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Enzymes of Ammonia Assimilation in Leaves of High and Low Protein Wheat During Grain Development

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Glutamine synthetase, glutamate synthase, glutamate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase have been assayed, during grain filling, in flag leaf and second leaf of a high protein wheat cv. Shera (11.6% protein) and a relatively low protein wheat cv C-306 (9.8% protein). At a stage around 24 days after anthesis, developmental patterns undergo a distinct change or a shift takes place in relative values for the two cultivars for most of the parameters studied. The relatively higher activities of glutamine synthetase and glutamate synthase in cv. Shera leaves compared with cv C-306 leaves might lead to more active assimilation of ammonium during pre-senescence phase, and generation and reassimilation of remobilized ammonium during post-senescence phase. Differences in specific activities of these enzymes between cv. Shera and C-306 leaves were most marked at 24 days after anthesis.

Key Words: ***Triticum aestivum*** **(Wheat), High protein, Grain development, Glutamate dehydrogenase, Glutamine synthetase, Glutamate synthase, Glutamate pyruvate transaminase, Glutamate oxaloacetate transaminase**

Introduction

Protein accumulation in developing cereal grains requires translocation of amino nitrogen mainly in the form of glutamine from the leaves (Tully & Hanson 1979). Leaves store most of the nitrogen during vegetative phase of plant growth and about four-fifth of total leaf nitrogen is generally present as protein (Fowden 1979). Leaf protein is mobilized during reproductive phase for nutrition of developing grains. The top leaves also photosynthesize and photorespire actively during most of the duration of grain development. Loss of ammonia through photorespiration may far exceed the ammonia replenished through nitrate reduction. To save the plant from total loss of its organic nitrogen, the photorespired ammonia must be reassimilated (Keys et al. 1978). Ammonia assimilation in leaves during reproductive phase is, therefore, a necessity to replenish organic nitrogen lost through photorespiration, and through mobilisation to the developing grains. A study of the enzymes of ammonia assimilation in leaves during grain filling may, therefore, be of interest. The present communication reports the activities of glutamate dehydrogenase, glutamine synthetase, NADH-glutamate synthase, glutamate pyruvate transaminase and glutamate oxaloacetate transaminase in the flag leaf and second leaf of a high protein (11.6% cv Shera) and a relatively low protein (9.8%, cv C. 306) wheat cultivars at different stages of grain development.

Materials and Methods

Plant Material

Plants of high protein wheat cultivar, Shera (11.6% protein) and a relatively low protein wheat

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cultivar, C-306 (9.8% protein) were raised in pots in the net house under identical environmental and fertility conditions. The ears were tagged on the day of anthesis. Flag leaf and second leaf were harvested at weekly intervals, beginning 10 days after anthesis, until maturity. The leaves were cut into small pieces and representative samples taken for analysis.

Enzyme Assays

Activities of glutamate dehydrogenase, glutamine synthetase, NADH-glutamate synthase, glutamate pyruvate transaminase and glutamate oxaloacetate transaminase were assayed according to standard methods described elsewhere (Garg et al. 1984). For assay of NADH-linked enzymes (Glutamate dehydrogenase, glutamate synthase and glutamate pyruvate transaminase), oxidation of NADH was monitored by decrease in absorbance at 340 nm. Controls without substrate were included to account for the endogenous unspecific NADH oxidation. Glutamine synthetase and glutamate oxaloacetate transaminase were assayed by colorimetric methods. Two extracts were prepared for each sample and each extract was assayed in duplicate.

Reaction mixtures for various enzymes consisted of the following:

Glutamate dehydrogenase, (*μmol*): Tris-HCl buffer, *p*H 7.6, 40; NH_4Cl, 300; α-ketoglutaric acid (neutral), 33; NADH, 2.0; and enzyme extract, 0.2ml.

Glutamine synthetase (*μmol*): Imidazole-HCl buffer, *p*H 7.5, 80; $MgCl_2$, 80; sodium glutamate, 100; $NH_2OH.HCl$ (freshly prepared in 0.5M NaOH), 100; ATP, 20; mercaptoethanol (1:100 v/v), 0.4ml and enzyme extract, 0.3ml.

Glutamate synthase (*μmol*): Tris-HCl buffer, *p*H 7.6, 50; glutamine, 40; KCl, 30; EDTA, 4.0; α-ketoglutaric acid neutral), 40; NADH, 2.0; mercaptoethanol (1:100 v/v), 0.2ml and enzyme extract, 0.2ml.

Glutamate pyruvate transaminase (*Alanine aminotransferase, μmol*): Tris -HCl buffer (*p*H 7.6), 60; α-ketoglutaric acid (neutral) 12.5; alanine, 100; pyridoxal phosphate, 2.0; NADH, 2.0; lactate dehydrogenase, 2 IU; enzyme extract, 0.2ml and H_2O 0.8ml.

Glutamate oxaloacetate transaminase (*Aspartate aminotransferase, μmol*): Phosphate buffer (*p*H 7.5), 40; α-ketoglutaric acid (neutral), 20; and aspartic acid (neutral), 150. Reaction was stopped by 0.5ml 2, 4 dinitrophenyl hydrazine (2mg/ml concentrated HCl, diluted to 100ml). After 10 min colour developed by 5ml 0.4N NaOH was measured at 550nm against reagent blank.

Total protein was determined by micro-Kjeldahl method (Horwitz 1965).

Results and Discussion

Flag leaf and second leaf of wheat remain green during most of the grain filling period and senesce only during later stages. However, the second leaf starts senescing earlier than flag leaf, as wheat leaves senesce sequentially upwards (Thiman 1980). Nitrogen metabolism changes at the onset of senescence from nitrogen assimilation to remobilization (Simpson & Dalling 1981), accompanied by the loss of assimilatory capacity (Kumar & Abrol 1990). Even varieties of the same species differ remarkably in senescence behaviour (Miksell & Paulson 1971). Results of the present investigation (tables 1-3 and figures 1-2) revealed that at a stage around 24 days after anthesis (DAA), developmental patterns underwent distinct change or a shift took place in relative values for the two varieties, for most of the parameters studied. The results could, therefore, be usefully discussed with respect to the two phases of leaf development viz. early phase (pre-senescence) and later phase (post-senescence).

During early phase, the green flag leaf and second leaf of wheat photosynthesize and photorespire actively. The ammonium produced by decarboxylation of glycine during photorespiration (Kumar & Abrol 1990) is assimilated predominantly by GS/GOGAT system (Wallsgrove et al. 1987) involving glutamine synthetase (GS) and glutamate synthase (GOGAT). Glutamine synthetase showed a decline during early phase of grain filling and the activity was slightly higher in

Table 1 *Specific activities of enzymes of glutamine metabolism in flag leaf of C-306 and relatively high protein wheat variety Shera during grain development**

Days after anthesis	Variety	Glutamine synthetase (μmoles γ-CHA formed/hr/mg protein)	Glutamate synthase (nmoles NADH oxid./min/ mg protein)	Glutamate dehydrogenase (nmoles NADH oxid./min/mg protein)	Glutamate pyruvate transaminase (nmoles NADH oxid./min/mg protein	Glutamate oxaloacetate transaminase (μmoles α-k.g. used/hr/mg protein
10	C-306	13.64±1.17	7.38±0.27	8.24±0.48	6.92±0.02	2.01±0.11
	Shera	10.02±0.039	6.54±0.51	5.73±0.37	7.19±0.31	0.19±0.01
17	C-306	7.98±0.29	5.14±0.14	11.43±0.14	12.09±0.93	0.82±0.02
	Shera	7.87±0.37	8.08±0.81	9.13±0.31	7.0±0.07	1.01±0.07
24	C-306	11.29±1.05	5.69±0.34	16.09±0.09	4.86±0.21	1.39±0.01
	Shera	17.88±1.51	8.84±0.41	18.83±0.37	7.95±0.35	1.33±0.04
31	C-306	3.54±0.07	12.25±0.51	27.59±0.71	11.94±0.51	0.75±0.01
	Shera	11.46±0.03	8.96±0.73	25.05±0.52	18.01±0.87	2.78±0.22
38	C-306	4.58±0.28	14.76±0.76	31.55±0.48	12.21±0.91	1.95±0.07
	Shera	4.12±0.14	20.20±0.98	30.98±0.09	13.33±0.73	1.92±0.06

* Values are mean of 4 replicates ±S D

Table 2 *Specific activity of enzymes of glutamine metabolism in second leaf of C-306 and relatively high protein wheat variety Shera during grain development**

Days after anthesis	Variety	Glutamine Synthetase (μmoles γ-CHA formed/hr/mg protein)	Glutamate synthase (nmoles NADH oxid./min/ mg protein)	Glutamate dehydrogenase (nmoles NADH oxid./min/mg protein)	Glutamate pyruvate transaminase (nmoles NADH oxid./min/mg protein	Glutamate oxaloacetate transaminase (μmoles α-k.g. used/hr/mg protein
10	C-306	28.77±0.47	8.49±0.73	10.72±0.21	6.83±0.33	1.06±0.03
	Shera	32.05±0.81	6.66±0.70	10.50±0.55	11.45±0.10	1.35±0.05
17	C-306	12.86±0.83	6.46±0.32	11.14±0.15	12.31±0.81	1.09±0.04
	Shera	13.21±0.74	6.09±0.18	10.99±0.41	6.81±0.31	0.82±0.02
24	C-306	10.52±0.35	6.31±0.11	12.44±0.42	9.60±0.34	1.46±0.06
	Shera	21.69±0.41	9.24±0.25	33.12±0.21	9.88±0.43	2.48±0.21
31	C-306	0.72±0.02	22.19±0.29	36.75±2.56	17.43±0.17	1.08±0.03
	Shera	10.44±0.35	9.43±0.39	32.59±2.11	15.49±0.51	2.28±0.17
38	C-306	0.57±0.02	14.04±0.44	44.12±2.57	17.76±0.58	2.28±0.09
	Shera	0.80±0.03	20.12±0.12	24.50±2.02	11.55±0.83	1.82±0.11

* Values are mean of 4 replicates ±S D

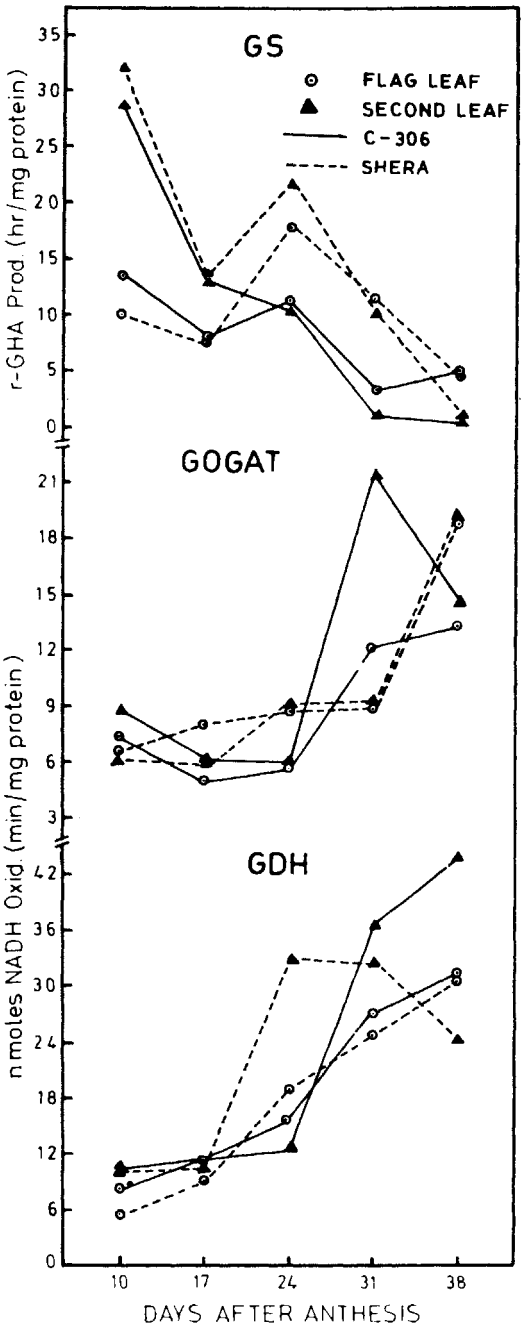

Figure 1 Glutamine synthetase (G S), Glutamate synthase activity (GOGAT) and Glutamate dehydrogenase (GDH) in C-306 and Shera wheat leaves during grain development

second leaf of Shera as compared to C-306 (figure 1, tables 1 & 2). Similarly GOGAT activity registered a slight decline in C-306 leaves, while it exhibited a rise in Shera, and the activity was higher in Shera as compared to C-306 more so in flag leaf than in second leaf (figure 1, tables 1-2). An increasing activity of GS and GOGAT in Shera leaves and relatively higher levels of these activities in Shera compared with C-306 could lead to enhanced assimilation of ammonium in Shera leaves. Glutamate dehydrogenase (GDH) predominantly a mitochondrial enzyme, believed to be important only in oxidative degradation of glutamate, is now known to be active in assimilation of ammonium in the mitochondria where ammonium is produced by decarboxylation of glycine (Lauiere & Daussant 1983, Caumaerts & Jacob 1985). Glutamate dehydrogenase increased during grain filling period and was substantially higher in second leaf of Shera, compared with C-306 (figure 1, tables 1 & 2). However, GDH activity was more or less similar in the flag leaf of the two varieties. A similar increase in GDH during grain filling has also been observed by Simpson and Dalling (1981). Transaminases are involved in exchange of α-amino nitrogen between glutamate and several other amino acids. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are the two major transaminases. GOT activity registered a decrease in flag leaf and slight increase in second leaf during early phase in C-306; while it exhibited a distinct rise in both flag leaf and second leaf of Shera. GOT activity was higher in flag leaf of C-306 (figures 1, tables 1 & 2). However, GDH 2). GPT activity during early phase showed a slight decline in flag leaf of C-306 and second leaf of Shera, where as it increased in second leaf of C-306 and flag of Shera and the activity was slightly higher in C-306 leaves. Since transaminases catalyze reversible reactions, evaluation of the role of these enzymes in nitrogen assimilation requires knowledge of the relative cellular concentration of substrates and corresponding products.

During later phase, when flag leaf and second leaf began to senesce, GS activity came down,

GOGATGDH/GOT and GPT activities increased except in second leaf of Shera where GDH and GOT showed a slight decline. Except for GOGAT and GPT activities, all the enzyme activities were relatively higher in Shera leaves as compared to C-306 (figures 1, 2, tables 1, 2). Two isoforms of GS have been reported in barley and rice leaves (Guiz et al. 1979, Mann et al. 1979) and the post-senescence GS activity is comprised mainly of cytosolic (GS_1) isoform and the overall decrease in GS activity could be result of loss of chloroplastidic isoform (GS_2) activity accompaning degeneration of chloroplasts during senescence. GDH may play a truely oxidative role participating actively in degradation of amino acids derived by hydrolysis of proteins during senescence (Miflin & Lea 1977).

The specific activities of major enzymes of ammonia assimilation GS and GOGAT were on the whole higher in Shera leaves as compared to

Table 3 *Dry matter and total protein in leaves of C-306 and relatively high protein wheat variety Shera during grain development**

Days after anthesis	Variety	Dry matter (mg/leaf blade)		Total protein (mg/leaf blade)	
		Flag leaf	Second leaf	Flag leaf	Second leaf
10	C-306	113.83±3.15	144.09±3.14	19.75±2.82	22.58±0.91
	Shera	131.64±8.73	158.23±4.34	24.17±1.28	20.19±0.35
17	C-306	126.17±4.76	169.73±4.30	20.87±1.78	24.68±1.63
	Shera	139.95±5.11	167.78±7.86	21.16±1.11	16.51±1.29
24	C-306	124.46±4.91	166.97±4.84	18.18±0.35	18.25±0.73
	Shera	137.95±3.24	172.52±2.52	18.05±0.97	13.44±0.25
31	C-306	135.98±2.40	184.77±7.35	9.56±0.07	7.74±0.33
	Shera	141.52±5.12	189.56±5.76	14.69±0.34	11.26±0.57
38	C-306	183.97±3.93	195.73±4.24	7.23±0.25	6.83±0.02
	Shera	202.16±5.26	218.45±2.18	10.31±0.28	10.96±0.05

* Values are mean of 4 replicates +S.D.

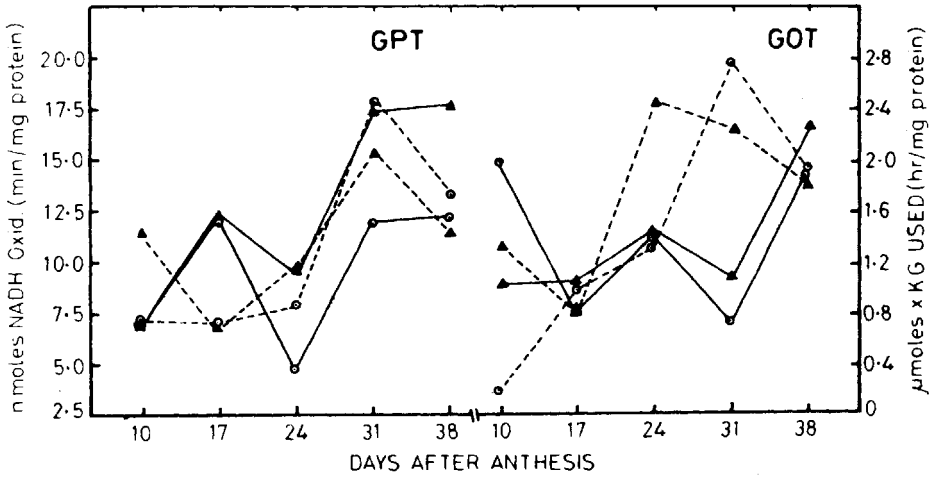

Figure 2 Glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) activity in C-306 and Shera wheat leaves during grain development

C-306 at both pre-senescence and post-senescence phases (table 1-2). The relatively higher activities of GS and GOGAT in Shera leaves compared with C-306 leaves might lead to more active assimilation of ammonium during early phase and generation and reassimilation of remobilized ammonium during later phase. In general, differences in specific activities between Shera and C-306 were most marked at 24 days when protein accumulation was active in the developing grain (table 1, 2).

Dry weight and total protein increased and decreased respectively in the two leaves of both the varieties during grain filling period (table 3); Shera leaves, particularly the flag leaves had substantially higher dry weight as compared to those of C-306. Flag leaf of Shera compared with C-306 had higher protein content, but it was relatively lower at early phase in second leaf of Shera, possibly due to higher nitrogen mobilization from the second leaf to developing grain.

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