Proc. Indian Acad. Sci. (Plant Sci.), Vol. 95, No. 2, September 1985, pp. 71–78.

Assimilate supply in relation to grain weight in wheat (*Triticum aestivum* L)

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MS received 15 December 1984; revised 23 May 1985

Abstract. The contents of various free sugars in large and small wheat grains were compared with those in normal grains at different stages of grain development. Their content paralleled grain weight only upto 14 days after anthesis (A + 14). On a dry weight basis, no positive correlations were observed between the concentrations of various free sugars and grain weight at any of the stages. Differences in grain weight were dependent mainly on the number of cells formed in the endosperm which in turn seemed to be regulated by the assimilate supply available to the grain during the first 14 DAA.

Keywords. *Triticum aestivum* L; free sugars; sucrose; starch; grain weight.

1. Introduction

Grain weight is an important component of yield in cereals including wheat (*Triticum aestivum* L), but the physiological and biochemical factors limiting grain size are still matters of conjecture. It is believed that either the source *via* the supply of precursors, or the sink by virtue of its capacity to accept assimilates and further converting them to starch are the main factors limiting starch accumulation and hence grain weight. However, the relative importance of source and sink in controlling grain weight in relation to yield is still controversial (Thorne 1974; Gieger 1976). Defoliation and shading (Asana *et al* 1969; Ford and Thorne 1975; Sofield *et al* 1977), altering grain number per ear (Bingham 1967; Bremner and Rawson 1978; Radley 1978) or a combination of both (Pinthus and Millet 1978; Jenner 1979, 1980) are the common techniques employed in studies designed to differentiate between the two. The primary assumption implied in defoliation is that it limits grain growth by reducing the supply of assimilates. Degraining, on the other hand, is assumed to cause large grains by increasing the supply of assimilates to the remaining grains. Because of the scarcity of information on the effects of these treatments on the levels of soluble carbohydrates in the grain, the validity of these assumptions could not be ascertained unequivocally. An attempt has, therefore, been made here to quantify various sugars in larger and smaller grains resulting from degraining and defoliation, and to compare the results with those obtained from normal grains in order to test the validity of the assumption that assimilate supply may limit starch accumulation and so control grain weight.

2. Materials and methods

Wheat plants (cv WH-157) were raised under field conditions, following recommended agronomic practices, in three replications (Kumar and Singh 1980, 1981). In all, there were nine plots; each treatment i.e., degraining, defoliation and control, being represented in three. At anthesis, ears from 150 plants from each replication were trimmed to eight central spikelets (degraining); the flag and penultimate leaves were removed from another 150 plants (defoliation). From each replication, duplicate samples were taken and individually analysed. Hence, each value included in the tables is a mean of six independent estimations.

The grain samples, harvested at weekly intervals from A + 7 until A + 42 were dried in an oven at 105 °C for 4 hr and then at 80 °C to constant dry weight. Only the central grains from each spikelet were used for further analysis. Soluble sugars were extracted by boiling the powdered sample in 80 % (v/v) ethanol (Cerning and Guilbot 1973). The method of Yemm and Willis (1954) was employed for the determination of total soluble sugars. Reducing sugars were estimated by Somogyi's modified method (Nelson 1944; Somogyi 1945). The content of nonreducing sugars was obtained as the difference between total and reducing sugars. Sucrose was estimated as reducing sugars after hydrolysis with invertase (Johnson *et al* 1964). Starch from the sugar-free pellet was estimated by the method of Clegg (1956). The number of endosperm cells was counted following the method of Rijven and Wardlaw (1966). Statistical analysis was performed by the methods described by Snedecor (1956).

3. Results and discussion

For the sake of convenience, heavier and lighter grains resulting from degraining and defoliation have respectively been referred to as H and L.

Dry weight increased continuously throughout grain development (table 1). It was greatest in H and least in L with control grains having intermediate values, indicating

Table 1. Dry weight of developing wheat grains of variety WH-157 under defoliation (L) degraining (H) and normal development (C)[a]

Days after anthesis	Dry weight (mg/grain) L	C	H
7	2·7 ±0·08	3·3 ±0·08	4·3 ±0·08
14	9·7 ±0·35	11·0 ±0·35	12·2 ±0·40
21	18·9 ±0·32	20·6 ±0·36	25·4 ±0·34
28	26·3 ±0·69	33·0 ±0·69	40·2 ±0·82
35	42·0 ±0·84	46·3 ±0·78	52·9 ±0·71
42	42·8 ±1·12	50·1 ±1·07	60·1 ±1·24

[a] values are mean ± S.E. from six independent estimations.

that both the rate of dry matter accumulation and final grain size were significantly ($P < 0.01$) altered by defoliation and degraining. At maturity, H grains had 20 % more and L 14 % less dry matter than the control.

Starch content grain^{-1} increased progressively throughout development (table 2); rapid starch synthesis commencing at 14 days after anthesis. Its amount was lowest in L and highest in H at all stages of development. The rate of starch accumulation during the linear phase of grain filling (days 14 to 35) for the three treatments, L, control and H respectively was 1·39, 1·55 and 1·79 mg grain^{-1} day^{-1}. However, in the last interval (days 35 to 42), the mean daily rate of starch accumulation was 0·1, 0·46 and 0·88 mg day^{-1} in three types, indicating that the treatments appeared to have affected the duration of grain filling. The responses also indicated the existence of a mechanism which could influence the capacity of endosperm to accumulate starch. Responses to degraining showed that grains had the capacity to grow longer than normal in intact ears, exhibiting the presence of unused growth potential. Bremner and Rawson (1978) also proposed the concept of unused growth potential in developing cereal grains.

Sucrose content grain^{-1} increased sharply at day 14 after anthesis, declining thereafter upto day 28 and then remaining almost constant till the end (table 3). Except at the 35 day stage, its content grain^{-1} was higher in H than in control. Control grains likewise had a higher sucrose content grain^{-1} than L apart from the 21-day stage. However, a significant positive correlation ($P<0.01$) between sucrose content and grain weight was observed only at 14 DAA. Sucrose per unit dry weight decreased progressively with maturity and at no stage after day 14 was there a significant positive correlation between sucrose concentration and grain weight.

Total soluble sugars content grain^{-1} was significantly higher in H than in control and L grains until the 14-day stage (table 4). After 14 days also, although the pattern was similar the differences were non-significant. Reducing sugars grain^{-1} followed a developmental pattern similar to that obtained for sucrose and total soluble sugars

Table 2. Starch content in developing wheat grains of variety WH-157 under defoliation (L) degraining (H) and normal development (C)[a]

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	0·47 ±0·01	0·60 ±0·02	0·83 ±0·02	172·1 ±3·67	182·2 ±5·06	191·3 ±4·56
14	3·11 ±0·09	3·64 ±0·11	4·12 ±0·12	321·6 ±9·55	332·5 ±10·26	337·1 ±9·91
21	12·10 ±0·17	13·40 ±0·20	17·00 ±0·47	641·3 ±9·05	652·0 ±10·06	669·8 ±18·56
28	19·66 ±0·27	24·80 ±0·32	30·37 ±0·63	748·1 ±10·66	751·6 ±9·56	754·9 ±15·81
35	32·34 ±0·80	36·17 ±0·74	41·66 ±0·81	770·1 ±19·05	781·3 ±15·95	788·1 ±15·30
42	32·97 ±0·47	39·36 ±0·78	47·86 ±1·24	770·3 ±10·90	785·1 ±15·61	797·2 ±20·61

[a] values are mean ±S.E. from six independent estimations.

Table 3. Sucrose content in developing wheat grains of variety WH-157 under defoliation (L), degraining (H) and normal development (C)[a]

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	0·60 ±0·01	0·76 ±0·02	1·04 ±0·01	219·6 ±2·10	232·6 ±4·15	241·6 ±2·31
14	1·25 ±0·01	1·37 ±0·04	1·50 ±0·03	129·2 ±1·12	125·4 ±3·07	122·8 ±2·82
21	1·03 ±0·03	0·96 ±0·07	1·24 ±0·06	54·5 ±1·53	46·5 ±3·10	48·9 ±2·35
28	0·58 ±0·03	0·75 ±0·03	0·83 ±0·03	22·2 ±1·15	22·8 ±0·87	20·6 ±0·90
35	0·69 ±0·05	0·76 ±0·05	0·62 ±0·05	16·5 ±1·06	16·5 ±0·95	11·8 ±0·97
42	0·68 ±0·07	0·80 ±0·08	0·94 ±0·09	15·9 ±1·61	15·9 ±1·96	15·7 ±1·66

[a] values are mean ±S.E. from six independent estimations.

Table 4. Total soluble sugars content in developing wheat grains of variety WH-157 under defoliation (L) degraining (H) and normal development (C)[a]

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	1·27 ±0·02	1·59 ±0·05	2·22 ±0·06	461·6 ±11·85	484·0 ±15·75	514·0 ±14·7
14	2·80 ±0·09	3·68 ±0·12	4·23 ±0·12	288·8 ±9·15	336·1 ±10·80	346·3 ±9·25
21	2·44 ±0·09	2·45 ±0·14	3·14 ±0·17	129·1 ±4·55	118·9 ±6·85	123·6 ±6·95
28	2·01 ±0·12	2·23 ±0·15	2·45 ±0·15	76·4 ±4·56	67·7 ±4·45	60·8 ±3·70
35	1·89 ±0·12	2·19 ±0·21	2·35 ±0·21	45·0 ±3·00	47·4 ±4·55	44·4 ±4·11
42	1·47 ±0·10	1·54 ±0·13	1·81 ±0·11	34·3 ±2·40	30·7 ±2·55	30·2 ±1·82

[a] values are mean ±S.E. from six independent estimations.

(table 5). Though at most of the stages, H grains had the greatest control intermediate and L the least quantities of non-reducing sugars grain^{-1} (table 6), the differences again were significant ($P < 0{\cdot}01$) only upto the 14-day stage. On a dry weight basis, parallelism between non-reducing sugars and grain size was obtained only on day 7. However, the differences between the treatments were non-significant.

The results reported above indicate that though the levels of total, reducing and non-reducing sugars grain^{-1} were altered as a result of defoliation or degraining, the

Table 5. Reducing sugars content in developing wheat grains of variety WH-157 under defoliation (L), degraining (H) and normal development (C)[a]

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	0·22 ±0·01	0·28 ±0·00	0·43 ±0·01	79·3 ±3·61	87·3 ±0·60	100·5 ±1·40
14	0·52 ±0·04	0·71 ±0·04	0·94 ±0·01	53·3 ±3·85	65·0 ±3·35	77·2 ±0·31
21	0·53 ±0·02	0·58 ±0·02	0·70 ±0·02	28·3 ±0·80	28·3 ±0·95	27·8 ±0·82
28	0·41 ±0·01	0·51 ±0·01	0·65 ±0·02	15·8 ±0·17	15·4 ±0·36	16·2 ±0·40
35	0·37 ±0·01	0·40 ±0·01	0·50 ±0·02	8·8 ±0·26	8·6 ±0·21	9·5 ±0·36
42	0·35 ±0·01	0·39 ±0·02	0·46 ±0·02	8·1 ±0·21	7·7 ±0·36	7·7 ±0·32

[a] values are mean ±S.E. from six independent estimations.

Table 6. Nonreducing sugars content in developing wheat grains of variety WH-157, under defoliation (L), degraining (H) and normal development (C)[a]

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	1·05 ±0·03	1·30 ±0·03	1·79 ±0·03	328·3 ±10·70	396·7 ±9·12	413·5 ±6·66
14	2·29 ±0·08	2·97 ±0·05	3·25 ±0·06	236·5 ±9·00	271·1 ±5·02	269·1 ±4·60
21	1·90 ±0·06	1·86 ±0·04	2·43 ±0·08	100·8 ±3·30	90·6 ±1·90	85·8 ±3·30
28	1·59 ±0·07	1·73 ±0·10	1·80 ±0·12	60·6 ±2·46	52·3 ±3·05	44·7 ±2·90
35	1·52 ±0·10	1·79 ±0·11	1·84 ±0·15	36·2 ±2·42	38·8 ±2·40	34·9 ±2·80
42	1·12 ±0·08	1·15 ±0·05	1·34 ±0·09	26·2 ±1·90	23·0 ±1·16	22·4 ±1·50

[a] values are mean ±S.E. from six independent estimations.

differences between treatments were significant only upto the 14-day stage. Sucrose is the principal form in which carbohydrates are translocated to the grain via the phloem (Porter 1962). Its level, therefore, is a more reliable and stable indicator for predicting altered assimilate supply. The pattern of sucrose content in different grain types was similar to that shown by other sugars, indicating that defoliation cuts down the supply of assimilates available for distribution to grains and that the reduction in the rate of grain filling and final grain weight following defoliation is due to a reduction in the supply of assimilates. The responses to removing spikelets are also a pointer in the same direction.

Since the % water content of the three grain types did not differ much throughout development, expression of the results on a dry weight basis was considered to reflect the behaviour of apparent concentrations of sugars quite accurately. On dry weight basis, at none of the stages, a significant positive correlation was obtained between grain weight and any of the sugars. Jenner (1979) also found apparent concentrations of sucrose in the endosperm of wheat to remain unaffected by degraining. Such a relationship invalidates the assumption that changes in the rate of starch accumulation and final grain size are attributable only to the altered inflow of assimilates into the grain. It can thus be concluded that the accumulation of starch in developing wheat grains is not limited at the level of supply of precursors. Additional support comes from the data on a per grain basis which show that significant alterations in the levels of various free sugars were observed only upto the 14-day stage, whereas rapid starch synthesis commenced from 14 days onwards.

Endosperm cell number in wheat is known to be fixed by A + 14 and the further increase in grain size is by cell expansion (Jennings and Morton 1963). Estimates of endosperm cell numbers were therefore made at the 28-day stage. Table 7 shows a significant positive correlation ($P < 0{\cdot}01$) between endosperm cell number and final grain dry weight. However, it is obvious from the cell weight data (table 7) that variation in grain weight is not accounted for entirely by variation in endosperm cell number.

Deposition of dry matter in the endosperm of cereal seeds is generally viewed as the product of the number of cells in the endosperm and weight per cell. Of these two components, cell division dominates the first half of the period of grain growth while growth in the second half results from the deposition of dry matter in cells produced in the first half. It is therefore not surprising that a positive relationship has been obtained here between final grain dry weight and endosperm cell number. A similar relationship has been found to be true in varietal (Brocklehurst 1977) and positional differences of grain (Singh and Jenner 1982) and in factors such as degraining (Brocklehurst 1977; Radley 1978) and shading (Wardlaw 1970), supporting the idea put forward earlier by Bingham (1969) that the capacity of the endosperm to accumulate dry matter is determined by the number of cells formed in the endosperm. From a detailed study of culturing the detached wheat ears in solutions of sucrose and glutamine or exposing the intact plants to high or low photon irradiance, Singh and Jenner (1984) also concluded that the rate of cell division in the endosperm and thus the capacity of the endosperm to

Table 7. Endosperm cell number and average cell weight in wheat grains of variety WH-157 on day 28[a]

Grain type	Endosperm cell number	Average cell weight (mg cell^{-1})
L (defoliated plants)	133600 ± 2660	197×10^{-6}
C (normal plants)	146200 ± 3220	226×10^{-6}
H (degrained plants)	162400 ± 2740	246×10^{-6}

[a] values are mean ± S.E. of six independent estimations.

accumulate dry matter during its formative phase, are responsive to variation in the supply of organic nutrients from the rest of the plant. However, they could not decipher the nature of the mechanism controlling the above process.

Putting all the information together, it could be proposed that the cell number in the wheat endosperm is a major factor controlling final grain dry weights. This number seems to be regulated through the supply of assimilates to the grain during the cell division phase which continues in wheat upto 14 days after anthesis. The endosperm cell number changes to match the amount of available assimilates and in turn determine the final grain weight.

Acknowledgements

One of the authors (RK) gratefully acknowledges the financial assistance from CSIR, New Delhi in the form of a fellowship.

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