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PATTERN OF DRY MATTER AND PROTEIN ACCUMULATION IN DEVELOPING WHEAT SEEDS AND THEIR RELATIONSHIP

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ABSTRACT

The kinetics of protein and carbohydrate accumulation during seed development showed major differences between as well as within genotypes over the stages. Variation in protein content in a variety at different stages of seed development is recorded. The ratio between carbohydrate and protein biosynthesis was, in general, narrow during the last phase of seed development when protein accumulation mainly occurs. In contrast, the 18–24 days period after anthesis was favourable for carbohydrate biosynthesis. Correlation between carbohydrates and protein (mg/seed) was significantly positive and between carbohydrates and protein content negative. However, it has been shown that both carbohydrates and protein decrease or increase simultaneously though the magnitude differs. Variation for protein biosynthesis at different stages may be utilised for the development of more efficient system of protein biosynthesis.

Key words: Wheat, *Triticum aestivum*, protein, carbohydrates, developing seed.

Plant breeders have made efforts to improve the protein content in wheat varieties. However, not much success has been achieved due to various constraints, including low genetic variability in relation to protein content in mature seeds. Although some wheat varieties have high protein percentage, in most of them the higher protein level is not due to real increase in protein accumulation but due to reduction in carbohydrate content in the seed. As a result, when these genotypes were used in breeding programmes, the protein content invariably decreased in well filled seeds. Negative correlation between protein content and grain yield has been reported [1]. However, possibility of combining high seed weight and high protein content has been indicated [2]. Most of the earlier work was based on the data obtained on mature seed. Seed protein at maturity is influenced by several physiological, biochemical events, and genetic differences in the patterns of protein accumulation during seed development. Therefore this study has been undertaken to understand the pattern of protein and carbohydrate accumulation in developing seeds.

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MATERIALS AND METHODS

Eight wheat genotypes differing in seed weight and plant height were grown under uniform fertility conditions (NPK 100, 60 and 40 kg/ha). All genotypes, except Mex. C.B. 116 and Olesen's Dwarf, took 37–40 days from anthesis to maturity. Mex. C.B. 116 and Olesen's Dwarf matured within 33–35 days from anthesis. On the first day of anthesis (0 day) each spike was tagged and dated. Only the ears tagged for anthesis date during one week period in all genotypes were utilised in the study to minimise the temperature caused variation, and harvested 10, 17, 24 and 31 days after anthesis and at maturity. The period of seed development was, thus, divided into four stages: I) 0–10 days after anthesis, II) 11–17 days, III) 18–24 days, IV) 25–31 days and V) 32 days–completed maturity. Samples of developing seeds were taken from the basal florets in the central region of each spike at every harvest. Fresh samples were either stored in liquid nitrogen or analysed immediately. Dry weight was determined by oven drying at 100°C till constant weight.

Table 1. Carbohydrate synthesis in developing seeds of wheat.

Genotype	Carbohydrate level (mg/seed) on different days after anthesis					
	0-10	11-17	18-24	25-31	32-maturity	maturity
Mex. C. B. 116	5.08	8.18	7.56	7.73	—	27.98
	(17.8)	(28.6)	(26.5)	(27.1)	—	
Olesen's Dwarf	2.85	6.85	3.01	8.66	—	21.37
	(13.3)	(32.1)	(14.1)	(40.5)	—	
Kalyan Sona	6.64	5.93	6.97	5.18	6.85	31.57
	(21.0)	(18.8)	(22.18)	(16.4)	(21.1)	
HD 1944	6.48	5.04	9.86	16.18	2.28	39.84
	(16.2)	(12.6)	(24.7)	(40.6)	(5.7)	
Sonalika	6.63	6.95	11.07	13.42	10.04	48.11
	(13.8)	(14.4)	(23.0)	(27.9)	(20.9)	
K-65	4.79	9.80	13.04	12.38	5.92	45.93
	(10.4)	(21.3)	(28.4)	(26.9)	(12.9)	
NP 880	5.61	4.77	17.13	20.13	0.42	48.06
	(11.6)	(10.1)	(35.4)	(42.0)	(0.8)	
Pusa 5-3	5.38	11.37	10.28	12.36	2.72	42.11
	(12.8)	(27.0)	(24.4)	(29.3)	(6.5)	

Note. Values in parentheses represent percentage of total carbohydrates at maturity.

Nitrogen content was determined by Kjeldahl method [3], and protein content obtained by multiplying N content with the factor of 5.75. Nonproteinous dry matter content was calculated by substracting the quantity of protein per seed from seed weight. Since the major part (95%) of the nonproteinous dry matter in a seed is carbohydrates [4, 5], it has been grossly taken as an index of carbohydrates accumulation.

RESULTS

Carbohydrate · accumulation. Total dry weight accumulation in the developing seed is shown in Fig. 1, and carbohydrate accumulation during respective periods of seed development in Table 1. Up to 17 days after anthesis, the smaller seeded varieties like Kalyan Sona, Olesen's Dwarf and Mex. C.B. 116 had accumulated 40.0–46.4% of the carbohydrate content recorded at maturity, while the bold seeded genotypes HD 1944, NP 880, Sonalika, K-65 and Pusa 5-3 accumulated only 21.7–39.8% carbohydrates during the same period. Kalyan Sona, in spite of having smaller seed, accumulated more carbohydrates within 10 days after anthesis than the bold seeded genotypes K-65, NP 880 and Pusa 5-3. NP 880 accumulated the highest quantity of carbohydrates per seed during IV stage after anthesis. The triple dwarf variety HD 1944 and double dwarf variety Kalyan Sona were almost similar in carbohydrates accumulation up to 17th day, however, the rate of carbohydrate biosynthesis was much higher in HD 1944 in the later period and, consequently, higher seed weight was obtained at maturity. NP 880 and Sonalika had almost same quantity of carbohydrates in mature seed, however, pattern of accumulation was different as NP 880 accumulated 99% of its final seed weight up to IV stage of seed development while Sonalika accumulated only 78% during the same period. The pattern for total dry matter accumulation also followed similar trend (Fig. 1).

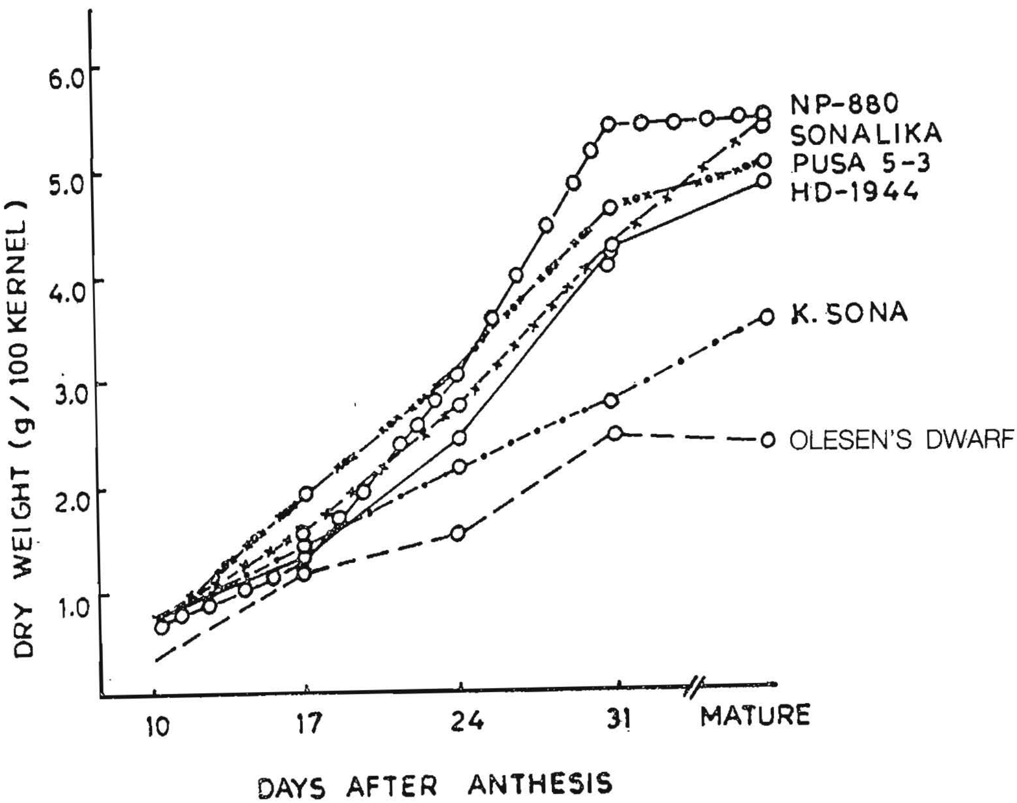

Fig. 1. Pattern of total dry matter accumulation during seed development.

Table 2. Protein synthesis in developing seeds of wheat

Genotype	Protein level (mg/seed) on different days after anthesis					
	0-10	11-17	18-24	25-31	32-maturity	maturity
Mex. C. B. 116	0.85	0.97	0.96	0.67	—	3.45
	(24.6)	(28.0)	(27.6)	(19.4)	—	
Olesen's Dwarf	0.60	1.28	0.24	1.17	—	3.29
	(18.2)	(38.9)	(7.4)	(35.5)	—	
Kalyan Sona	0.89	0.64	0.43	0.69	1.34	3.99
	(22.3)	(16.1)	(10.8)	(17.3)	(33.5)	
HD 1944	0.93	0.66	1.36	1.62	0.45	5.02
	(18.6)	(13.1)	(27.0)	(32.2)	(7.0)	
Sonalika	0.97	0.75	1.13	1.38	1.88	6.11
	(15.9)	(12.2)	(18.4)	(22.6)	(30.8)	
K-65	0.68	0.98	1.13	1.64	1.93	6.36
	(10.2)	(15.4)	(17.8)	(25.8)	(32.2)	
NP 880	0.87	0.72	1.30	3.10	0.64	6.63
	(12.7)	(10.5)	(19.0)	(45.3)	(12.2)	
Pusa 5-3	0.85	1.48	1.20	3.07	1.31	7.91
	(10.7)	(18.7)	(15.2)	(38.8)	(16.6)	

Note. Values in parentheses represent percentage of total proteins at maturity.

Protein synthesis. The kinetics of protein synthesis in spring wheat showed considerable variation (Table 2). Kalyan Sona accumulated 38% of total protein, while NP 880 only 23% during the first 17 days after anthesis. Pusa 5-3 and NP 880 accumulated, respectively, 38.8 and 45.3% of the total protein in matured seed during IV stage. Kalyan Sona, Sonalika and K-65 accumulated more than 30% of total protein after 32 days of anthesis till maturity.

Table 3 shows the variation in protein content at different stages of seed development. Pusa 5-3 had 15.8% protein at maturity but only 11.5% 24 days after anthesis. Sonalika had 12.8% on 10th day and 11.3% at maturity. The ratio of carbohydrates per unit of protein biosynthesis during development indicated variation not only within a variety over stages but also between the varieties. Interestingly, in all varieties the ratio between carbohydrates and protein synthesis was narrow (0.6:1–6.3:1) during the V stage of seed development, which is mainly responsible for protein accumulation. On the other hand, the III stage was favourable for carbohydrate synthesis, as the ratio was 5.8:1–16.2:1.

Relationship between carbohydrate and protein synthesis. The correlation between carbohydrate and protein levels (mg/seed) during development was positive and highly significant ($r = 0.78$–0.93, $P = 0.05$–0.01) at all stages. On the other hand, correlation between protein and carbohydrate content was negative ($r = -0.85$) and significant at the first stage of development (Table 4).

Table 3. Protein content in developing seeds of different wheat genotypes

Genotype	Protein content (%) on different days after anthesis				
	10	17	24	31	maturity
Mex. C. B. 116	14.3 (6.0)	12.1 (6.4)	11.8 (7.9)	10.8 (11.5)	14.0 —
Olesen's Dwarf	17.4 (4.7)	16.3 (5.3)	14.3 (12.3)	13.4 (7.4)	14.8 —
Kalyan Sona	11.9 (7.4)	10.9 (9.2)	9.1 (16.2)	9.7 (7.5)	11.2 (5.1)
HD 1944	12.6 (6.9)	11.6 (7.6)	10.6 (7.2)	9.9 (5.9)	12.2 (6.3)
Sonalika	12.8 (6.8)	12.2 (9.0)	10.3 (5.8)	10.0 (5.6)	11.3 (5.3)
K-65	12.5 (7.0)	10.3 (9.9)	9.2 (11.5)	10.0 (7.5)	12.2 (5.1)
NP 880	13.5 (6.4)	12.5 (7.8)	9.2 (13.1)	11.1 (6.5)	12.4 (0.6)
Pusa 5-3	13.5 (6.4)	12.2 (7.7)	11.5 (8.5)	14.4 (4.0)	15.8 (2.0)

Note. Values in parentheses are the units of carbohydrates increased per unit increase in protein during the same period.

DISCUSSION

The study of carbohydrate accumulation in wheat varieties at different developmental stages revealed that the bold seeded variety K-65 accumulated lesser carbohydrates (4.79 mg/seed) at the I stage of seed development than NP 880 (5.61 mg/seed) but the former variety accumulated twice as much carbohydrates (9.8 mg/seed) as variety NP 880 (4.77 mg/seed) at the II stage, i.e. 17 days after anthesis. Among all the genotypes tested, variety Pusa 5-3 had the highest rate of carbohydrate accumulation (11.37 mg/seed) at the II stage while NP 880 (4.77 mg/seed) showed the lowest carbohydrate accumulation rate during the same period. However, NP 880 had the highest carbohydrate accumulation at III and IV stages (17.13 mg/seed and 20.13 mg/seed, respectively). Similarly, genotype- and stage-dependent variation for protein accumulation was also recorded. Variety K-65 accumulated less proteins per seed (0.68 mg/seed) during the I stage than NP 880 (0.87 mg/seed), while at the II stage the former variety accumulated substantially more proteins than the latter (0.98 and 0.72 mg/seed, respectively). HD 1944 and Sonalika accumulated more proteins (0.93 and 0.97 mg/seed) than K-65, NP 880 and Pusa 5-3 (0.68, 0.87 and 0.85 mg/seed, respectively) at I stage, while at IV stage protein accumulation was much higher in NP 880 (3.10 mg/seed) and Pusa 5-3 (3.07 mg/seed) than in variety HD 1944 and Sonalika (1.62 and 1.38 mg/seed, respectively). The differential rates of carbohydrate and protein synthesis and accumulation in wheat varieties with different seed size and protein content indicates that protein and carbohydrate synthesis is controlled by different mechanisms at various developmental stages. Differences in protein synthesis pattern during development have been reported in corn [6] and wheat [7]. In general, protein content increased at maturity in all the varieties in comparison to stages III and IV of grain development which indicates higher rate of protein accumulation from 31st day after anthesis to maturity. It is known [8] that rate of protein synthesis is an important parameter ultimately affecting seed protein content.

Table 4. Simple correlation coefficients between carbohydrate and protein accumulation at various stages of wheat seed development

Character pair	Values of r on different days after anthesis				
	10	17	24	31	maturity
Carbohydrates vs. protein per seed	0.931**	0.784*	0.803*	0.781*	0.868**
Total dry matter per seed vs. protein percentage	−0.852**	−0.481	−0.644	−0.029	−0.283

*, **Significant at 5% and 1% levels, respectively.

This study also showed positive relationship between carbohydrates and protein accumulation per seed in absolute terms. Further, the pattern of protein and carbohydrate accumulation in individual varieties over different stages of seed development showed that the rate of carbohydrate synthesis is also maximum when protein synthesis rate is the highest and vice-versa, indicating the absence of any antagonism between the two biochemical processes. For example, during IV stage of seed development, varieties HD 1944, NP 880 and Pusa 5-3 accumulated maximum protein (1.62, 3.10 and 3.07 mg/seed, respectively) as well as carbohydrates (16.18, 20.13 and 12.36 mg/seed, respectively). Similarly, variety HD 1944, NP 880 on 17th day and Pusa 5-3 on 10th day after anthesis had the lowest levels of carbohydrates (5.04, 4.77 and 5.38 mg/seed, respectively) as well as protein (0.66, 0.72 and 0.85 mg/seed, respectively).

Thus, it may be concluded that carbohydrate and protein accumulations are coordinated phenomena. The negative correlation between seed size and protein content (% basis) was reported as a function of the fact that increase in seed size is commonly associated with disproportionately large deposition of carbohydrates relative to protein [2]. However, this study was based on observations on mature seed only. It has also been reported that light intensity [9], CO_2 supplementation [10, 11], and nitrogenous fertilizer application [1] affect nitrogen and carbon assimilation in the same direction. However, despite positive relationship between total carbohydrate and protein accumulation per seed through the entire development, negative correlation between protein content (% basis) and seed size has been demonstrated by the present study.

The question arises: how does this negative correlation occur? Our results show that the rate of increase in carbohydrate accumulation per unit increase in protein accumulation varies at different stages in the same genotype and at the same stage between genotypes, even though there is parallelism between accumulation rate of both protein and carbohydrates. At stage I (0–10 days) the carbohydrate : protein ratio was narrow (4.7:1–7.4:1) which resulted in high protein percentage (11.9–17.4%). In contrast, at III and IV stages of seed development, the carbohydrate accumulation was comparatively much higher than protein and, consequently, it resulted in wider ratio (5.3:1–16.2:1) and low protein content (9.1–14.3%). At V stage, again, carbohydrate accumulation per unit protein is low (0.6:1–6.3:1) and protein percentage increases (11.2–15.8%). The present study reveals that negative correlation between seed weight and protein content (% basis) is a result of disproportionate deposition of carbohydrates and proteins. However, there is no antagonism between these two products of biochemical processes.

This study, therefore, shows that wheat genotypes differ in their rate of increase in carbohydrate and protein accumulation at different stages in the same genotype. In addition, it has been shown that both carbohydrates and proteins decrease or increase in a coordinated manner, though their magnitudes differ. The genotypic differences for protein biosynthesis at various stages of seed development may be utilised in breeding to develop varieties with more efficient system of protein synthesis and accumulation in the seed.

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