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Nitrogenous compounds and protease activity in developing testa-pericarp and endosperm of high and low protein wheat (*Triticum aestivum* L.)

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Abstract. Nitrogenous compounds such as total protein, true protein, soluble protein, non-protein nitrogen, total amides, ammonium, nitrate, nitrite, free amino acids and neutral and acid protease activities were estimated in a high protein wheat cv Shera (12·8% protein) and a relatively low protein wheat cv C-306 (10·4% protein) at different stages of grain development. All the nitrogenous compounds studied, as well as protease activities, were located in both testa-pericarp and endosperm. Developmental patterns and relative levels were, more or less, similar when expressed on per organ basis, or on dry weight basis. In the testa-pericarp; dry weight, total protein, soluble protein, true protein, amide, ammonium, nitrate and nitrite contents increased during development, and were higher in Shera, compared with cv C-306. Non-protein nitrogen content decreased during pericarp development, and was higher in cv Shera, compared with cv C-306. Free amino acid content and protease activities decreased in developing pericarp, and were lower in cv Shera, compared with cv C-306. Similar developmental patterns and relative levels of nitrogenous compounds and protease activities were found in endosperm. It is suggested that both testa—pericarp and endosperm make qualitatively similar contributions to the accumulation of nitrogenous compounds in developing wheat grain; higher protease activity in the endosperm of C-306 is not responsible for lower protein accumulation, and nitrate and nitrite are assimilated mainly in the green pericarp of the wheat grain. It appears that differences in protein contents of Shera and C-306 wheat arise primarily from differences in translocation of nitrogenous solutes from the phloem sap to the peduncle and pericarp, enroute endosperm.

Keywords. *Triticum aestivum* L.; wheat; pericarp; endosperm; development; nitrogenous compounds; protease.

1. Introduction

Developing cereal grains derive most of their nitrogen supply from the leaves (Neals *et al* 1963; Nair and Abrol 1978). The assimilates are loaded into the phloem stream, and pass via the peduncle (Wardlaw and Moncur 1976) into the pericarp, enroute endosperm where storage proteins are synthesized. Nitrogenous compounds present in the phloem sap are metabolised extensively in the developing grain to provide amino acids in the right proportions for the synthesis of grain protein (Kolderup 1980). A small fraction of the synthesized protein may also be degraded by proteases present in the developing grain (Perez *et al* 1973; Gupta *et al* 1976). Thus, the contents of nitrogenous compounds in the developing grains remain in a state of flux.

Changes in the contents of nitrogenous compounds in the developing cereal grains

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of high and low protein cultivars have been reported (Perez *et al* 1973; Donovan *et al* 1977; Donovan 1979; Sen and Mehta 1980; Rana *et al* 1984). However, very little is known about the compartmentalization of these changes between developing testa-pericarp and endosperm of cereal grains. The present communication reports changes in the contents of nitrogenous compounds and neutral and acid protease activities in developing testa-pericarp (hereafter referred to as pericarp) and endosperm of a high protein cultivar Shera (12·8% protein) and a relatively low protein cultivar C-306 (10·4% protein) of wheat (*Triticum aestivum* L.). The nitrogenous compounds included here are total protein (crude protein), true protein, soluble protein, non-protein nitrogen, total amides, ammonium, total free amino acids, nitrate and nitrite.

2. Materials and methods

2.1 *Plant material*

Wheat plants (cv C-306 and Shera) were raised in pots under identical environmental and fertility conditions. Earthen pots (30 cm dia) were filled with farm soil (sandy loam) mixed with farm yard manure in the proportion of 5:1. Fertilizer solution (16 g urea and 10 g KH_2PO_4/l) was applied first before sowing and then 15 days after sowing (125 ml/pot). After thinning, each pot contained 5 plants. Hoagland solution, containing N, P, K and other minerals was applied (0·5 l/pot) at an interval of 20 days during plant development. Ears were harvested at 10, 17, 24, 31 and 38 days after anthesis (DAA). At each sampling, 20 ears were taken, grains removed and composite sample obtained. Embryos were removed from the grains, and pericarp and endosperm separated and stored in liquid nitrogen until further use. Separation of pericarp from endosperm was not feasible at 10 and 38 DAA, hence whole grains were used for analysis. At other stages of grain development, separation of pericarp from endosperm was complete.

2.2 *Chemical composition*

Total protein (Crude protein, total NX 5·7) (Horwitz 1980), soluble protein (Lowry *et al* 1951), ammonium (Wriston 1970), amide (Varner *et al* 1953) and nitrite and nitrate (Grover *et al* 1978) were determined following the standard procedures. True protein was separated from non-protein nitrogen by precipitation with Stutzer's reagent (Popli 1972), and the precipitated protein analyzed for nitrogen by micro-Kjeldahl's method. Non-protein nitrogen was estimated as a difference between total protein nitrogen and corresponding true protein nitrogen.

2.3 *Enzyme assays*

Neutral and acid protease activities were extracted at 0–4°C, from fresh plant material with 10 ml of 0·05 M phosphate buffer (pH 7·5) containing 0·01 M cysteine and 35 mM EDTA. The activity was assayed as described by Nair *et al* (1978), except that acetate buffer was replaced by citrate buffer, and incubations were carried out at 40°C. Extraction and assay conditions for the enzyme were standardized.

Each value in the tables and figures is the mean ± SE of 4 independent estimations.

3. Results

Since, it was not feasible to separate pericarp from endosperm at 10 and 38 DAA, the values reported for these two stages are for the whole grain, and have been published earlier (Rana *et al* 1984).

Developmental patterns for various parameters studied were more or less similar in C-306 and Shera wheat. Dry weight, total protein, soluble protein, true protein, amide, ammonium, nitrate and nitrite contents increased during endosperm development (figures 1 and 2). Non-protein nitrogen (NPN) content increased to peak value at 24 DAA and then declined (figure 2a). Free amino acid content and protease activities decreased until 31 DAA, followed by a slight increase (figures 2c and 3). However in Shera endosperm, amino acid content continued to decrease until maturity (figure 2c). In the pericarp, dry weight, total protein, soluble protein and true protein contents increased during grain development, particularly between 24 and 31 DAA (figure 1). Amide, nitrate and nitrite contents recorded consistent increase throughout pericarp development (figures 2b, e, f). NPN and ammonium

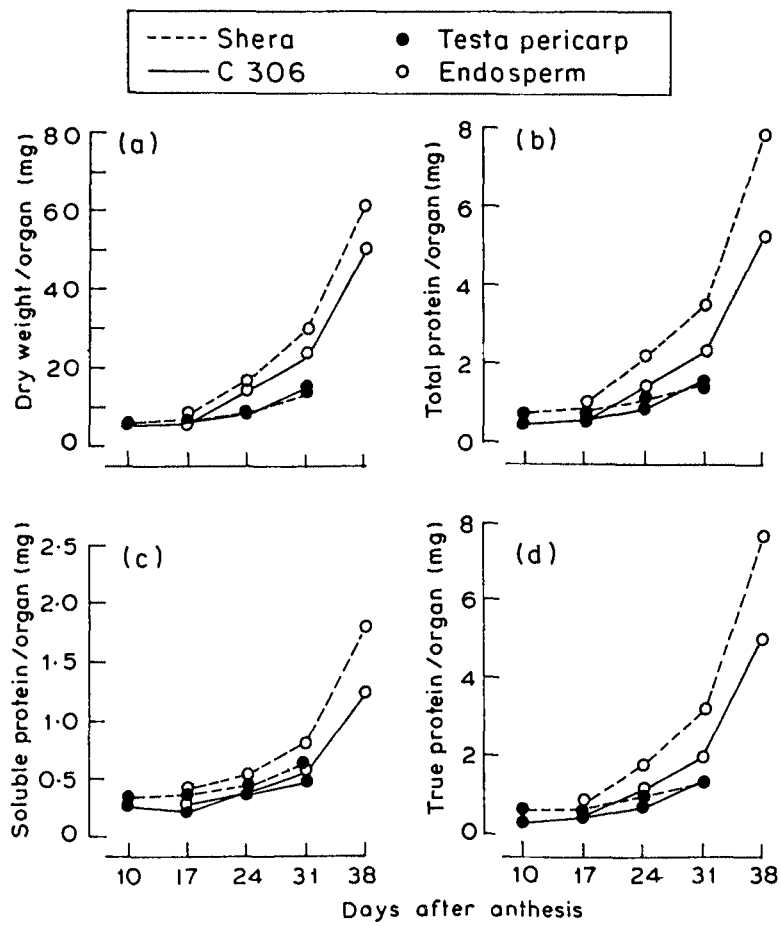

Figure 1. Dry weight (a), total protein (b), soluble protein (c) and true protein (d) contents in developing testa-pericarp and endosperm of Shera and C-306 wheat.

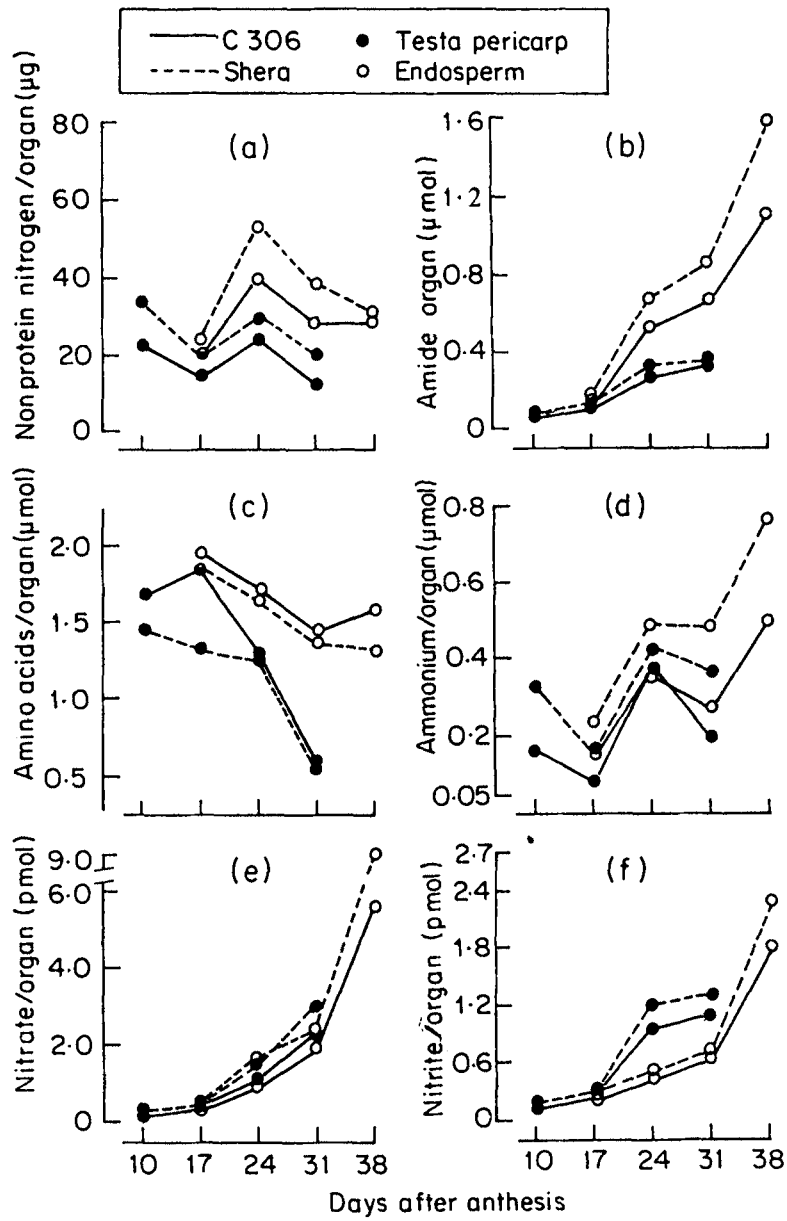

Figure 2. Non protein nitrogen (**a**), amide (**b**), amino acids (**c**), ammonium (**d**), nitrate (**e**) and nitrite (**f**) contents in developing testa-pericarp and endosperm of Shera and C-306 wheat.

contents followed a zigzag pattern with resultant slight decrease in NPN and slight increase in ammonium content (figures 2a,d). Amino acid content and protease activities declined during pericarp development (figures 2c and 3).

Dry weight, total protein, soluble protein, true protein, NPN, amide, ammonium, nitrate and nitrite contents per organ were higher in Shera as compared to C-306

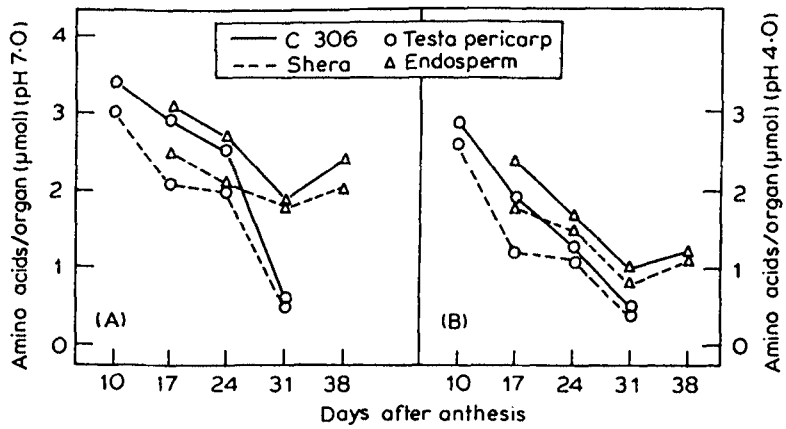

Figure 3. Neutral protease (**a**) and acid protease (**b**) activities in developing testa-pericarp and endosperm of Shera and C-306 wheat.

(figures 1 and 2). Amino acid content and protease activities per organ were lower in Shera, as compared to C-306 (figures 2c and 3). Except for nitrate and nitrite contents, all the nitrogenous compounds studied and protease activities were higher in endosperm, as compared to pericarp of either cultivar (figures 1–3). Nitrate and nitrite contents were higher in pericarp as compared to endosperm (figures 2e, f).

On dry weight basis, true protein and nitrate concentrations increased in the endosperm (tables 1, 3). Soluble protein, NPN, ammonium and free amino acid concentrations and protease activities decreased during endosperm development (tables 1–4). Amide concentration increased to a peak value at 24 DAA, followed by a decline (table 2), whereas, total protein and nitrite concentrations (tables 1 and 3) did not change much during endosperm development. The different parameters followed similar developmental patterns in the pericarp, as in the endosperm with few differences (tables 1–4). Ammonium and nitrite concentrations increased to peak value at 24 DAA followed by a decline (table 3). None of the parameters showed any significant varietal differences in developmental patterns.

Endosperm as well as pericarp of Shera contained lower concentration of free amino acids and lower protease activities, as compared to C-306 (tables 2 and 4). Concentrations of all other nitrogenous compounds were higher in Shera as compared to C-306 (tables 1–3). Average concentrations of soluble protein, NPN, free amino acids, ammonium, nitrate and nitrite, as also the protease activities were higher in pericarp as compared to endosperm of either variety (tables 1–4). Amide concentration was relatively lower in pericarp (table 2). Whereas, true protein concentration was higher in pericarp as compared to endosperm in Shera, it did not differ much in C-306 (table 1). On the other hand, total protein concentration in pericarp and endosperm was almost at the same level in either cultivar (table 1).

4. Discussion

All the nitrogenous compounds estimated in the present study, as well as protease activities were found to be present both in the pericarp and endosperm. This

Table 1. Total protein, true protein and soluble protein in developing testa-pericarp and endosperm of C-306 and Shera wheat.

Days after anthesis	Variety	Total protein (g/100 g dry wt)		True protein (mg/g dry wt)		Soluble protein (mg/g dry wt)	
		Testa-pericarp	Endosperm	Testa-pericarp	Endosperm	Testa-pericarp	Endosperm
10	C-306	9·46 ± 0·35	—	64·86 ± 0·52	—	53·92 ± 0·86	—
	Shera	12·46 ± 0·73	—	93·36 ± 0·23	—	53·17 ± 2·45	—
17	C-306	10·00 ± 0·34	10·00 ± 0·92	84·48 ± 0·42	85·32 ± 0·36	39·43 ± 1·32	43·27 ± 1·40
	Shera	12·01 ± 0·45	12·80 ± 0·64	101·49 ± 0·40	111·60 ± 6·04	49·61 ± 2·28	55·08 ± 2·76
24	C-306	11·92 ± 0·73	10·78 ± 1·06	101·28 ± 0·31	82·86 ± 1·23	41·46 ± 0·69	26·00 ± 0·44
	Shera	12·78 ± 0·16	13·97 ± 0·32	110·00 ± 0·31	121·84 ± 0·33	46·72 ± 1·48	34·27 ± 0·74
31	C-306	11·21 ± 0·92	9·90 ± 0·20	106·43 ± 0·74	91·30 ± 0·30	32·98 ± 1·04	23·80 ± 0·55
	Shera	11·58 ± 0·12	12·27 ± 0·59	109·02 ± 0·33	115·73 ± 0·40	49·20 ± 1·59	28·99 ± 0·65
38	C-306	—	10·40 ± 0·22	—	101·47 ± 0·33	—	24·41 ± 0·53
	Shera	—	12·82 ± 0·05	—	125·94 ± 0·33	—	29·06 ± 1·53

Table 2. Non-protein nitrogen, amide and free amino-acids in developing testa-pericarp and endosperm of C-306 and Shera wheat.

Days after anthesis	Variety	Non-protein nitrogen (mg/g dry wt)		Amide (μ mol/g dry wt)		Free amino acids (μ mol/g dry wt)	
		Testa-pericarp	Endosperm	Testa-pericarp	Endosperm	Testa-pericarp	Endosperm
10	C-306	4·25 ± 0·04	—	11·58 ± 1·10	—	324·00 ± 2·86	—
	Shera	5·65 ± 0·02	—	13·29 ± 1·20	—	243·00 ± 2·05	—
17	C-306	2·41 ± 0·06	3·06 ± 0·05	17·24 ± 0·77	17·74 ± 1·54	316·00 ± 1·73	316·00 ± 2·88
	Shera	2·99 ± 0·08	3·20 ± 0·16	22·39 ± 0·96	24·00 ± 2·17	197·00 ± 2·06	247·00 ± 1·50
24	C-306	3·08 ± 0·09	2·79 ± 0·12	34·62 ± 0·78	37·86 ± 3·63	167·00 ± 0·90	122·00 ± 0·50
	Shera	3·33 ± 0·02	3·35 ± 0·20	35·56 ± 3·28	42·93 ± 8·44	139·00 ± 0·50	104·00 ± 0·50
31	C-306	0·85 ± 0·06	1·21 ± 0·04	23·57 ± 2·58	28·70 ± 8·01	42·00 ± 0·20	62·00 ± 0·20
	Shera	1·50 ± 0·04	1·33 ± 0·04	26·32 ± 3·81	30·06 ± 1·81	41·00 ± 0·30	47·00 ± 1·73
38	C-306	—	0·55 ± 0·04	—	21·97 ± 3·63	—	31·00 ± 0·33
	Shera	—	0·49 ± 0·03	—	25·61 ± 12·30	—	21·00 ± 0·57

Table 3. Ammonium nitrate and nitrite in developing testa-pericarp and endosperm of C-306 and Shera wheat.

Days after anthesis	Variety	Ammonium (μ mol/g dry wt)		Nitrate (p mol/g dry wt)		Nitrite (p mol/g dry wt)	
		Testa-pericarp	Endosperm	Testa-pericarp	Endosperm	Testa-pericarp	Endosperm
10	C-306	32·81 ± 1·26	—	45·30 ± 2·53	—	33·37 ± 2·34	—
	Shera	54·82 ± 2·31	—	46·25 ± 5·14	—	37·88 ± 1·45	—
17	C-306	13·79 ± 0·83	22·53 ± 0·75	67·37 ± 5·63	52·93 ± 2·11	45·54 ± 2·52	39·37 ± 0·01
	Shera	23·88 ± 2·69	30·67 ± 2·85	78·39 ± 7·31	61·91 ± 2·82	47·26 ± 6·12	45·54 ± 1·78
24	C-306	48·71 ± 0·20	25·72 ± 2·36	142·16 ± 4·09	65·75 ± 4·05	127·00 ± 4·08	29·68 ± 2·10
	Shera	47·78 ± 8·46	30·93 ± 3·85	163·93 ± 4·01	98·26 ± 2·23	132·00 ± 0·00	33·41 ± 1·64
31	C-306	14·29 ± 0·44	11·73 ± 0·77	165·90 ± 16·57	82·35 ± 1·78	77·40 ± 7·48	24·00 ± 0·03
	Shera	27·82 ± 1·99	16·78 ± 0·47	231·68 ± 13·23	84·50 ± 4·66	101·00 ± 4·31	24·04 ± 1·78
38	C-306	—	9·91 ± 0·60	—	110·59 ± 15·35	—	35·97 ± 2·73
	Shera	—	12·39 ± 1·17	—	145·11 ± 5·86	—	37·37 ± 2·27

Table 4. Neutral protease and acid protease in developing testa-pericarp and endosperm of C-306 and Shera wheat.

Days after anthesis	Variety	Neutral protease (μ mol amino-acids/g dry wt)		Acid protease (μ mol amino acids/g dry wt)	
		Testa-pericarp	Endosperm	Testa-pericarp	Endosperm
10	C-306	646·00 ± 4·20	—	561·00 ± 0·75	—
	Shera	495·00 ± 6·95	—	427·00 ± 0·90	—
17	C-306	498·00 ± 2·12	496·00 ± 2·15	332·00 ± 0·80	385·00 ± 1·12
	Shera	316·00 ± 3·20	333·00 ± 8·14	182·00 ± 0·40	236·00 ± 1·50
24	C-306	315·00 ± 0·78	194·00 ± 0·60	167·00 ± 0·83	119·00 ± 0·80
	Shera	218·00 ± 0·93	134·00 ± 0·90	120·00 ± 0·90	95·00 ± 0·25
31	C-306	42·00 ± 1·14	84·00 ± 1·10	36·00 ± 1·26	42·00 ± 0·36
	Shera	39·00 ± 1·10	63·00 ± 1·80	32·00 ± 1·31	31·00 ± 2·22
38	C-306	—	47·00 ± 1·20	—	24·00 ± 0·99
	Shera	—	33·00 ± 1·60	—	18·00 ± 0·80

indicates that both pericarp and endosperm make qualitatively similar contribution to the accumulation of nitrogenous compounds in the developing wheat grain.

The pericarp remains green for about three fourth of the duration of grain development and contributes substantially to carbon and nitrogen metabolism of developing grain. Several enzyme activities have been demonstrated in green pericarp of developing wheat grains (Kumar *et al* 1982; Garg *et al* 1985). Glutamine is the major amide and glutamate is the major nonamide amino acid in the phloem sap feeding the developing grain (Simpson and Dalling 1981). Green pericarp of the developing grain possesses glutamine synthetase activity and thereby, can synthesize glutamine from glutamic acid. Earlier work in this laboratory has shown that wheat endosperm (cv C-306 and Shera) lack glutamine synthetase activity (Garg *et al* 1985). Duffs and Rosei (1978) have also indicated that the cellular conditions in the photosynthetic green pericarp of barley grain are more favourable for glutamine synthetase activity. This partly explains the observed increase in the level of amide (glutamine) in the pericarp, upto 31 DAA (figure 2b).

An additional factor responsible for increased levels of amide (figure 2b) might be increased translocation from the vegetative plant. However, Mikesell and Paulson (1971) have reported slight decrease in amino acid translocation from the phloem stream at grain maturity in wheat.

A sharp increase in nitrate and nitrite contents in the pericarp at 24 DAA (figure 2e, f) suggests that these inorganic forms of nitrogen are metabolised mainly in green pericarp and accumulate in this component of the grain, after the green chlorophyll of the pericarp starts disappearing.

Average protease activities were slightly lower in pericarp, compared with endosperm on per organ basis (figure 3), but on dry weight basis (table 4), pericarp possessed nearly two fold protease activity compared with endosperm. Since, pericarp, compared with endosperm, accounts for much more soluble protein, as a proportion of dry weight (table 1), it indicates that proteases in the developing grain might be concerned with the proteolysis of soluble enzyme proteins, rather than the insoluble storage proteins.

A comparison of pericarp and endosperm for average values of the various nitrogenous compounds and protease activities should provide an insight into the compartmentalization of these metabolites and enzyme activities. Such a comparison reveals that endosperm contribution towards the respective nitrogenous compounds and protease activity is much more than pericarp. Contribution of endosperm to dry weight, total protein and amide content of developing grain was nearly 3 fold as compared to that of pericarp. Excluding the last stage of grain development i.e. 38 DAA (when pericarp merges with the endosperm), and calculating the relative average values of nitrate and nitrite in the pericarp and endosperm of the developing grain; it can be seen that pericarp contribution to nitrate and nitrite contents is relatively higher, compared with endosperm. Average nitrite content in the pericarp is nearly two fold, as compared to that in the endosperm even though, pericarp contributes only one third of the dry matter of the developing grain. However, differences in nitrate and nitrite contents between the two cultivars or between the pericarp and endosperm of the same cultivar may not be of great consequence, because of their very low contents (p mol) compared with the contents (μ mol) of other nitrogenous compounds such as amide, free amino acids and ammonium.

Comparison of average values of nitrogenous compounds and protease activities,

further reveals that both pericarp and endosperm of Shera, compared with C-306 wheat, have higher values for most of these parameters, except for free amino acids and protease activity. Although, there is no direct evidence to show that proteases in developing cereal grains degrade synthesized storage proteins, Perez *et al* (1973) and Gupta *et al* (1976) have provided indirect evidence in this direction. Whereas, average values for acid protease and neutral protease activities are relatively higher in the pericarp and endosperm of C-306, as compared to Shera; endosperm of both varieties have higher average activities when compared to pericarp. However, on dry weight basis, pericarp possessed nearly two fold protease activity, as compared to endosperm. Therefore, higher protease activity in the endosperm of C-306, as compared to Shera, does not appear to make a substantial contribution in degrading the synthesized storage protein in C-306 endosperm, leading to decreased accumulation of protein. On the other hand, since both pericarp and endosperm of Shera, compared with C-306 have higher levels of soluble protein, both on per organ basis, as well as dry weight basis; higher protease activity in C-306 also does not appear to be associated with the proteolysis of soluble protein. It may thus be inferred that proteases *in vivo* in the developing grain may be in an inactive form and might become active during extraction of the enzyme. Cereal grains have been reported to contain protease inhibitors (Boisen 1983).

Notwithstanding the varietal differences in the levels of enzyme activities associated with ammonia assimilation, in the developing pericarp and endosperm of Shera and C-306 wheat (Garg *et al* 1985), it appears that similar factors operate in the two component parts of the grain, resulting in higher accumulation of nitrogenous compounds including protein, in Shera as compared to C-306. The most probable factor might be higher translocation of nitrogenous solutes from the phloem sap to the pericarp. Thence these nitrogenous compounds remain at a higher level in Shera, as compared to C-306, as these compounds enter the endosperm from the pericarp. However, the situation seems to be different with free amino acids which are lower in Shera, compared with C-306. Possibly, it may be due to higher utilization of free amino acids for protein synthesis in Shera. This is supported by the observation that both total protein and soluble protein contents were higher in Shera, compared with C-306 (figures 1b, d).

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References

Boisen S 1983 Protease inhibitors in cereals. Occurrence, properties, physiological role and nutritional influence; *Acta Agric. Scand.* **33** 369–381

Donovan G R 1979 Relationship between grain nitrogen, nonprotein nitrogen and nucleic acids during wheat grain development; *Aust. J. Plant Physiol.* **6** 449–457

Donovan G R, Lee J W and Niu R D 1977 Compositional changes in developing grains of high and low protein wheats. I Chemical compositions; *Cereal Chem.* **54** 638–645

Duffus C M and Rosie R 1978 Metabolism of ammonium ion and glutamate in relation to nitrogen supply and utilization during grain development in barley; *Plant Physiol.* **61** 570–574

Garg N, Singh R and Batra V I P 1985 Enzymes of glutamine and asparagine metabolism in testa-pericarp and endosperm of developing wheat grain; *Phytochemistry* **24** 1663–1666

Grover H L, Nair T V R and Abrol Y P 1978 Nitrogen metabolism in the upper three leaf blades of wheat at different soil nitrogen levels I. Nitrate reductase activity and contents of various nitrogenous constituents; *Physiol. Plant.* **42** 287–292

Gupta R K, Tiwari O P, Gupta A K and Das H K 1976 Synthesis and degradation of proteins during wheat endosperm development; *Phytochemistry* **15** 1101–1104

Horwitz W 1980 *Official Methods of Analysis of the Association of Official Analytical Chemists* 13th edition (Washington: ADAC)

Kolderup F 1980 Interconversion of amino acids in maturing wheat grains; in *Proc. Int. Symp. Seed Protein Imp. Cereal and grain legumes*, Norway, Vol. 1, pp 187–203

Kumar R, Ram H and Singh R 1982 Some of the metabolic activities of immature pericarp in developing wheat grains; *Natl. Acad. Sci. Lett.* **5** 153–155

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with Folin-phenol reagent; *J. Biol. Chem.* **193** 265–275

Mikesell M E and Paulson G M 1971 Nitrogen translocation and the role of individual leaves in protein accumulation in wheat grain; *Crop. Sci.* **11** 919–922

Nair T V R and Abrol Y P 1978 Nitrate assimilation and mobilization of reduced nitrogen from upper three leaf blades to the developing wheat grains; *Indian J. Plant Physiol.* **21** 41–47

Nair T V R, Grover H L and Abrol Y P 1978 Metabolism of upper three leaf blades of wheat at different soil nitrogen levels. II Protease activity and mobilization of reduced nitrogen to developing grains; *Physiol. Plant* **42** 293–300

Neals T F, Anderson M J and Wardlaw I F 1963 The role of leaves in the accumulation of nitrogen by wheat during ear development; *Aust. J. Agric. Res.* **14** 725–736

Perez C M, Cagempang G B, Esmama B V, Nonserrate R V and Juliano B O 1973 Protein mobilization in leaves and developing grains of rice differing in grain protein content; *Plant Physiol.* **51** 537–542

Popli S 1972 *Biochemical Evaluation of some important varieties of Bajra (Pennisetum typhoideum)*, M. Sc. Thesis, Haryana Agricultural University, Hisar

Rana K, Singh R and Batra V I P 1984 Nitrogenous compounds and protease activity in developing grains of high and low protein wheats; *Plant Physiol. Biochem.* **11** 108–117

Sen K and Mehta S L 1980 Protein and enzyme levels during development in high lysine barley mutant; *Phytochemistry* **19** 1323–1328

Simpson R J and Dalling M J 1981 Nitrogen redistribution during grain growth in wheat (*Triticum aestivum* L.). III Enzymology and transport of amino acids from senescing flag leaves; *Planta* **151** 11–17

Varner J E, Bulen W A, Vaneck S and Burnell R C 1953 Determination of ammonium, amide, nitrite and nitrate nitrogen in plant extracts; *Anal. Chem.* **25** 1528–1529

Wardlaw I F and Moncur L 1976 Source, sink and hormonal control of translocation in wheat; *Planta* **128** 93–100

Wriston J C Jr 1970 Asparagenase; *Methods Enzymol.* **17** 732–736