Bot. Bull. Academia Sinica (1990) **31:** 149-161

Ammonia assimilation and glutamine metabolism related to protein accumulation in developing rice grains

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(*Received November 14, 1989; Accepted February 8, 1990*)

Abstract. Glutamine synthetase, ferredoxin- and NADH-dependent glutamate synthase, glutamate dehydrogenase, glutamate pyruvate transaminase and glutamate oxaloacetate transaminase in the intact rice grain and its hull, testa-pericarp and endosperm were assayed during grain development. All enzymes appeared high activities except the glutamate dehydrogenase which is absent in all tissues. Obviously, the glutamine synthetase-glutamate synthase cycle is the only pathway to operate ammonia assimilation in the developing rice grain. In the first crop season, ammonia assimilation operated more active, transamination took place earlier, protein accumulation was more rapid; and the content of free ammonium, total amides and glutamine were at low levels and of free amino acids was at high level in the developing grain when compared with that in the second crop season. Ammonia assimilation in the hull, testa-pericarp and endosperm are mainly via the co-operation of glutamine synthetase and ferredoxin-dependent glutamate synthase in both crop seasons. Transamination in the hull and the testa-pericarp operated actively only in the second crop season at early stage of grain development; but in the endosperm, it operated actively in both crop seasons at later stage of grain development. In the first crop season, protein accumulation into the endosperm was more rapid, the free amino acids in the endosperm was at high level, total amides and glutamine in all tissues at early stage of grain development were at high levels when compared with that in the second crop season.

Key words: Ammonia assimilation; Developing rice grain; Glutamate synthase; Glutamate pyruvate transaminase; Glutamate oxaloacetate transaminase; Glutamine metabolism; Glutamine synthetase; Hull; Endosperm; *Oryza sativa*; Protein accumulation; Teata-pericarp.

Introduction

The primary assimilation of ammonia into organic nitrogen does take place in the grain in situ, and amino acids derived from leaves are transformed within the grain to provide the appropriate types of amino acids in the correct proportions for the synthesis of grain proteins (Duffus and Rosie, 1978). Glutamine and glutamate are two of the major amino acids in the phloem exudate of leaves (Tully and Hanson, 1979) feeding the maturing cereal grain. Studies of biosynthesis and accumulation of storage proteins in developing rice seeds had been reported (Yamagata *et al.*, 1982; Luthe, 1983). Enzymes of glutamine metabolism in the developing wheat grain as well as in the testa-pericarp and the endosperm of the developing grain had also been demonstrated (Garg *et al.*, 1984; 1985). The results indicated that ammonia assimilation in the developing wheat grain takes place by the glutamate dehydrogenase pathway in the endosperm, and by both the glutamate dehydrogenase and the glutamime synthetase-glutamate synthase pathways in the testa-pericarp. But, it is still not known with certainty as to which pathway of ammonia assimilation is operative in the developing rice grain.

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Some reports indicated that rice variety with high content of protein in the grain was due to the high content of free amino acids in the developing grain resulting to accumulate more protein in the grain (Cruz *et al.*, 1970; Perez *et at.*, 1973). However, the biosynthesis of free amino acids in the developing grain is intimately related to ammonia assimilation and glutamine metabolism in the grain. Therefore, variations in the activity of enzymes of ammonia assimilation and glutamine metabolism in the developing rice grain can evaluate the ability of protein biosynthesis of the developing grain. Rice is a major crop in Taiwan and harvested twice a year. The growth pattern of rice plants is different between crop seasons mainly due to differences of weather conditions in different crop seasons (Wu *et al.*, 1975). Thus, weather conditions may also influence the biochemical changes in the developing rice grain resulting the difference of ammonia assimilation and glutamine metabolism between crop seasons during grain development. The present paper reports the activity of some enzymes in relation to ammonia assimilation and glutamine metabolism as well as the content of various nitrogenous compounds in the developing rice grain during development. In order to emphasize the compartmentalization of the maturing grain, these enzyme activities and nitrogenous compounds in hull, testa-pericarp and endosperm of the developing grain were also studied individually. And differences of ammonia assimilation, glutamine metabolism and protein accumulation in the developing grain between crop seasons will be discussed to evaluate the efficiency of protein biosynthesis in the developing grain.

Materials and Methods

Plant Material

Rice variety of *Oryza sativa* L., cv. Hsinchu 56 was grown in the paddy field of the Institute of Botany, Academia Sinica which is located in Nankang, Taipei, Taiwan. Field management including basal and top dressings of fertilizer and irrigations followed the general methods used by farmers, and ammonium sulfate was used as the nitrogen fertilizer. The first crop season started in mid-March and ended in mid-July with flowering stage in early June. The second crop season started in mid-August and ended in mid-December with the flowering stage in late October. During the flowering stage, the ears were tagged on the day of anthesis and harvested at 4- or 5-day intervals. Harvesting was started from 5 days after anthesis until the end of grain maturity. The harvested developing grains were stored in liquid nitrogen until enzyme assay and chemical analysis were performed within two months. Tissues including hull, testa-pericarp and endosperm were separated from the grain just before the enzyme extraction and chemical analysis, and the embryo was discarded. The testa-pericarp included pericarp, tegmen and aleurone layer of the grain.

Chemicals

Amino acids, α-ketoglutarate, pyridoxyl phosphate, ATP, NADH, lactate dehydrogenase and malate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, Mo. USA). Nessler's reagent, sodium dithionite and other chemicals were analytical grade either from Merck (Darmstadt, FRG) or Wako Puro Chemical Industries (Tokyo, Japan). Ferredoxin was prepared from spinach leaves according to Yocum (1982). The ratio of A_{420}/A_{275} was greater than 0.48. The concentration of ferredoxin was determined depend upon the molar extinction coefficient of 9600 M^{-1} cm^{-1} at 420 nm.

Enzyme Extraction

Unless otherwise stated, all operations were carried out at 4°C. A minimum number of 40 intact developing grains or 80 individual tissues were ground with liquid nitrogen in a mortar and pastle until fine powder, then homogenized completely with an appropriate amount of 0.05 M Tris-HCl buffer (pH 7.5) comtaining 2 mM 2-mercaptoethanol and 2 mM EDTA. The homogenate was clarified by centrifugation at 12,000 x g for 30 minutes. The clear supernatant was collected and used as the enzyme extract for various assays after measuring the volume.

Enzyme Assay

Glutamine synthetase was assayed by the biosynthetic reaction which was based on the release of inorganic phosphate in the presence of ammonium chloride (Shapiro and Stadtman, 1970). The assay mixture contained 50 mM Imidazole-HCl buffer (pH 7.0), 7.5 mM ATP, 100 mM sodium glutamate, 50 mM NH_4Cl, 50 mM $MgCl_2$ and 0.1 ml of enzyme extract in a total volume of 0.4 ml. The reaction was started by adding

the enzyme solution, and the enzyme extract was omitted in the blank test. After incubation at 30°C for 15 minutes, the reaction was terminated by adding 3.6 ml of ferrous sulfate reagent (0.8% $FeSO_4 \cdot 7H_2O$ in 0.015 N H_2SO_4, prepared freshly), followed by adding 0.3 ml of ammonium molybdate reagent [6.6% $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 7.5N H_2SO_4]. The absorbance at 660 nm was measured after several minutes. Sodium dihydrogen phosphate was used as standard. One unit of glutamine synthetase activity is defined as the amount of enzyme catalyzing the release of 1.0 μmole of inorganic phosphate per minute at 30°C.

Ferredoxin- and NADH-dependent glutamate synthase activities were assayed by estimating the production of L-glutamate in the presence of L-glutamine and α-ketoglutarate. The ferredoxin-dependent glutamate synthase was assayed according to Suzuki and Gadal (1982). The assay mixature contained 22.5 mM K, Na - phosphate buffer (pH 7.3), 5 mM L-glutamine, 5 mM α-ketoglutarate, 0.02 mM ferredoxin, 9 mM sodium dithionite (16 mg of sodium dithionite dissolved in 1.0 ml of 190 mM $NaHCO_3$) and 0.1 ml of enzyme extract in a total volume of 1.0 ml. The reaction was started by adding sodium dithionite, then incubated at 30°C for 20 minutes, and the enzyme extract was omitted in the blank test. The NADH-dependent glutamate synthase was assayed according to Matoh *et al.* (1980). The assay mixture contained 50 mM K, Na - phosphate buffer (pH 7.3), 10 mM L-glutamine, 10 mM α-ketoglutarate, 0.6 mM NADH and 0.1 ml of enzyme extract in a total volume of 1.5 ml. The reaction was started by adding NADH, then incubated at 30°C for 20 minutes, and the enzyme extract was omitted in the blank test. The reaction was terminated by placing the assay tubes in boiling water for 1 minute. For determination of the glutamate produced, the assay mixture was centrifuged at 2,000 x g for 10 minutes to remove the precipitate and the supernatant was directly charged on a resin AG1 x 8 column (acetate form, 200-400 mesh, 8 x 30 mm). After washing with 5 ml of water, the glutamate was eluted from the column with 1.0 M acetic acid. The first 5 ml of the eluent was collected and the glutamate was determined by the ninhydrin procedure according to Moore and Stein (1954). The absorbance at 570 nm was measured, and L-glutamate was used as standard. One unit of glutamate synthase activity is defined as the amount of enzyme catalyzing the formation of 1 μmole of glutamate per minute at 30°C.

Glutamate dehydrogenase, glutamate pyruvate transaminase and glutamate oxaloacetate transaminase were assayed by mornitoring changes in absorbance at 340 nm due to oxidation of NADH to NAD^+. Glutamate dehydrogenase and glutamate pyruvate transaminase were assayed according to Garg *et al.* (1984). For glutamate dehydrogenase, the assay mixture contained 70 mM Tris-HCl buffer (pH 7.6), 16.5 mM α-ketoglutarate, 150 mM NH_4Cl, 0.11 mM NADH and 0.2 ml of enzyme extract in a total volume of 2.0 ml. For glutamate pyruvate transaminase, the assay mixture contained 28 mM Tris-HCl buffer (pH 7.6), 5.8 mM α-ketoglutarate, 46.5 mM L-alanine, 2 units lactate dehydrogenase, 9.3 mM pyridoxyl phosphate, 0.1 mM NADH and 0.2 ml of enzyme extract in a total volume of 2.15 ml. Glutamate oxaloacetate transaminase was assayed according to Bergmeyer *et al.* (1983). The assay mixture contained 94.5 mM K, Na-phosphate buffer (pH 7.4), 35.5 mM L-aspartate, 6.8 mM α-ketoglutarate, 0.2 mM NADH, 23 units malate dehydrogenase and 0.2 ml of enzyme extract in a total volume of 3.18 ml. The reaction was started by adding NADH, and incubated at 30°C. The α-ketoglutarate was omitted in the blank test in all cases. The reaction rate was measured by mornitoring changes in absorbance at 340 nm. One unit of the enzyme activity is defined as the amount of enzyme catalyzing the oxidation of 1 μmole of NADH per minute at 30°C.

Determination of Total Protein

The total nitrogen content in the intact developing rice grain and its individual tissues were determined with the Micro-Kjeldahl method (Williums, 1984). The total protein content per grain or per individual tissue was calculated from the Kjeldahl nitrogen by multiplying the factor 5.95 (Juliano, 1972).

Determination of Free Ammonium, Free Amino Acids, Total Amides and Glutamine

A minimum number of 40 intact developing grains or 80 individual tissues were gently ground to powder with liquid nitrogen in a mortar and pastle, then homogenized completely with 10 ml of 80% ethanol. The homogenate was filtered through a sintered-glass funnel, and the residue was washed three times with 20 ml portions of 70% ethanol. The filtrates and washings

were combined and concentrated under reduced pressure below 45°C in a rotary evaporator to a volume less than 10 ml. The pigments in the extracts was removed by adding active carbon, and clarified by filtration through a sintered-glass funnel with celite bed. The filtrates was collected in a 25-ml volumetric flask and made it to the volume with water.

Free ammonium, free amino acids and total amides in the clarified extracts were separated and determined according to the procedure of Henderlong and Schmidt (1966). A Dowex-50 x 8 column (8 x 60 mm, Na^+-form, 200 - 400 mesh) was saturated with 0.2 M K, Na-phosphate buffer at pH 7.4. The clarified extracts was then passed through the column, and washed with deionized water allowing the free ammonium to be absorbed on the resin while the free amino acids, amides and neutral substances were eluted togather with water and collected in a beaker (about 30 ml). The free ammonium was then eluted from the column with 1 N KCl and the first 10 ml of the eluent was collected. The content of free ammonium was estimsted by the direct nesslerization procedure (Lang, 1958) with NH_4Cl as standard. The eluent in the beaker was adjusted to pH 2.2 with HCl, then passed through an another Dowex-50 x 8 column (8 x 60 mm, H^+-form 200 -400 mesh, saturated with 0.2 M sodium citrate buffer, pH 2.2) and washed with deionized water allowing free amino acids and amides to be absorbed on the resin while the neutral substances was washed away with water. Free amino acids and amides were then eluted from the column with 0.2 M K, Na-phosphate buffer (pH 7.4) containing 2 N KCl and the first 10 ml of the eluent was collected. The content of free amino acids was determined according to Moore and Stein (1954) with L-leucine as standard. The content of total amides, glutamine and asparagine were determined with the modified procedure of differential acid-hydrolysis according to Henderlong and Schmidt (1966). The content of free ammonium, free amino acids, total amides, glutamine and asparagine in the sample were calculated as nmole per grain or per individual tissue.

Results

The Activity of Enzymes in Developing Rice Grains

Glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in the developing rice grain and its individual tissues were assayed during grain development up to 35 days after anthesis (DAA) in the first crop season and up to 30 DAA in the second crop season. Measurable activity was recorded in all tissues of the developing grain and at all stages during grain development. Results are expressed as total enzyme activity per grain or per individual tissue.

The variation of GS, ferredoxin - and NADH - dependent GOGAT, GPT and GOT activities in the intact grain are shown in Figures 1, 2 and 3 during grain development. The GS (Fig. 1) had high activity between 5 to 20 DAA and peaked at 10 DAA in the first crop season; however, the GS had high activity between 5 to 25 DAA and peaked at 20 DAA in the second crop

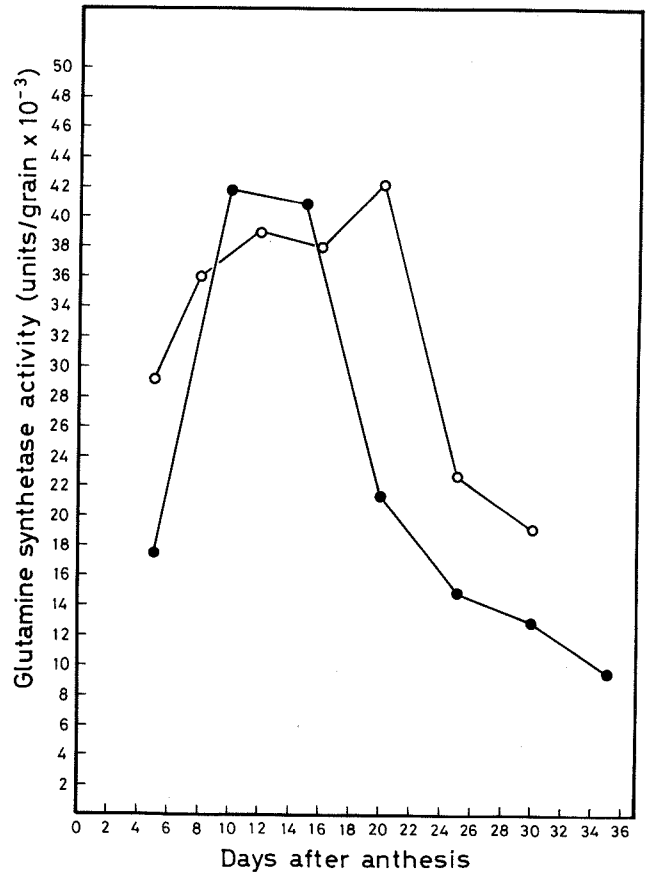

Fig. 1. Changes of glutamine synthetase activity in the intact developing rice grain of Hsinchu 56 in the first crop season (●) and the second crop season (○).

season. The activities of both the ferredoxin- and the NADH-dependent GOGAT (Fig. 2) elevated and declined twice during grain development in the first crop season. The first one appeared between 5 to 20 DAA and peaked at 10 DAA. and the second one appeared between 25 to 35 DAA and peaked at 30 DAA, Both the ferredoxin- and NADH-dependent GOGAT also elevated and declined twice of their activities during grain development in the second crop season. The first one of the ferredoxin-dependent GOGAT appeared between 5 to 12 DAA and peaked at 8 DAA, and the second one appeared between 16 to 25 DAA and peaked at 20 DAA. The first one of the NADH-dependent GOGAT appeared between 5 to 20 DAA and peaked at 8 DAA, and the second one appeared between 20 to 30 DAA and peaked at 25 DAA. The activities of both the GPT and the GOT (Fig. 3) were very high at 5 DAA and then declined gradually during the whole course of grain development in the first crop season. In contrast, both the GPT and the GOT showed their high activities between 5 to 20 DAA in the second crop season, but the GPT peaked at 15 DAA and the GOT peaked at 8 DAA. On the other hand, no any GDH activity was detacted in all the tissues of the developing grain.

The variation of GS, ferredoxin- and NADH-dependent GOGAT, GPT and GOT activities in hull, testa-pericarp and endosperm are shown in Figures 4, 5, 6 and 7 during grain development. The GS (Fig. 4) in

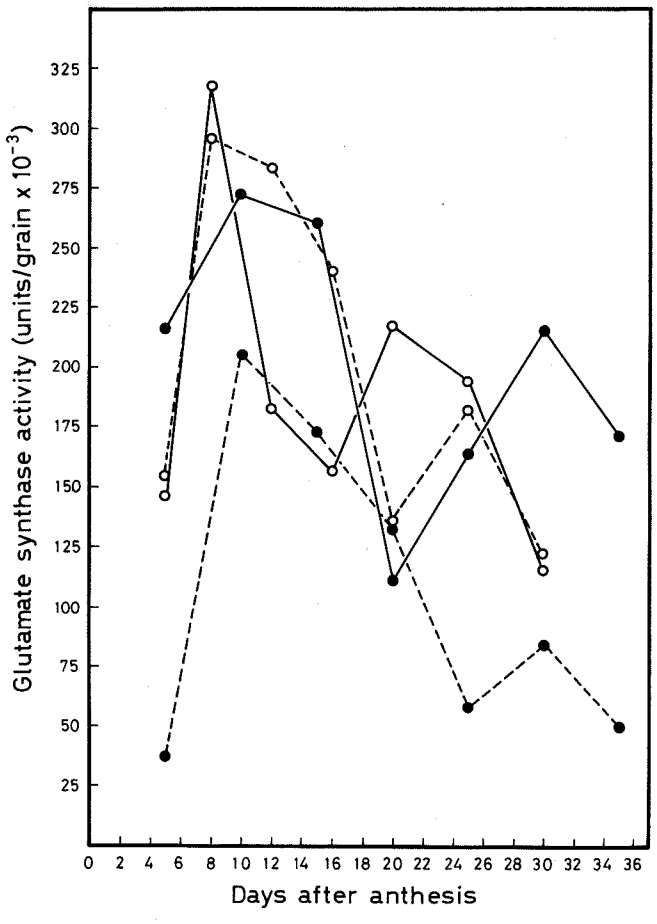

Fig. 2. Changes of ferredoxin-dependent glutamate synthase (——)and NADH-dependent glutamate synthase (----) activities in the intact developing rice grain of Hsinchu 56 in the first crop season (●) and the second crop season (○).

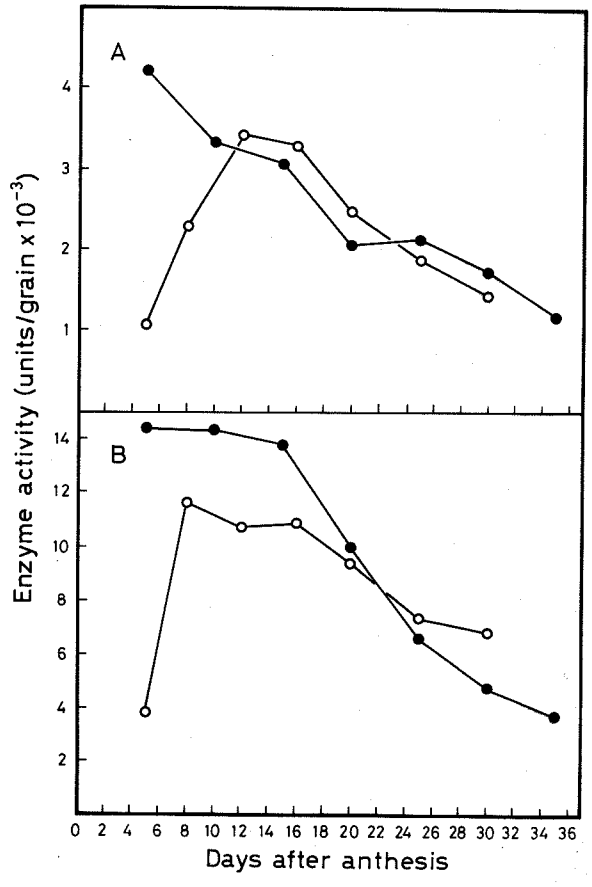

Fig. 3. Changes of glutamate pyruvate transaminase (A) and glutamate oxaloacetate transaminase (B) activities in the intact developing rice grain of Hsinchu 56 in the first crop season (●) and the second crop season (○).

the hull had very high activity at 5 DAA and then declined rapidly with the advancement of grain development either in the first or in the second crop season. In the testa-pericarp, the GS had high activity between 10 to 20 DAA and peaked at 15 DAA in the first crop season; but the GS had high activity between 8 to 25 DAA and peaked at 12 DAA in the second crop season. In the endosperm, the GS had very high activity at 10 DAA and then declined gradually with the advancement of grain development in the first crop season; but the GS had high activity between 16 to 25 DAA and peaked at 20 DAA in the second crop season. The ferredoxin-dependent GOGAT (Fig. 5) in the hull had high activity between 15 to 30 DAA and peaked at 20 DAA, and the activity of NADH-dependent GOGAT was very low during grain development in the first crop season; however, the ferredoxin-dependent GOGAT had high activity between 5 to 25 DAA and peaked at 8 DAA, and the NADH-dependent GOGAT had high activity at 8 DAA and then declined rapidly with the advancement of grain development in the second crop season. In the testa-pericarp, the ferredoxin-dependent GOGAT had high activity between 10 to 25 DAA and peaked at 20 DAA, and the NADH-dependent GOGAT had high activity between 15 to 30 DAA and peaked at 25 DAA in the first crop season; however, the ferredoxin-dependent GOGAT had high activity between 5 to 25 DAA and peaked at 12 DAA, and the NADH-dependent GOGAT had high activity at 8 DAA and declined rapidly with the advancement of grain development in the

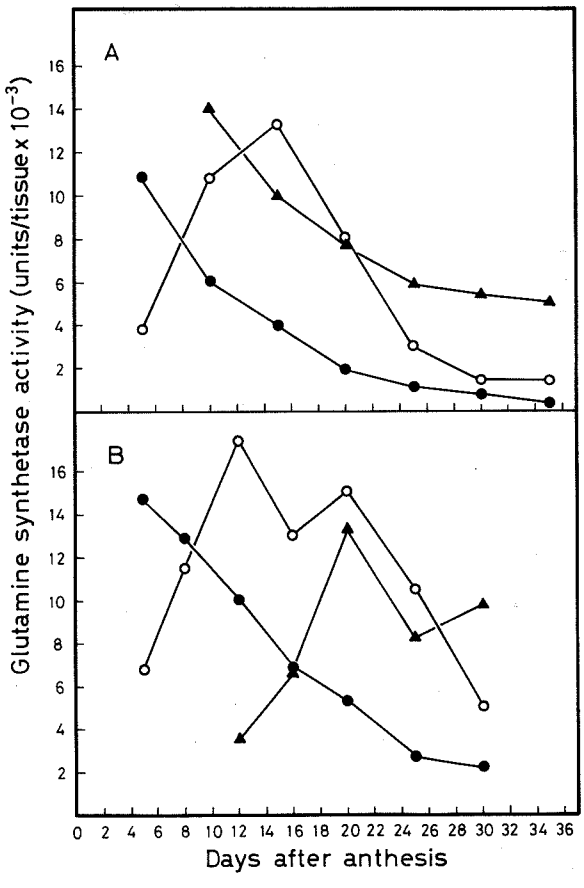

Fig. 4. Changes of glutamine synthetase activity in hull (●), testa-pericarp (○) and endosperm (▲) of the developing rice grain of Hsinchu 56 in the first crop season (A) and the second crop season (B).

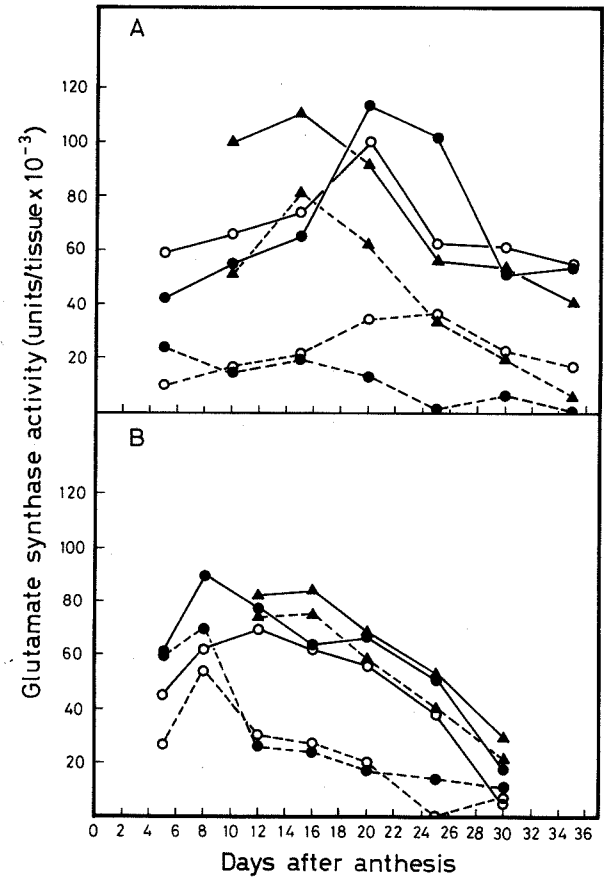

Fig. 5. Changes of ferredoxin-dependent glutamate synthase (——) and NADH-dependent glutamate synthase (----) activities in hull (●), testa-pericarp (○) and endosperm (▲) of the developing rice grain of Hsinchu 56 in the first crop season (A) and the second crop season (B).

second corp season. In the endosperm, both the ferredoxin- and the NADH-dependent GOGAT showed their high activities between 10 to 20 DAA and peaked at 15 DAA in the first corp season; however, both the ferredoxin- and the NADH-dependent GOGAT showed their high activities at 16 DAA and then declined rapidly with the advancement of grain development in the second crop season. The activity of GPT (Fig. 6) in the hull was very low during the whole course of grain development in the first crop season; however, the GPT had high activity between 8 to 20 DAA and peaked at 12 DAA in the second crop season. In the testa-pericarp, the activity of GPT eleveted and declined twice during grain development in the first crop season. The first one appeared at 5 DAA, and the second one appeared between 20 to 35 DAA and peaked at 25 DAA. However, the GPT had high activity between 8 to 20 DAA and peaked at 12 DAA in the second crop season. In the endosperm, the GPT had high activity between 20 to 35 DAA and peaked at 25 DAA in the first crop season; however, the GPT had high activity between 12 to 30 DAA and peaked at 16 DAA in the second crop season. The activity of GOT (Fig. 7) in the hull was very high at 5 DAA, and then declined gradually with the advancement of grain development in the first crop season; however, the GOT had high activity between 8 to 20 DAA and peaked at 12 DAA in the second crop season. In the testa-pericarp, the activity of GOT eleveted and declined twice during grain development in the first crop season. The first one appeared at 5 DAA, and the second one appeared between 20 to 35 DAA and peaked at 25 DAA. However, the GOT had high activity

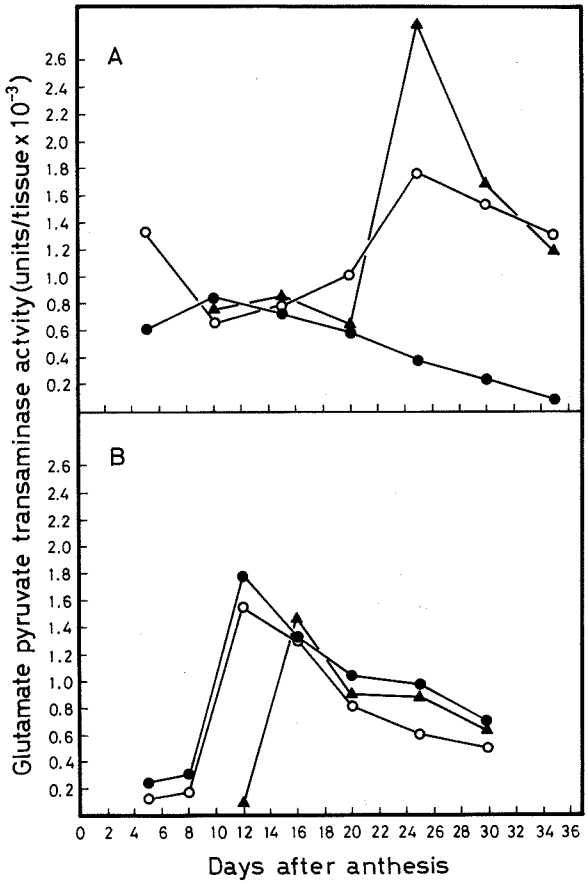

Fig. 6. Changes of glutamate pyruvate transaminase activity in hull (●), testa-pericarp (○) and endosperm (▲) of the developing rice grain of Hsinchu 56 in the first crop season (A) and the second crop season (B).

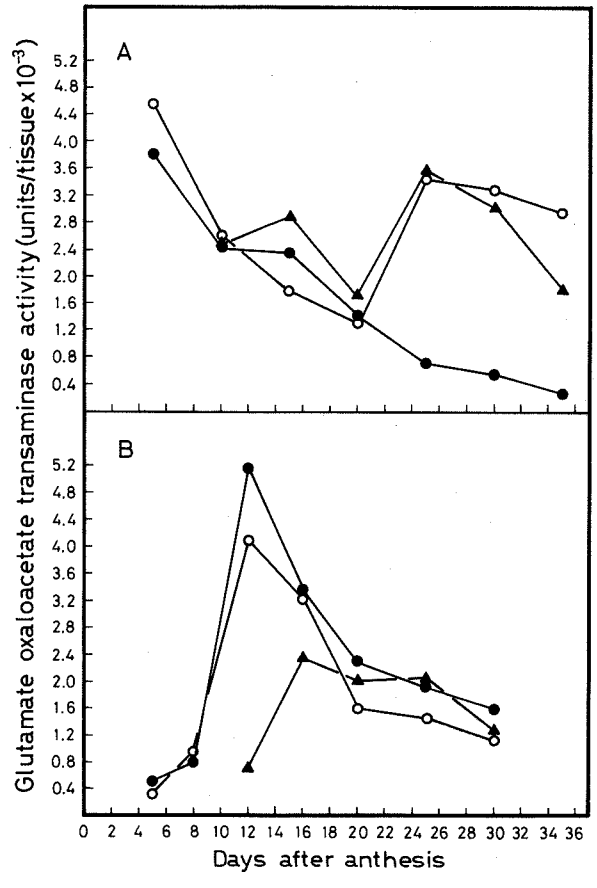

Fig. 7. Changes of glutamate oxaloacetate transaminase activity in Hull (●), testa-pericarp (○) and endosperm (▲) of the developing rice grain of Hsinchu 56 in the first crop season (A) and the second crop season (B).

bettween 8 to 20 DAA and peaked at 12 DAA in the second crop season. In the endosperm, the activity of GOT also elevated and declined twice during grain development in the first crop season. The first one appeared between 10 to 20 DAA and peaked at 15 DAA, and the second one appeared between 20 to 35 DAA and peaked at 25 DAA. However, the GOT had high activity between 12 to 30 DAA and peaked at 16 DAA in the second crop season.

The Content of Nitrogenous Compounds in Developing Rice Grains

The content of total protein in the developing grain are shown in Table 1. In the intact developing grain, the protein content increased to the highest level at 20 DAA in the first crop season, and was at 25 DAA in the second crop season during the whole course of grain development. The protein content in the hull was very low, however, it showed a high level in the early stage of grain development. The protein content in the testa-pericarp was also very low, but it still showed a peak of high level at 20 DAA. The variation of protein content in the hull and the testa-pericarp during grain development did not have significant difference between crop seasons. The protein content in the endosperm increased to the highest level at 20 DAA in the first crop season, and was at 25 DAA in the second crop season.

The content of free ammonium, free amino acids, total amides and glutamine in the intact developing grain are shown in Table 2. The content of free ammonium showed a high level in the early stage of grain development. However, the level of free ammonium in the developing grain of the first crop season was much lower than that of the second crop season. The content of free amino acids maintained at a constant level after anthesis until to the later stage of grain development, and the level of free amino acids in the developing grain of the first crop season was much higher than that of the second crop season. The content of total amides and glutamine maintained at constant levels after anthesis until the middle stage of grain develop-

Table 1. *The content of total protein in developing rice grains of Hsinchu 56*
Each value in this table represents the mean of triplicate analysis.

Days after anthesis	Intact[1] grain (mg/grain)	Tissues of rice grain[2]		
		Hull	Testa-pericarp[3]	Endosperm
		(..... mg/tissues .....)		
		The first crop season		
5	0.3525	0.1748	0.0106[4]	----
10	0.8359	0.1362	0.1341	0.2852
15	1.5081	0.1224	0.1832	0.8576
20	2.0131	0.1061	0.2305	1.3674
25	1.9319	0.0873	0.1842	1.4004
30	1.9179	0.0873	0.1610	1.3215
35	1.9033	0.0759	0.0870	1.5437
		The second crop season		
5	0.2824	0.1745	0.0538[4]	----
8	0.5142	0.1766	0.2923[4]	----
12	0.6886	0.1513	0.1794	0.0352
16	1.6304	0.1408	0.2013	0.3676
20	1.6651	0.1120	0.2831	1.0635
25	1.9427	0.0804	0.1946	1.2663
30	2.0783	0.0744	0.1311	1.5518

[1]40 grains were used in each analysis.
[2]80 tissues were used in each analysis.
[3]In the testa-pericarp included pericarp, tegmen and aleurone layer.
[4]Endosperm was included in the testa-pericarp.

Table 2. *The content of free ammonium, free amino acids, total amides and glutamine in developing rice grains of Hsinchu 56*

40 Grains were used in each analysis, and each value in this table represents the mean of triplicate analyis.

Days after anthesis	Free ammonium	Free amino acids	Total amides	Glutamine
	(nmole/grain)			
		The first crop season		
5	0.065	46.938	1.019	1.024
10	0.123	43.743	1.063	1.028
15	0.072	46.448	1.062	1.060
20	0.049	43.268	1.041	1.087
25	0.036	42.675	1.108	1.155
30	0.053	31.070	1.329	1.316
35	0.040	28.938	1.057	1.049
		The second crop season		
5	0.084	19.607	1.155	1.167
8	0.122	22.438	1.195	1.175
12	0.218	22.997	1.193	1.199
16	0.154	21.110	1.182	1.150
20	0.135	21.844	1.271	1.214
25	0.127	20.341	1.201	1.214
30	0.045	11.603	1.967	1.985

ment, and then increased their levels markedly in the later stage of grain development. However, the levels of total amides and glutamine in the developing grain of the first crop season were much lower than that of the second crop season.

The content of free ammonium, free amino acids, total amides and glutamine in hull, testa-pericarp and endosperm are shown in Table 3. The content of free ammonium in all tissues showed a high level in the early stage of grain development in both crop seasons. The content of free amino acids in all tissues maintained at a constant level throughout the entire developing stage of grain development . Howéver, the level of free amino acids in the endosperm in the first crop season was higher than that in the second crop season. In the first crop season, the content of total amides and the glutamine in all tissues showed high levels in the early stage of grain development. However, in the second crop season, the content of total amides and glutamine in the hull and the testa-pericarp showed high levels in the later stage of grain development, and in the endosperm they showed high levels in the early stage of grain development.

Discussion

Free ammonium is present in the developing rice grain during the early stage of grain development and could be utilized by GS. The nitrogen redistribution lies in favor of glutamate synthesis through the action of GOGAT. Amino acids and amides play important roles in the metabolism of nitrogen in the developing grain since they are the precussors of storage proteins in the grain. The accumulation of proteins into the endosperm of the developing rice grain is greatest in the early stage of grain development and the increase in protein content begins as early as 4 DAA (Juliano, 1972; Yamagata *et al.*, 1982). Thus, changes in activities of some enzymes related to ammonia assimilation and glutamine metabolism as well as the content of nitrogenous compounds in the developing rice grain were examined from the earliest stage of grain development. We observed that both the hull and the testa-pericarp of the developing rice grain contained chlorophyll, and changed their color from pale green to bright green in the early stage of grain development. The

Table 3. *The content of free ammonium, free amino acids, total amides and glutamine in the tissues of developing rice grain of Hsinchu 56*

80 Tissues were used in each analysis, and each value in this table represents the mean of triplicate analysis.

Days after anthesis	Free ammonium			Free amino acids			Total amides			Glutamine		
	Hull	Testa-pericarp	Endosperm	Hull	Testa-pericarp	Endosperm	Hull	Testa-pericarp	Endosperm	Hull	Testa-pericarp	Endosperm
	(nmole/tissue)											
	The first crop season											
5	0.028	0.016[1]	---	11.988	11.638[1]	---	0.757	0.727[1]	---	0.746	0.704[1]	---
10	0.037	0.033	0.036	11.568	12.337	14.976	0.671	0.813	0.696	0.664	0.725	0.699
15	0.033	0.024	0.066	11.342	14.644	18.576	0.706	0.533	0.786	0.691	0.555	0.765
20	0.036	0.027	0.030	11.598	12.512	12.442	0.522	0.666	0.655	0.511	0.655	0.643
25	0.030	0.024	0.026	11.809	11.621	13.141	0.678	0.622	0.686	0.674	0.687	0.685
30	0.030	0.017	0.026	11.425	13.351	14.504	0.661	0.711	0.760	0.647	0.704	0.751
35	0.020	0.014	0.014	11.394	13.124	12.442	0.533	0.651	0.584	0.530	0.630	0.583
	The second crop season											
5	0.029	0.014[1]	---	12.966	12.652[1]	---	0.598	0.623[1]	---	0.584	0.608[1]	---
8	0.030	0.043[1]	---	13.805	12.477[1]	---	0.620	0.599[1]	---	0.611	0.589[1]	---
12	0.040	0.035	0.014	12.198	11.726	11.796	0.632	0.616	0.656	0.614	0.608	0.652
16	0.026	0.017	0.021	11.254	12.390	11.149	0.622	0.639	0.622	0.626	0.632	0.606
20	0.024	0.016	0.024	11.495	12.442	11.953	0.652	0.646	0.648	0.650	0.648	0.644
25	0.021	0.016	0.019	11.621	10.205	10.965	0.668	0.631	0.600	0.671	0.633	0.617
30	0.016	0.012	0.013	11.058	10.730	10.537	0.732	0.689	0.548	0.728	0.680	0.539

[1]Endospern was included in the testa-pericarp.

chlorophyll disappered in the later stage of grain development. Some enzyme activities involved in CO_2 fixation and carbon metabolism in the chlorophyll-containing testa-pericarp of the immature barley grain had been demonstrated (Duffus and Rosie, 1973). The testa-pericarp also exhibited several enzyme activities associated with nitrogen metabolism (Duffus and Rosie, 1978). Nutbeam and Duffus (1978) also demonstrated that the green testa-pericarp of the immature cereal grain is active in photosynthesis. Certainly, both the green hull and the green testa-pericarp of the developing rice grain should also be active in photosynthesis and supplied the carbon skeletons required for the ammonia assimilation and amino acids transformation in the developing grain. Activities of GS, feredoxin- and NADH-dependent GOGAT, GDH, GPT and GOT in the intact developing rice grain as well as in hull, testa-pericarp and endosperm were assayed during grain development. The results showed that GS, ferredoxin- and NADH-dependent GOGAT, GPT and GOT showed very high activities in the early stage of grain development up to 20 DAA, but no any GDH activity was detacted in all tissues of the developing grain during grain development. The lack of GDH indicated that the GS/GOGAT cycle is the only pathway to operate ammonia assimilation in the developing rice grain that is different from the mode to operate ammonia assimilation in the developing wheat grain (Garg *et al.*, 1985).

In the intact developing rice grain, the appearance of the high activity of GS (Fig. 1) completely matches the appearance of major peaks of ferredoxin- and NADH-dependent GOGAT activities (Fig. 2) in the early stage of grain development in the first crop season. However, the appearance of the high activity of GS only partially matches the appearance of major peaks of the two GOGAT activities in the second crop season. Therefore, the ammonia assimilation in the developing grain via the pathway of GS/GOGAT cycle in the first crop season should be more effective than that in the second crop season. The appearance of high activities of GPT and GOT in the early stage of grain

development (Fig. 3) indicated that the transamination in the developing grain operated actively in the early stage of grain development, but transamination in the first crop season took place earlier than that in the second crop season. It offers an advantage in the biosynthesis of proteins in the developing grain during the first crop season for a rapid accumulation of storage proteins inside the developing grain. The protein accumulation (Table 1) in the developing grain of the first crop season reaches its highest level about 5 days earlier than that of the second crop season. The content of free ammonium, total amides and glutamine (Table 2) in the developing grain of the first crop season were much lower than that of the second crop season, but the content of free amino acids in the developing grain of the first crop season was much higher than that of the second crop season. These results support the notion that the ammonia assimilation was more effective and the transamination took place earlier in the developing grain of the first crop season as compared with that in the second crop season.

In the hull of the developing rice grain, the appearance of high activities of GS (Fig. 4) and ferredoxin-dependent GOGAT (Fig. 5) indicated that the ammonia assimilation in the hull is mainly via the co-operation of GS and ferredoxin-dependent GOGAT in the early stage of grain development. The ferredoxin-dependent GOGAT is the main enzyme in the hull for the biosynthesis of glutamate in both crop seasaons. Both the GPT and the GOT in the hull (Figs. 6 and 7) showed low activities during grain development in the first crop season, but they showed high activities in the early stage of grain development in the second crop season. Obviously, the transamination in the hull of the developing grain in the early stage of grain development in the first crop season operated less active than that in the second crop season. The produced glutamine and glutamate in the hull might be translocated into the endosperm for the biosynthesis of storage proteins, or transaminated through GPT and GOT and then incorporated into soluble enzyme proteins immediately in the earliest stage of grain development. This might be the reason why the protein content in the hull (Table 1) was very low during grain development and the content of the total amides and glutamine in the hull (Table 3) showed high levels in the early stage of grain development in the first crop season. Therefore, the hull plays an important role in ammonia assimilation and glutamine metabolism in the early stage of grain development.

In the testa-pericarp of the developing rice grain, the appearance of the high activity of GS (Fig. 4) completely matches the high activity of ferredoxin-dependent GOGAT (Fig. 5), but does not match the high activity of NADH-dependent GOGAT during grain development in both crop seasons. Thus, the ammonia assimilation in the testa-pericarp is also mainly via the co-operation of GS and ferredoxin-dependent GOGAT in the middle stage of grain development. Furthermore, the activity of NADH-dependent GOGAT was much lower than that of ferredoxin-dependent GOGAT in the developing grain. Therefore, the ferredoxin-dependent GOGAT is also the main enzyme in the testa-pericarp for the biosynthesis of glutamate in both crop seasons. The appearance of high activities of GPT and GOT in the testa-pericarp (Figs. 6 and 7) indicated that the process of transamination in the testa-pericarp operated actively in the later stage of grain development in the first crop season; however, it operated actively in the early stage of grain development in the second crop season. The low level of protein content in the testa-pericarp (Table 1) indicated that the produced glutamine and glutamate in the testa-pericarp might also be translocated into the endosperm, or transaminated and incorporated into soluble enzyme proteins in the early stage of grain development. The content of total amides and glutamine in the testa-pericarp (Table 3) showed high levels in the early stage of grain development in the first crop season, and they showed high levels in the later stage of grain development in the second crop season. These results were consistant with the appearance of high activities of GPT and GOT in the testa-pericarp of the developing grain in both crop seasons.

In the endosperm of the developing rice grain, the appearance of the high activity of GS (Fig. 4) matches high activities of ferredoxin- and NADH-dependent GOGAT (Fig. 5) in the early stage of grain development in the first crop season; however, the high activity of GS matches high activities of ferredoxin- and NADH-dependent GOGAT in the middle stage of grain development in the second crop season. Therefore, the ammonia assimilation in the endosperm operated actively in the early stage of grain development in first crop season, but it operated actively in the middle stage of grain development in the second crop season. Conse-

quently, leading the accumulation of proteins into the endorsperm in the first crop season reaches the highest level about 5 days earlier than that in the second crop season (Table 1). Both the ferredoxin- and NADH-dependent GOGAT are the same important enzymes in the endosperm for the biosynthesis of glutamate in both crop seasons. The appearance of high activities of GPT and GOT in the endosperm (Figs. 6 and 7) indicated that the transamination in the endosperm operated actively in the later stage of grain development in both crop seasons. The content of free amino acids, total amides and glutamine in the endosperm (Table 3) of the first crop season were higher than that in the second crop season. They were also the effective factors to promote the accumulation of proteins into the endosperm in the first crop season. The increment of protein content in the endosperm was consistant with the increment of protein content in the intact grain. It indicated that most of the protein in the developing grain is accumulated into the endosperm.

On the other hand, the amount of glutamine in the intact developing rice grain and its tissues was almost equal to the amount of total amides at the same developing stage during grain development in both crop seasons, and no any asparagine was observed in all tissues at any developing stage. It indicated that all of the amides in the developing grain is the glutamine.

Acknowledgements. This work was financially supported by the National Science Concial under Grant No. NSC76-0201-B001-30 and Academia Sinica of the Republic of China.

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發育中之水稻種子中氨之同化和麩醯胺之代謝與蛋白質蓄積之關係

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於發育中之水稻種子及其穀殼、種皮和胚乳中之麩醯胺合成酶，依賴 Ferredoxin 及依賴 NADH 之麩氨酸合成酶、麩氨酸去氫酶、丙氨酸轉氨酶和天冬氨酸轉氨酶之活性，經測試後發現於種子發育期間，其體素中沒有麩氨酸去氫酶之活性；而其他各種酶之活性則很強。顯然麩醯胺合成酶和麩氨酸合成酶連鎖環之催化作用，是發育中之水稻種子中進行氨同化作用之唯一途徑。於兩期稻作間，以第一期作的發育中之種子中，其氨同化作用之進行較爲活躍，轉氨基作用發生得較早。蛋白質蓄積之速度亦較快；種子中游離態氨、總醯胺及麩醯胺之含量較低，而游離氨基酸之含量則較高。無論爲一期作或二期作，於穀殼、種皮及胚乳中，氨同化作用之進行，主要皆依循麩醯胺合成酶和依賴 Ferredoxin 之麩氨酸合成酶連鎖環之催化途徑。於穀殼及種皮中，僅於第二期作時，在種子發育初期，其中轉氨基作用較爲活躍；可是於胚乳中，無論爲一期作或二期作，在種子發育後期，其轉氨基作用都很活躍。第一期作之種子，其胚乳中蛋白質蓄積之速度較快，游離氨基酸之含量亦較高；於種子發育初期，其各體素中之總醯胺及麩醯胺之含量亦較高。