lower levels of starch and a higher level of total, reducing and non-reducing sugars.

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