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SEASONAL VARIATIONS IN BIOSYNTHESIS OF AMIDES DURING RICE GROWTH[1,2]

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Abstract

In the first crop season, both the glutamate synthase and glutamate dehydrogenase in leaves and sheaths showed the highest activity at tillering and a higher activity at flowering, and the glutamate synthase still remained a higher activity at harvesting. The glutamine synthetase in leaves showed higher activities at the beginning of tillering and flowering. In the second crop season, the glutamate synthase in leaves and sheaths showed higher activities at the beginning of tillering, booting and ripening to harvesting. The glutamate dehydrogenase in leaves and sheaths showed higher activities at tillering and flowering. The glutamine synthetase in leaves of Tainung 62 showed a higher activity at the end of tillering only, and of Ai-chaw-wu-chien at the beginning of tillering and booting. The glutamine synthetase in sheaths showed a higher activity at flowering, and the glutamate synthase and glutamine synthetase in roots showed higher activity at tillering regardless of crop seasons. The glutamate dehydrogenase in roots showed a higher activity at tillering in the first crop season, and at booting in the second crop season. The asparagine synthetase in leaves, sheaths and roots of the first crop season maintained a rather constant activity during rice growth; however, in leaves and sheaths of the second crop season, it showed a very high activity at flowering. In the first crop season, the total amides and glutamine content in leaves of Tainung 62 showed higher levels at tillering and flowering, and of Ai-chaw-wu-chien at tillering and ripening. The asparagine content in leaves of Tainung 62 showed higher levels at tillering and ripening, and of Ai-chaw-wu-chien at flowering and ripening. The total amides and glutamine contents in sheaths showed the highest level at booting. The total amides and glutamine contents of Tainung 62 as well as the total amides and asparagine contents of Ai-chaw-wu-chien in roots showed higher levels at the beginning of tillering and booting. In the second crop season, the total amides and asparagine contents in leaves showed higher levels at tillering and flowering. The glutamine content in leaves of Tainung 62 showed a higher level at tillering only, and of Ai-chaw-wu-chien from ripening to harvesting. The total amides and glutamine contents in sheaths showed a very high level at booting and a higher level at

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ripening. The asparagine content in sheaths of Tainung 62 showed a higher level at booting only, and of Ai-chaw-wu-chien at booting and harvesting. The total amides and glutamine contents in roots of Tainung 62 showed a higher level at booting, and the total amides and asparagine contents of Ai-chaw-wu-chien showed a higher level at tillering. The free ammonium content in leaves showed two higher levels at tillering and flowering in the first crop season, and at tillering and ripening in the second crop season.

Introduction

Much attention has been focused on the assimilation of ammonium by plants since ammonium plays an important role in nitrogen metabolism. There are many examples about the conspicuous increase of glutamic acid and glutamine in higher plants and microorganisms when ammonium or nitrate is assimilated (Yemm and Folks, 1958). The early studies indicated that the ammonium salts assimilated by plants were stored mostly as amides, and some of the ammonium nitrogen was transformed and incorporated into organic compounds already in the root system, especially, into the amide group of glutamine and asparagine (Kretovich, 1965). It is generally considered that the main route of nitrogen assimilation in higher plants occurred via the reductive amination of α-ketoglutarate to form glutamate by the catalysis of glutamate dehydrogenase. Moreover, the ammonium could be incorporated into glutamate to form glutamine by the catalysis of glutamine synthetase. Recently, the presence of glutamate synthase in higher plants has been demonstrated. The synthesis of glutamate is not entirely mediated by glutamate dehydrogenase (Lea and Miflin, 1974). Some investigations proved that glutamine and asparagine are usually predominant as transport and storage forms of nitrogen in higher plants (Oji and Izawa, 1972; Pate, 1973). Kanamori and Matsumoto (1974) reported that a large amount of asparagine was synthesized in rice seedling, and the biosynthesis of asparagine was intimately related to the presence of glutamine. Yoneyama and Kumazawa (1974) reported that a high level of asparagine content was found in rice seedlings when seedlings grew in the culture mdium containing ammonium nitrogen. Marwaha *et al.* (1976) also reported that the contents of asparagine and glutamine were very high both in roots and shoots of rice seedlings while they were cultivated in the medium containing ammonium nitrogen. Therefore, the biosynthesis of amides is very important in relation to the assimilation, translocation, storage and metabolism of nitrogen in rice plants during growth. Lewis (1975) indicated that amides influence rice growth and the developing grains directly. Rice is a major crop in Taiwan and it is harvested twice a year. The growth patterns of rice plants are different between the two crop seasons due to difference of weather conditions, and

the grain yield per unit area in the second crop season is always about 25% lower than that in the first crop season. Yuan and Shieh (1980) reported that the variations of amide nitrogen content in leaves of rice plants during growth were different between the two crop seasons. It might indicate that the variations of nitrogen assimilation, translocation, storage and metabolism during rice growth may also be different between the two crop seasons. However, little is known about the variations of enzyme systems in biosynthesis of amides and the contents of glutamine and asparagine in rice plants during growth. This paper reports the changes in activities of glutamate synthase, glutamate dehydrogenase, glutamine synthetase and asparagine synthetase as well as the contents of total amides, glutamine and aspasagine in leaves, sheaths and roots during rice growth.

Materials and Methods

Plant materials

Rice plants were grown at the farm of the Institute of Botany, Academia Sinica located in Nankang, Taipei, Taiwan. The first crop season started in the middle of March and ended in the middle of July, and the second crop season started in the middle of August and ended in the middle of December. Two rice varieties of Tainung 62 (Japonica type) and Ai-chaw-wu-chien (Indica type) were employed. Rice seedlings at four leaves stage were transplanted to paddy field with 5 to 7 seedlings as one hill and with a planting density of 20×25 cm (20 hills/m^2). The basal dressing of fertilizer (N_2:P_2O_5:K_2O=30:50:25 kg/ha) was supplied on the surface within 4 cm. The time of fertilizer treatment for top dressing depended upon the leaf-age index of rice plants. Ammonium nitrogen (N_2=35 kg/ha) was supplied for top dressing in the early growth stage at leaf-age index before 69. The top dressing was made on the 30th day after transplanting in the first crop season, and on the 20th day in the second crop season. Ammonium nitrogen (N_2=35 kg/ha) and potassium salt (K_2O=25 kg/ha) were supplied for top dressing in the later growth stage at leaf-age index after 92. The top dressing was made on the 81th day after transplanting in the first crop season, and on the 68th day in the second crop season. The drying treatment in the middle growth stage was at leaf-age index between 69 to 92. During that time, water supply was stopped until the surface of paddy soil showed cracks of 1 to 2 cm in width.

Sampling and treatment of samples

The sampling was started from the fourth week after transplanting in the first crop season and from the third week in the second crop season.

Thereafter, the sampling was made at every two- or three-week intervals throughout the growth period of rice plants. Samplings were usually made between 10 to 12 A.M. The sampling method was that the whole hill was scooped out from the paddy soil and eight hills were taken as one sample. Leaves, sheaths and roots were collected, cleaned with distilled water and cut into 5 to 10 mm in length. One part of the sample was placed in a plastic bag and kept below 5°C for determination of enzyme activities as soon as possible. Another part of the sample (10 grams) was soaked in 100 ml of 80% ethanol for determination the contents of total amides, glutamine and asparagine.

Extraction of enzymes

The glutamate synthase was extracted with 0.05 M Tris-HCl buffer (pH 7.5) containing 1mM EDTA-2Na and 10 mM 2-mercaptoethanol according to the procedure of Sodek *et al.* (1977). The glutamate dehydrogenase was extracted with 0.066 M Tris-HCl buffer (pH 8.0) containing 5×10^{-5}M cysteine and 0.1% Triton X-100 following the method reported previously (Yuan and Shieh, 1980). The glutamine synthetase was extracted with 0.05 M Tris-HCl buffer (pH 7.5) containing 2 mM EDTA-2Na and 1 mM 2-mercaptoethanol according to Kanamori and Matsumoto (1972). The asparagine synthetase was extracted with 0.1 M Tris-HCl buffer (pH 7.5) containing 15% (w/v) glycerol and 25 mM 2-mercaptoethanol according to Lea and Fowden (1975). The sample (10 grams) was homogenized with 50 ml of the extracting buffer individually in an ice bath using a Polytron homogenizer at 10,000 rpm for two minutes. The homogenate was squeezed through four layer of cheesecloth. The extract was centrifuged for 20 minutes at 12,000×g. An aliquot of 5 ml was then passed through a column of Sephadex G-25 (1.5×16 cm) equilibrated with the extracting buffer and the protein was eluted with the same buffer. The pretein fraction was collected in a volume of 16 ml and designated as the enzyme extract.

Enzyme assays

The glutamate synthase activity in the enzyme extract was assayed according to the procedure of Wallsgrove *et al.* (1977). The reaction mixture consisted of 50 mM Tris-HCl buffer (pH 7.5), 10 mM α-ketoglutarate, 10 mM L-glutamine, 125 μg methyl viologen, 10 mM α-aminooxyacetate and up to 0.15 ml of enzyme solution in a total volume of 0.5 ml. The reaction was started by the addition of 0.1 ml of reductant containing 16 mg $Na_2S_2O_4$ and 16 mg $NaHCO_3$ ml^{-1}. After incubation at 30°C for 15 minutes, the reaction was stopped by adding 1 ml of 95% ethanol followed by vigorous shaking to oxidize the methyl viologen and dithionite. All reactions were carried out

with a minus reductant blank. The glutamate formed was isolated by paper chromatography with a solvent system of n-butanol: acetic acid: water (4:1:1, v/v). The glutamate was located by the aid of guiding authentic glutamate and eluted from the chromatogram with distilled water. The glutamate content in the eluate was determined according to Moore and Stein (1954). One unit of glutamate synthase was defined as the amount of enzyme catalyzing synthesis of 1.0 nmole of glutamate per minute at 30°C.

The glutamate dehydrogenase activity in the enzyme extract was determined by measuring the initial rate of NADH oxidation at 340 nm at 30°C with a Gilford 250 spectrophotometer following the procedure reported previously (Yuan and Shieh, 1980). One unit of glutamate dehydrogenase was defined as the amount of enzyme causing a change of 0.01 absorbance at 340 nm per minute.

The glutamine synthetase activity in the enzyme extract was assayed according to Kanamori and Matsumoto (1972). The assay system consisted of 0.5 ml of 0.2 M Tris-HCl buffer (pH 7.5), 0.2 ml of 0.05 M ATP (pH 7.0), 0.5 ml of Na-glutamate, 0.1 ml of 1.0 M $MgSO_4$, 0.3 ml of 0.1 M NH_2OH (pH 7.0; freshly prepared), 0.1 ml of 0.1 M cysteine and 1.0 ml of enzyme solution. The final volume was completed to 3.0 ml with distilled water. The reaction was started by adding glutamate, and the glutamate was omitted in the blank test. After incubation at 30°C for 15 minutes, the γ-glutamate hydroxamate formed was determined by adding 1.0 ml of ferric chloride reagent (equal volume of 10% $FeCl \cdot 6H_2O$ in 2N HCl, 24% TCA, and 6N HCl were mixed together) and measuring absorbance of the reaction mixture (after centrifugation) at 540 nm with a Gilford 250 spectrophotometer, and the commercial γ-glutamate hydroxamate was used as standard. One unit of glutamine synthetase was defined as the amount of enzyme catalyzine the formation of 1.0 nmole of γ-glutamate hydroxamate per minute at 30°C.

The assay of asparagine synthetase activity in the enzyme extract was based on Lea and Fowden (1975) with some modifications. The reaction mixture consisted of 0.125 ml of 0.2 M Tris-HCl buffer (pH 8.0), 0.025 ml of 0.02 M aspartate, 0.02 ml of 0.05 M L-glutamine, 0.01 ml of 0.05 M ATP, 0.01 ml of 0.5 M $MgCl_2$, 0.01 ml of 1.25 M 2-mercaptoethanol, 0.01 ml of 0.1 M α-aminooxyacetate and up to 0.05 ml of enzyme solution in a total volume of 0.25 ml. The reaction was started by adding aspartate, and the aspartate was omitted in the blank test. After incubation at 37°C for 30 minutes, the reaction was stopped by adding 0.5 ml of 95% ethanol. The asparagine formed was isolated by paper chromatography with a solvent system of n-butanol: acetic acid: water (4:1:1, v/v). The asparagine was located by the aid of guiding authentic asparagine and eluted from the chromatogram with distilled water. Because of the eluate was still

contaminated with a small amount of glutamine, the asparagine content in the eluate was determined according to Henderlong and Schmidt (1966) with a modified procedure of differential acid-hydrolysis. Two separate aliquotes of the same eluate were analyzed as follows: In the first aliquot, the total amide-nitrogen was determined as the amount of ammonium-nitrogen released after hydrolysis in 1 N H_2SO_4 at 100°C for 3 hours in a sealed tube. In the second aliquot, the glutamine amide-nitrogen represented the ammonium-nitrogen released after hydrolysis in phosphate-borate buffer at pH 6.5 for 2 hours at 100°C in a sealed tube (the ratio of sample to 0.1 M potassium phosphate-borate buffer is 1:2 in volume). Since the amide group of asparagine was not hydrolyzed in phosphate-borate buffer or in subsequent nesslerization step, the asparagine amide-nitrogen was equal to the difference between the total amide-nitrogen and the glutamine amide-nitrogen. The ammonium-nitrogen released during each hydrolysis was estimated by the direct nesslerization procedure (Lang, 1958). After adding of Nessler's reagent to the hydrolysate, maximal colour development was achieved in a 10 minutes, and the absorbance was measured at 412 nm with a Gilford 250 spectrophotometer. One unit of asparagine synthetase was defined as the amount of enzyme catalyzing synthesis of 1.0 nmole of asparagine per minute at 30°C.

The activities of glutamate synthase, glutamate dehydrogenase, glutamine synthetase and asparagine synthetase in samples are calculated as units per gram fresh weight of sample.

Determination of free ammonium, total amides, glutamine and asparagine

Ten grams of sample which was soaked in 100 ml of 80% ethanol as described before was homogenized for 3 minutes using a Polytron homogenizer at 10,000 rpm, and then filtered through a sintered-glass funnel. The residue was washed three times with 40 ml portions of 70% ethanol. The extracts and washings were combined and concentrated under reduced pressure below 40°C in a rotary eveporator, and the final volume was adjusted to 25 ml with water. The precipitates was removed by centrifugation. The pigments in the extracts were removed by adding active carbon. The solution was then clarified by filtration using a sintered-glass funnel with celite bed.

Free ammonium and amides in the clarified extracts were isolated and determined according to the procedure of Henderlong and Schmidt (1966). A Dowex-50×8 column (1.5×4 cm, Na^+-form, 200–400 mesh) was prepared and saturated with 0.2M sodium potassium phosphate buffer at pH 7.4 (Hutchinson and Labby, 1962). An aliquot (10 ml) of the extracts was passed through the column allowing the free ammonium to be absorbed on the resin while the amides, amino acids and some neutral substances were eluted together

with deionized water and collected in a beaker (about 50 ml). The ammonium was then eluted from the column with 25 ml of 1 N KCl and washed with deionized water. The eluate was collected into 50-ml volumetric flask and made it to volume with deionized water. The content of ammonium in the eluate was estimated by the direct nesslerization procedure (Lang, 1958). The eluate, containing the amides, amino acide and neutral substances, was adjusted to pH 2.2 with 0.5 N HCl, then passed through another Dowex-50×8 column (1.5×4 cm, H^+-form, 200–400 mesh, saturated with 0.2 M citrate buffer, pH 2.2) allowing amides and amino acids to be absorbed on the resin while the neutral substances were washed away with deionized water. Amides and amino acids were then eluted from the column with 25 ml of 0.2 M sodium potassium phosphate buffer at pH 7.4 containing 2 N KCl. The eluate was collected into a 25-ml volumetric flask and made it to volume. The content of total amides, glutamine and asparagine in the eluate were estimated with the modified procedure of differential acid-hydrolysis (Henderlong and Schmidt, 1966). The content of free ammonium, total amides, glutamine and asparagine in samples were calculated as μmole per gram fresh weight of sample.

Results

Variations of glutamate synthase activity during rice growth

The glutamate synthase activities in leaves, sheaths and roots of Tainung 62 and Ai-chaw-wu-chien are shown in Fig. 1. Obviously, no significant difference in the glutamate synthase activity could be found between rice varieties. In the first crop season, the glutamate synthase activity in leaves and sheaths showed the highest level at the beginning of tillering, and decreased to a very low level at the booting stage, then increased to a higher level from flowering through ripening until harvesting. In the second crop season, the glutamate synthase activity in leaves and sheaths showed a higher level at the beginning of tillering. Although the activity decreased slightly at the tillering stage, it increased to a higher level again at the booting stage. Thereafter, the activity decreased to a very low level at the flowering stage and then increased to a higher level again form ripening to harvesting. In roots, the glutamate synthase activity showed a higher level at the tillering stage regardless of crop seasons. According to crop seasons, the glutamate synthase activity in leaves and sheaths at the tillering stage of the first crop season was much higher than that of the second crop season.

Variations of glutamate dehydrogenase activity during rice growth

The glutamate dehydrogenase activity in leaves, sheaths and roots of Tainung 62 and Ai-chaw-wu-chien are shown in Fig. 2. In the first crop

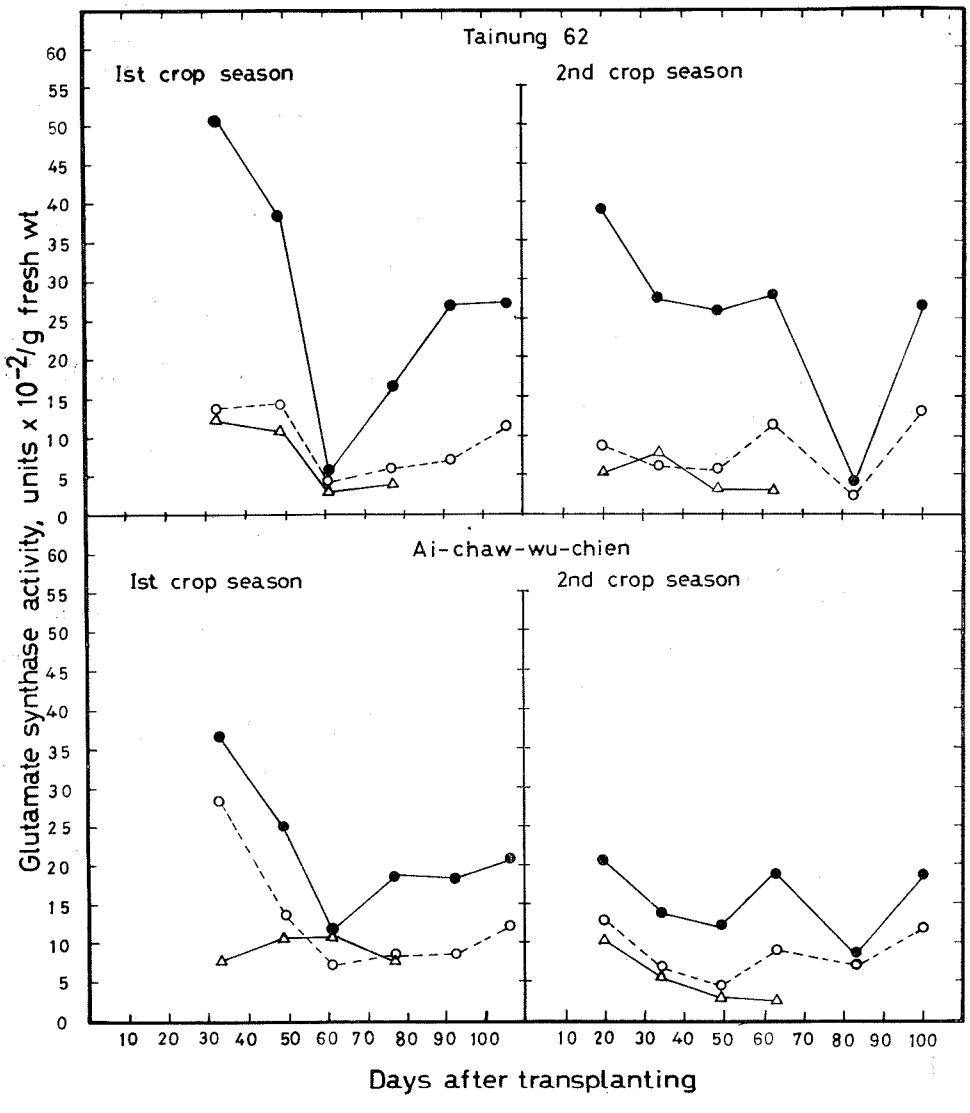

Fig. 1. Glutamate synthase activities in leaves (—●—●—), sheaths (--○--○--) and roots (—△—△—) of Tainung 62 and Ai-chaw-wu-chien during growth.

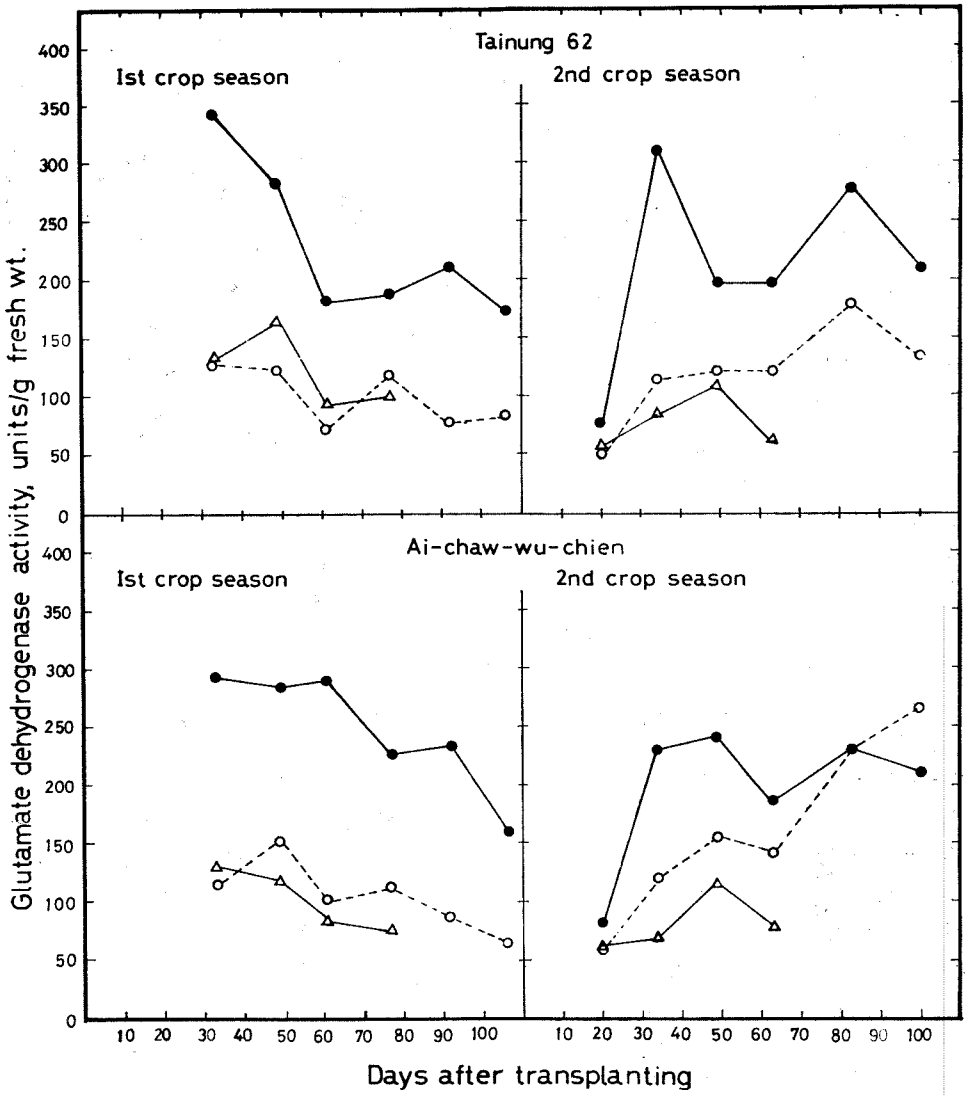

Fig. 2. Glutamate dehydrogenase activities in leaves (—●—●—), sheaths (--○---○----) and roots (-△—△—) of Tainung 62 and Ai-chaw-wu-chien during growth.

season, the glutamate dehydrogenase activity in leaves and sheaths showed a higher level at the tillering stage, and decreased to a lower level at the booting stage, then increased slightly at flowering; afterwards, the activity decreased to a lower level from ripening to harvesting. The glutamate dehydrogenase activity in roots showed a higher level at the tillering stage. In the second crop season, the glutamate dehydrogenase activity in leaves and sheaths showed a very low level at the beginning of tillering, however, the activity increased rapidly to a very high level at the tillering stage, but decreased to a lower level at booting, then increased to a higher level again at the flowering stage. Although the activity decreased slightly after flowering, it still maintained relatively high level through ripening to harvesting. The glutamate dehydrogenase activity in roots showed a higher level at the booting stage. The glutamate dehydrogenase activity in roots of the first crop season was higher than that of the second crop season in the early growth stage, however, the activity in sheaths of the first crop season was much lower than that of the second crop season in the later growth stage.

Variations of glutamine synthetase activity during rice growth

The glutamine synthetase activity in leaves, sheaths and roots of Tainung 62 and Ai-chaw-wu-chien are shown in Fig. 3. In the first crop season, the glutanine synthetase activity in leaves of Tainung 62 showed a higher level at the tillering stage, and decreased to a lower level at booting, then increased to a higher level again at the flowering stage. However, the glutamine synthetase activity in leaves of Ai-chaw-wu-chien showed a higher level at the beginning of tillering, and decreased to a lower level at the end of tillering, then increased to a higher level at the booting and flowering stages. After flowering, the glutamine synthetase activity decreased to a lower level from ripening to harvesting regardless of rice varieties. In the second crop season, the glutamine synthetase activity in leaves of Tainung 62 showed a lower level at the beginning of tillering, and increased to a higher level at the end of tillering, then decreased to a lower level with the advancement of rice growth until harvesting. However, the glutamine synthetase activity in leaved of Ai-chaw-wu-chien showed a higher level at the beginning of tillering, and decreased to a lower level at the end of tillering, then increased to a higher level again at the booting stage; thereafter, it decreased to a lower level from flowering until harvesting. The glutamine synthetase activity in sheaths showed a lower level at tillering, and increased gradually to reach the highest level at flowering, then decreased to a lower level at harvesting regardless of crop seasons and rice varieties. The glutamine synthetase activity in roots showed a higher level at tillering also regardless of crop seasons and rice varieties.

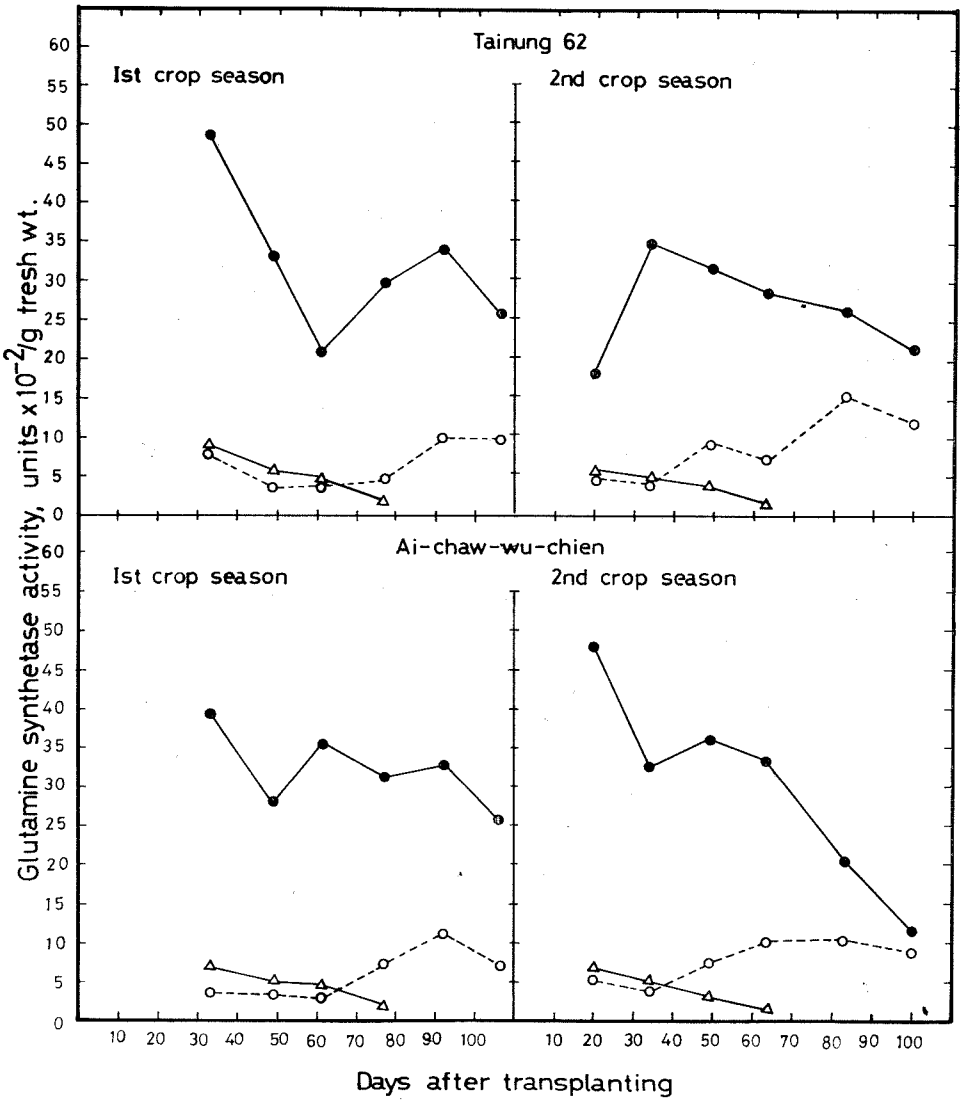

Fig. 3. Glutamine synthetase activities in leaves (—●—●—), sheaths (--○---○--) and roots (—△—△—) of Tainung 62 and Ai-chaw-wu-chien during growth.

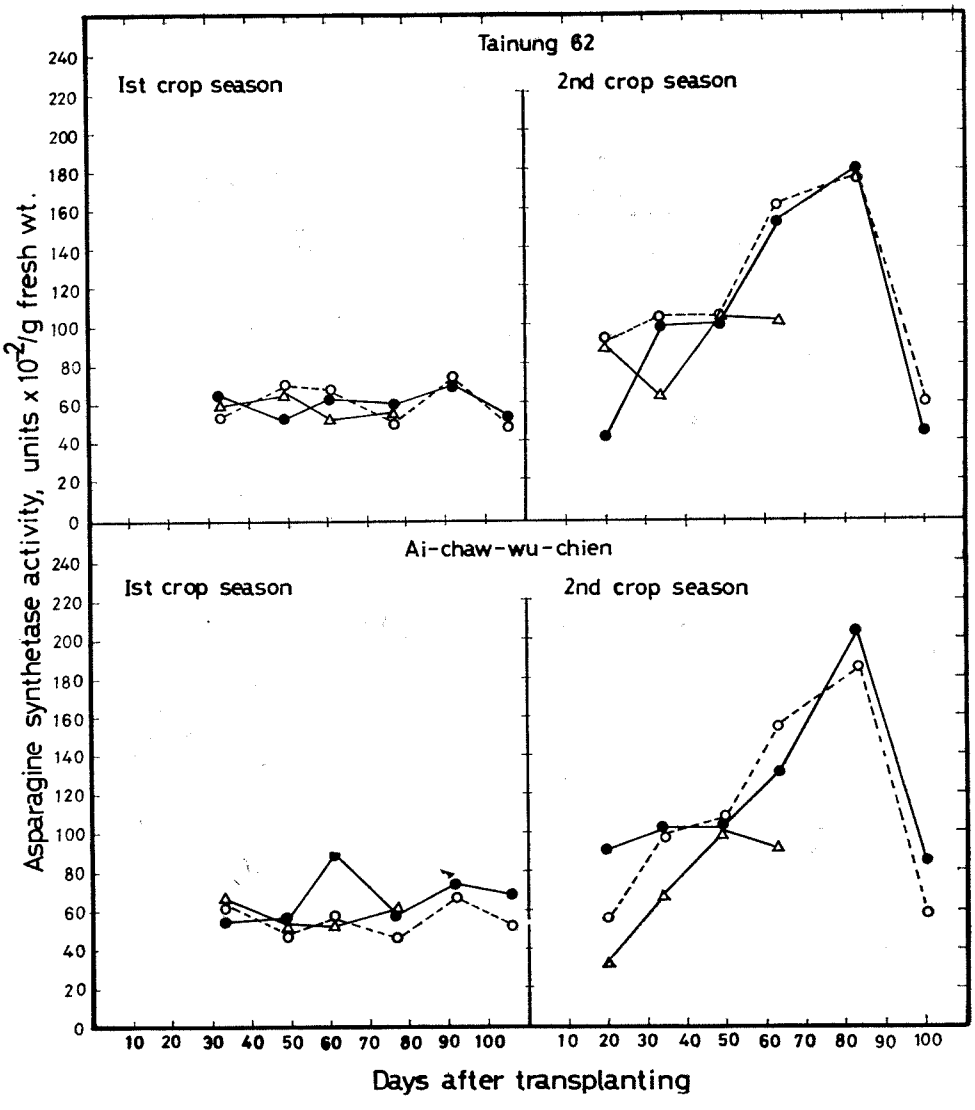

Fig. 4. Asparagine synthetase activities in leaves (—●—●—), sheaths (--○---○--) and roots (—△—△—) of Tainung 62 and Ai-chaw-wu-chien during growth.

Variations of asparagine synthetase activity during rice growth

The asparagine synthetase activity in leaves, sheaths and roots of Tainung 62 and Ai-chaw-wu-chien are shown in Fig. 4. In the first crop season, the asparagine synthetase activity in leaves, sheaths and roots maintained a rather constant level during rice growth. In the second crop season, the asparagine synthetase activity in leaves and sheaths showed a lower level at the tillering stage, and increased rapidly to a very high level through the booting stage to reach the highest level at flowering, then decreased to a lower level from ripening to harvesting. The asparagine synthetase activity in roots showed a higher level at the beginning of booting. however, the asparagine synthetase activity in leaves, sheaths and roots of the second crop season was much higher than that of the first crop season at the booting and flowering stages.

Changes of free ammonium and amides content during rice growth

The changes of free ammonium content in leaves, sheaths and roots are shown in Fig. 5. In the first crop season, the free ammonium content in leaves and sheaths showed higher levels at the tillering and ripening stages. However, in the second crop season, the free ammonium content in leaves showed higher levels at the tillering and flowering stages, and in sheaths at the flowering stage only. The free ammonium content in roots showed a higher level at the tillering stage. However, the free ammonium content in roots of the first crop season was much higher than that of the second crop season.

The changes of total amides, glutamine and asparagine contents in levaes are shown in Fig. 6. In the first crop season, the total amides and glutamine contents of Tainung 62 showed a higher level at the tillering stage, and decreased slightly at booting, then increased to a higher level again at flowering; thereafter, it decreased to a lower level from ripening to harvesting. The total amides and glutamine contents of Ai-chaw-wu-chien also showed a higher level at the tillering stage, and decreased slightly at the booting stage through flowering, then increased to a higher level at ripening; afterwards, it decreased to a lower level at harvesting. The aspargine content of Tainung 62 showed a higher level at the tillering stage, and decreased gradually to a lower level at flowering, then increased to reach a higher level at the ripening stage; afterwards, it decreased to a lower level at harvesting, The asparagine content of Ai-chaw-wu-chien showed lower levels at the tillering and booting stages, then increased to a higher level at the flowering stage; afterwards, it decreased to a lower level from ripening to harvesting. In the second crop season, the total amides and asparagine contents of Tainung 62 showed a higher level at the tillering stage, and decreased to a lower level at

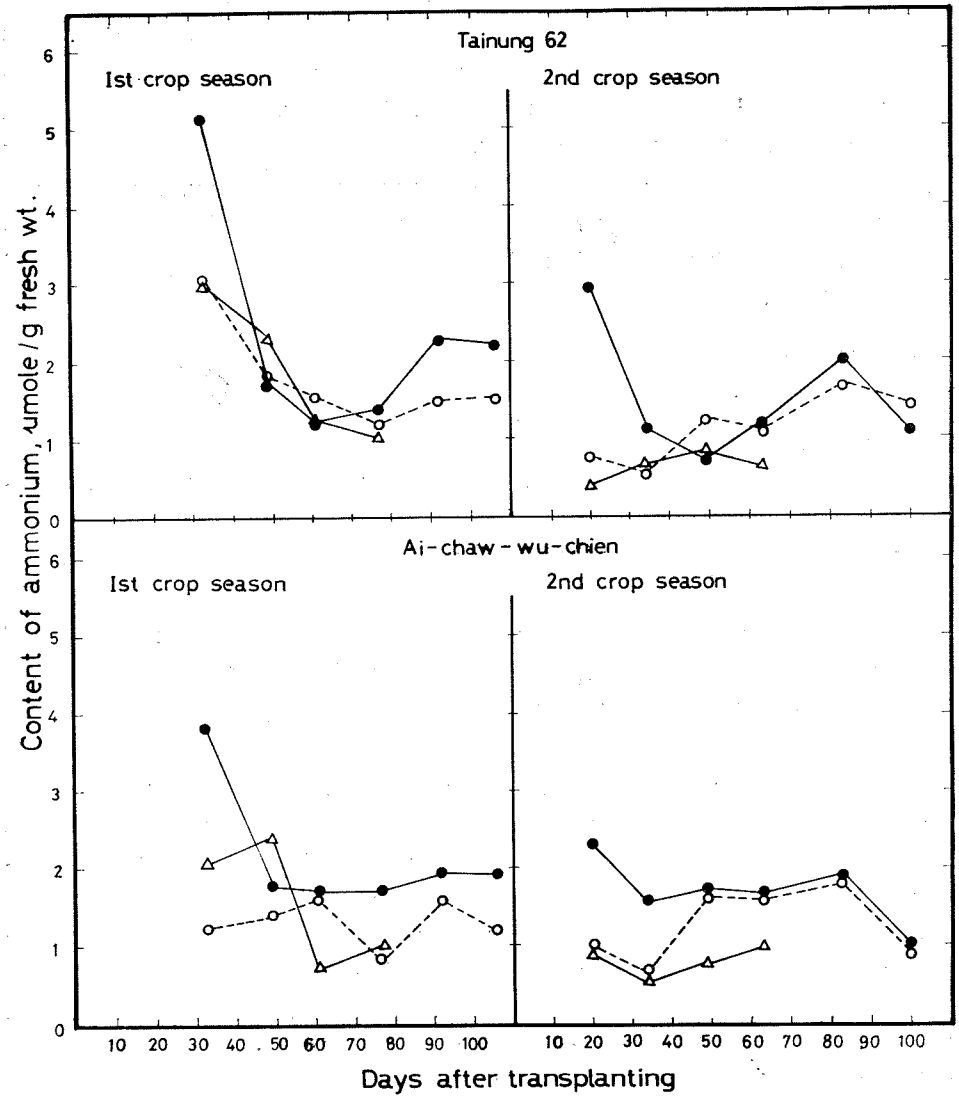

Fig. 5. Content of free ammonium in leaves (—●—●—), sheaths (--○---○---) and roots (—△—△—) of Tainung 62 and Ai-chaw-wu-chien during growth.

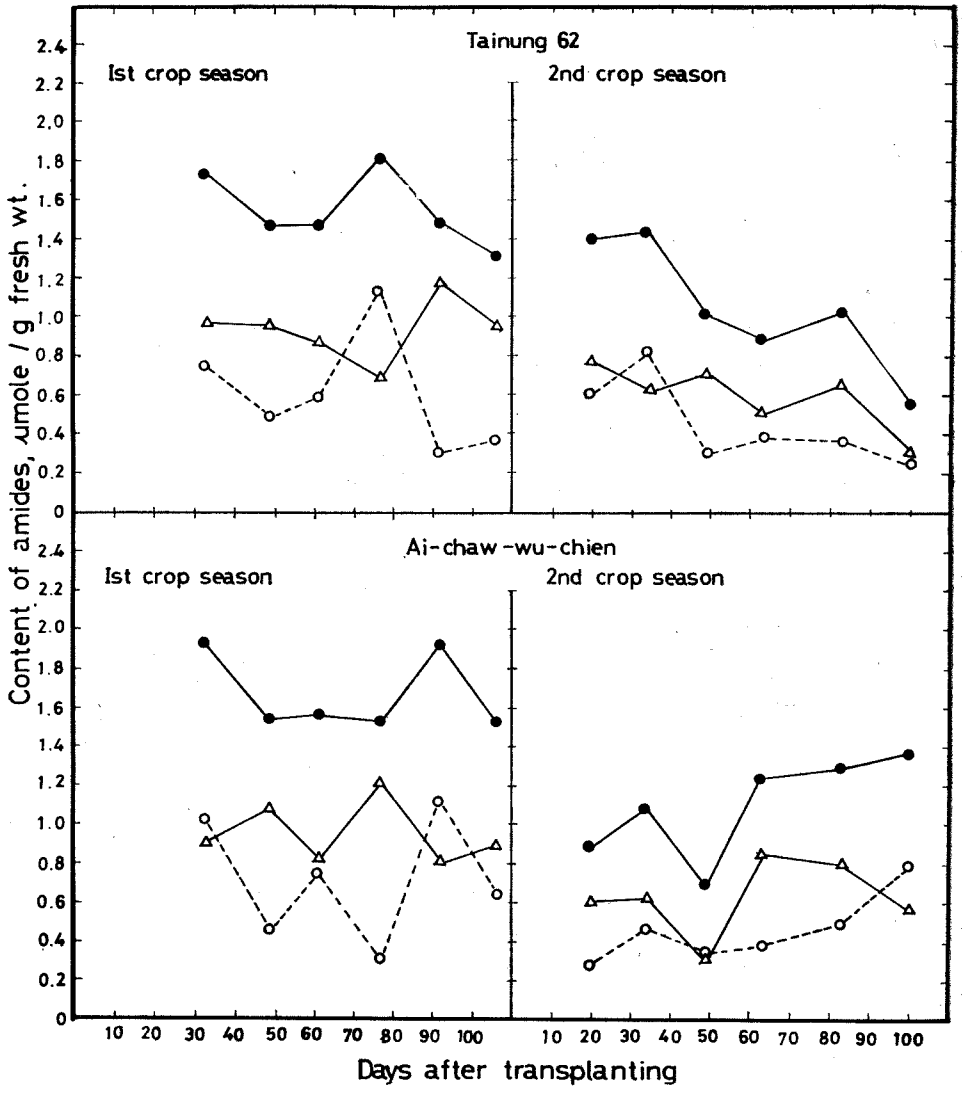

Fig. 6. Content of total amides (—●—●—), glutamine (--○---○---) and asparagine (—△—△—) in leaves of Tainung 62 and Ai-chaw-wu-chien during growth.

at booting, then increased slightly to a little bit higher level at the ripening stage; afterwards, it decreased to a very low level at harvesting. Total amides and asparagine content of Ai-chaw-wu-chien also showed a higher level at the tillering stage, and decreased to a lower level at booting, then increased to a very high level at the flowering and ripening stages; thereafter, the total amides content increased continuously to reach the highest level and the asparagine content decreased to a lower level at harvesting. The glutamine content of Tainung 62 showed a higher level at the tillering stage; however, it decreased to a lower level with the advancement of rice growth. The glutamine content of Ai-chaw-wu-chien showed a lower level at the tillering and booting stages; however, it increased gradually to a higher level from flowering through ripening until harvesting.

The changes of total amides, glutamine and asparagine contents in sheaths are shown in Fig. 7. In the first crop season, the total amides and glutamine contents of Tainung 62 and Ai-chaw-wu-chien showed a lowe level at the tillering stage, and increased to the highest level at booting, then decreased to a lower level at flowering. At the ripening stage, the total amides and glutamine contents of Tainung 62 still remained in a lower level until harvesting; however, those of Ai-chaw-wu-chien increased slightly to a little bit higher level, then decreased to a lower level at harvesting. The asparaging content of Tainung 62 and Ai-chaw-wu-chien showed a little bit higher level at the beginning of tillering, then decreased gradually to a lower level with the advancement of rice growth until ripening. At the harvesting stage, the asparagine content of Tainung 62 still remained at a lower level; however, of Ai-chaw-wu-chien increased to a higher level again. In the second crop season, the total amides and glutamine contents of Tainung 62 and Ai-chaw-wu-chien showed a lower level at the tillering stage, and increased to a very high level at the booting stage, then decreased to a lower level at flowering, and then increased again at the ripening stage. At the harvesting stage, the total amides and glutamine contents of Tainung 62 decreased to a lower level; however, those of Ai-chaw-wu-chien still remained at a higher level. The asparagine content showed a lower level at the tillering stage, and increased to a higher level at the booting stage, then decreased slightly to a lower level at the flowering and ripening stages. At the harvesting stage, the asparagine content of Tainung 62 decereased continuously to a very low level; however, of Ai-chaw-wu-chien increased to a higher level again.

The changes of total amides, glutamine and asparagine contents in roots are shown in Fig. 8. In the first crop season, the total amides and glutamine contents of Tainung 62 as well as the total amide and asparagine contents of Ai-chaw-wu-chien showed higher levels at the beginning of tillering and the

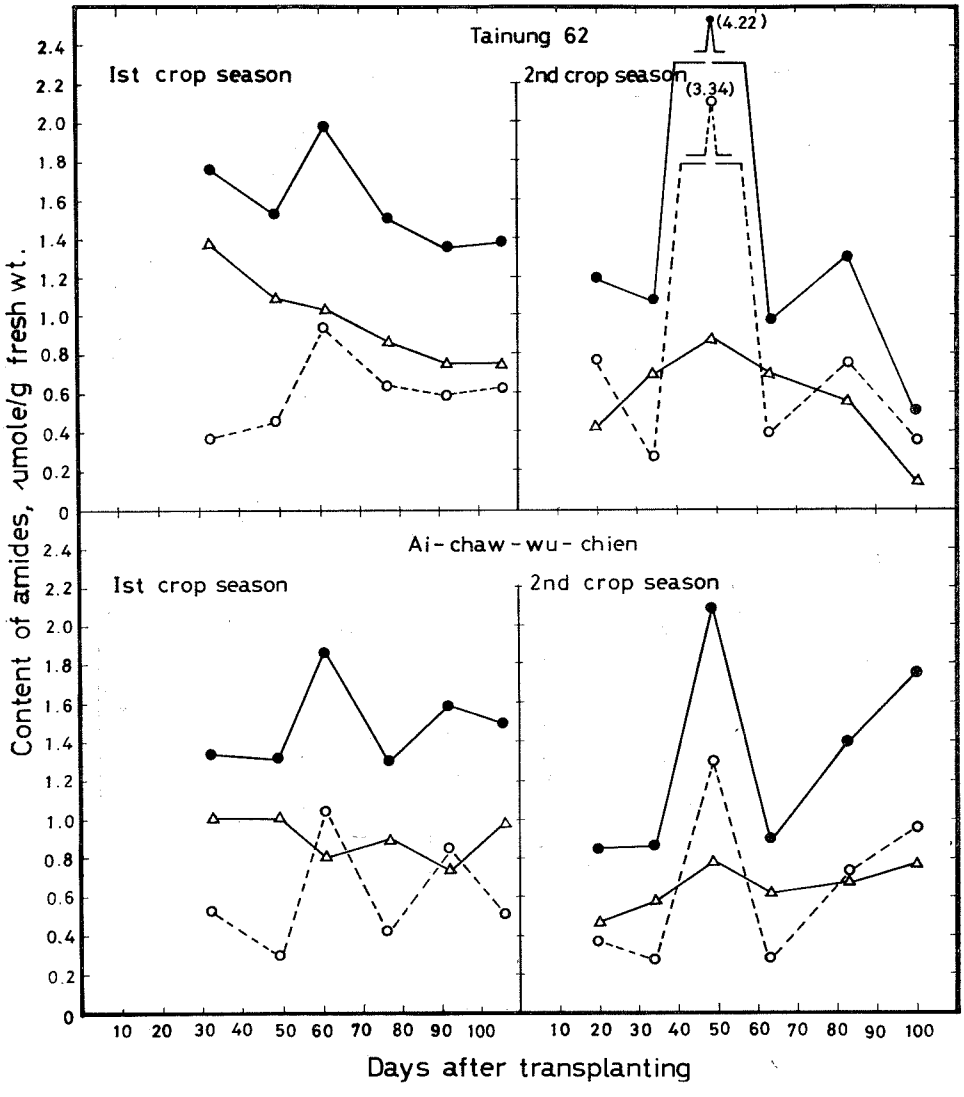

Fig. 7. Content of total amides (—●—●—), glutamine (--○---○---) and asparagine (—△—△—) in sheaths of Tainung 62 and Ai-chaw-wu-chien during growth.

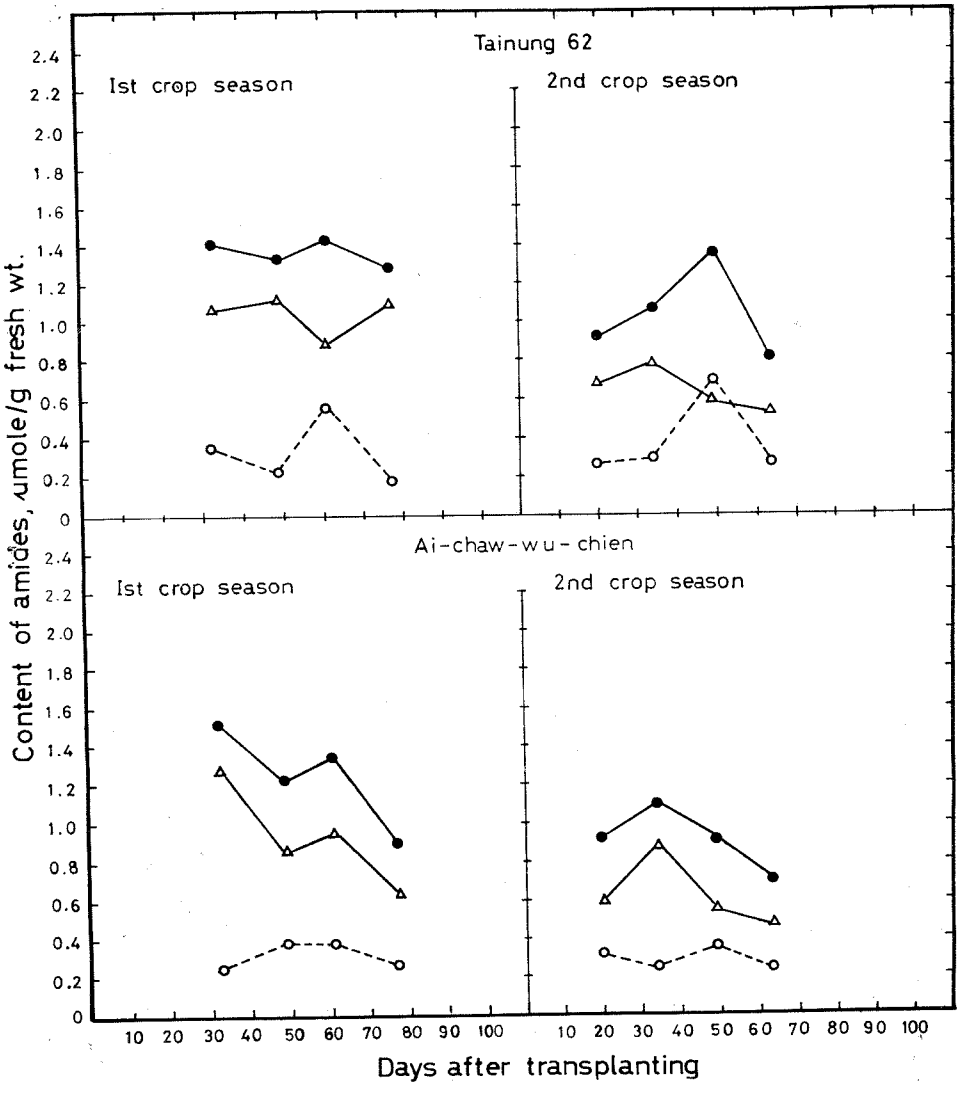

Fig. 8. Content of total amides (—●—●—), glutamine (--○---○--) and asparagine (—△—△—) in roots of Tainung 62 and Ai-chaw-wu-chien during growth.

booting stages. The asparagine content of Tainung 62 showed higher levels at the tillering and flowering stages. The glutamine content of Ai-chaw-wu-chien showed a higher level at the booting stage only. In the second crop season, the total amides and glutamine content of Tainung 62 showed a higher level at the booting stage, and the asparagine content showed a higher level at the tillering stage. The total amides and asparagine content of Ai-chaw-wu-chien showed a higher level at the booting stage.

Discussion

The transport and incorporation of nitrogen in plants involve many complex but predictable processes. During the span of the plant life, the developing leaves constitute a major sink for nitrogen translocated from roots. The amides, glutamine and asparagine, are usually predominant as transport and storage forms of nitrogen (Oji and Izawa, 1972). Glutamine is a key nitrogen donor for a number of nitrogenous compounds, and is now thought to redistribute nitrogen from the amide group to amino acids through the glutamate synthase system (Miflin and Lea, 1976), and asparagine is a main storage form of nitrogen in rice plants (Marwaha *et al.*, 1976). Currently, two enzymes have been considered for primary importance in the process of ammonium assimilation of higher plants. The glutamate dehydrogenase was originally implicated to be the most important enzyme of ammonium assimilation in root nodules. Since the discovery of glutamate synthase, the possibility of another important ammonium assimilation system was recognized. The glutamate dehydrogenase catalyzes the reductive amination of α-ketoglutarate to form glutamate, while the glutamate synthase transfers the amide group from glutamine to α-ketoglutarate converting both of them to glutamate. Glutamate synthase and glutamine synthetase may work in a cyclic manner in that the end product of either may be used as substrate for each other. Duke and Ham (1976) reported that the addition of nitrogen in the form of urea decreased the activities of glutamate dehydrogenase and glutamate synthase in root nodules of soybean, the same addition greatly enhanced the root glutamate dehydrogenase activity. It may be possible that either the glutamate dehydrogenase or the glutamate synthase could account for the total ammonium assimilation in soybean under appropriate physiological conditions.

Although nitrogen metabolism in rice plants has been extansively studied, however, little is known about the nitrogen transport within plants and the types of nitrogenous compounds transport from one organ to another. Muhammad and Kumazawa (1974) mentioned that the ammonium is mainly converted into organic nitrogen after absorption into rice plants, particularly,

into amides and amino acids in roots. Part of them were utilized for protein synthesis while the bulks transported to the shoot immediately. The question arises as to how the amide nitrogen is made available for amino acid biosynthesis in the case of glutamine. Lewis (1975) suggested that the glutamate synthase reaction may be the key to this scheme. The glutamate so formed may provide the amino group for the biosynthesis of other amino acids via transaminase reaction. The importance of glutamate synthase is that, in conjunction with glutamine synthetase, it forms a system of incorporating ammonium, present in low concentration, into α-amino nitrogen owing to the favourable Km of glutamine synthetase. However, under conditions of higher ammonium concentration, the glutamate dehydrogenase could be an important enzyme in ammonium assimilation.

Our results indicated that the activities of glutamate synthase, glutamate dehydrogenase, glutamine synthetase and asparagine synthetase as well as the content of total amides, glutamine and asparagine in rice plants varied according to crop seasons and growth stages. The appearence of higher levels of these enzyme activities in rice plants at different growth stages indicated that the variations of these enzyme activities during rice growth are different between the two crop seasons. According to the consistancy in variations of glutamate synthase and glutamine synthetase activities in leaves, sheaths and roots indicated the close relationship between those two enzymes. The results also seemed to support the suggestions of Miflin and Lea (1976) that the primary pathway for glutamate production from ammonium in rice plants may be mainly via glutamate synthase and glutamine synthetase system.

The glutamate dehydrogenase activity in leaves and sheaths showed its higher levels around the tillering and flowering stages regardless of crop seasons. Judging from the time of sampling, it was found that the appearence of higher levels of glutamate dehydrogenase activity seemed to coincide with the time of top dressings. Kanamori *et al.* (1972) mentioned that the NADH-dependent glutamate dehydrogenase activity in roots of rice seedlings was an inducible enzyme. According to the assay method in this study, the NADH was used as the reducing substrate, therefore, the appearence of higher levels of glutamate dehydrogenase in leaves and sheaths during rice growth might he induced by supplying of ammonium nitrogen as top dressings. Furthermore, in respect to ammonium detoxification within plant cells, Shepard and Thurman (1973) suggested that the stimulatory effects of ammonium upon glutamate dehydrogenase activity may offer a mechanism whereby the plant can increase the rate of removal of ammonium to avoid toxic effect.

Glutamine serves as a building block of protein and as a nitrogen donor

in various biosynthetic pathways, and its synthesis is an important pathway for the utilization of ammouium. Thus, the glutamate synthetase places at the point of divergence of several metabolic pathways, particularly, in the regulation of nitrogen metabolism. On the other hand, the glutamate synthase appears to be specific for glutamine as nitrogen donor, therefore, the glutamate synthase provides a mechanism by which the amide nitrogen of glutamine is converted into the amino nitrogen of glutamate. In the first crop season, the glutamine content in leaves of Tainung 62 showed higher levels at the tillering and flowering stages, and that of Ai-chaw-wu-chien showed higher levels at the tillering and ripening stages; however, the glutamine content in sheaths of Tainung 62 showed a higher level at the booting stage only, and that of Ai-chaw-wu-chien showed a higher level at the booting and ripening stages. In the second crop season, the glutamine content in leaves of Tainung 62 showed a higher level at the tillering stage only, and that of Ai-chaw-wu-chien showed a higher level from flowering through ripening until harvesting; however, the glutamine content in sheaths of Tainung 62 showed a very high level at the booting stage and a higher level at the ripening stage, and that of Ai-chaw-wu-chien showed a very high level at the booting stage and a higher level from ripening to harvesting. According to the results shown in Fig. 3, it was found that the changes of glutamine content in leaves and sheaths seemed to coincide with the variations of glutamine synthetase activity during rice growth.

The demonstration of glutamine-dependent asparagine synthetase in soybean (Streeter, 1973), rice seedlings (Kanamori and Matsumoto, 1974), luping seedlings (Rognes, 1975; Lea and Fowden, 1975) and corn roots (Stulen and Oaks, 1977) confirmed that the biosynthesis of asparagine in higher plants is via the direct formation from asparate with glutamine as the preferred donor for amide group nitrogen. In the first crop season, the asparagine content in leaves of Tainung 62 showed higher levels at the tillering and ripening stages, and that of Ai-chaw-wu-chien showed a higher level at the flowering stage only; however, the asparagine content in sheaths of Tainung 62 showed a little bit higher level at the beginning of tillering, and that of Ai-chaw-wu-chien showed a higher level at the beginning of tillering and the harvesting stages. In the second crop season, the asparagine content in leaves of Tainung 62 showed higher levels at the tillering and ripening stages, and that of Ai-chaw-wu-chien showed higher levels at tillering and from flowering to ripening; however, the asparagine content in sheaths of Tainung 62 showed a higher level at the booting stage only, and that of Ai-chaw-wu-chien showed higher levels at the booting and harvesting stages. According to the results shown in Fig. 4, the asparagine synthetase in leaves and sheaths maintained

a rather constant activity in the first crop season during rice growth; but, it showed a very high activity from booting through flowering to harvesting in the second crop season. The great defference in variation of asparagine synthetase activity in leaves and sheaths between two crop seasons during rice growth might indicate the physiological difference of rice plants between two crop seasons. And it might also indicate that the nitrogen metabolism is in the different pattern between two crop seasons. The appearence of very high activity of asparagine synthetase in leaves and sheaths in the second crop season did not result in the accumulation of a large amount of asparagine. Atkins *et al.*, (1975) demonstrated that all of the ^{14}C-labeled asparagine was translocated into seeds of white lupin when the labeled asparagine was fed to its fruiting shoots through the transpiration stream. Therefore, it was possible to synthesize a large amount of asparagine in leaves and sheaths of rice plants due to the presence of very high activity of asparagine synthetase; however, the synthesized asparagine was translocated to developing grains already. The lower content of glutamine in leaves and sheaths in the later growth stage of the second crop season seemed to meet this case. On the other hand, the very high content of glutamine in sheaths at the booting stage in the second crop season decreased rapidly while the asparagine synthetase increased to a very high activity. The glutamine might be consumed for synthesizing asparagine and translocated to developing grains. Furthermore, the glutamine synthetase activity in leaves and sheaths in the later growth stage of the first crop season was higher then that of the second crop season; therefore, it should have more glutamine to be synthesized in the later growth stage of the first crop season. It was recognized that the synthesis of amides in the later growth stage is mainly for translocation of nitrogen to developing grains. In the present case, it could be postulated that the translocation of nitrogen from leaves and sheaths of rice plants to its developing grains might be mainly in the form of glutamine in the first crop season and in the form of asparagine in the second crop season.

The free ammonium content in leaves showed two higher levels in the early and the later growth stages. It was almost consistant with the variations of glutamate dehydrogenase activity as well as the time of top dressings during rice growth. However, a part of free ammonium presented in leaves in the later growth stage might come from asparagine due to the deamination of asparaginase. The variations of total amides, glutamine and asparegine content in leaves, sheaths and roots are very complex. The most important factors may be the variations of glutamate synthase, glutamine synthetase and asparagine synthetase activities during rice growth. The accumulation of glutamine and asparagine in leaves, sheaths and roots of rice plants are the

results due to the reactions of these enzymes.

Glutamine to glutamate conversion has been shown to be the major mechanism of utilizing free ammonium. Lea and Fowden (1975) suggested that free ammonium could arise from oxidation deamination of amino acids released during proteolysis in senescent leaves. Therefore, the glutamine synthetase could then readily produce glutamine for direct export or subsequent amide metabolism due to its high affinity for free ammonium. Glutamine is an extremely reactive metabolite which lies at the center of cellular nitrogen metabolism and donates amide nitrogen to numerous substrates. However, the obvious importance of the other amides, asparagine, has not been ignored. Millard *et al.* (1975) mentioned that asparagine is a more efficient nitrogen source than glutamine for the production of proteins and nucleic acids in the cultured pea cotyledons. Recent studies into the mechanism on the action of asparagine synthetase suggested that glutamine and asparagine are the physiological substrates (Lea and Fowden, 1975; Streeter, 1973). The operation of this glutamine requiring enzyme would add importance to the suggested roles of glutamine synthetase activity in leaves and sheaths of rice plants.

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水稻生長期間醯胺生合成之季節性變異

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一期作時，葉片和葉鞘中麥氨酸合成酶 (Glutamate synthase) 和麥氨酸脫氨酶 (Glutamate dehydrogenase) 之活性於分蘖期爲最高，於開花期亦有較高之活性；而麥氨酸合成酶於收穫期仍有很高的活性。葉片中麥醯胺合成酶 (Glutamine synthetase) 之活性於分蘖初期和開花期爲較高。二期作時，葉片和葉鞘中麥氨酸合成酶之活性於分蘖初期、孕穗期、成熟期及收穫期都很高；而麥氨酸脫氫酶之活性於分蘖期和開花期爲較高。臺農62葉片中麥醯胺合成酶之活性於分蘖末期較高；而矮脚烏尖中則於分蘖初期和孕穗期爲較高。葉鞘中麥醯胺合成酶之活性於開花期較高。根部之麥氨酸合成酶和麥醯胺合成酶之活性於分蘖期較高，且與季節無關。根部之麥氨酸脫氫酶，於一期作時於分蘖期有較高之活性，而於二期作時則於孕穗期有較高之活性。一期作時，葉片、葉鞘和根部之天門冬醯胺合成酶 (Asparagine synthetase) 之活性於水稻生長期間相當穩定；而二期作時，葉片和葉鞘中天門冬醯胺合成酶之活性於開花期特別高。一期作時，臺農62葉片中醯胺總量 (Total amides) 和麥醯胺 (Glutamine) 之含量於分蘖期和開花期爲較高，而於矮脚烏尖中則於分蘖期和成熟期爲較高。臺農62葉片中天門冬醯胺 (Asparagine) 之含量於分蘖期和成熟期爲較高，而於矮脚烏尖中則於開花期及成熟期爲較高。葉鞘中醯胺總量和麥醯胺之含量於孕穗期爲最高。臺農62根部之醯胺總量和麥醯胺之含量以及矮脚烏尖根部之醯胺總量和天門冬醯胺之含量於分蘖初期和孕穗期爲較高。二期作時，葉片中醯胺總量和天門冬醯胺之含量於分蘖期和開花期爲較高。臺農62葉片中麥醯胺之含量於分蘖期較高，而於矮脚烏尖中則自成熟期至收穫期爲較高。葉鞘中醯胺總量和麥醯胺之含量於孕穗期爲最高，其次爲成熟期。臺農62葉鞘中天門冬醯胺之含量於孕穗期爲較高，而矮脚烏尖則於孕穗期和收穫期爲較高。臺農62根部之醯胺總量和麥醯胺之含量於孕穗期爲較高；矮脚烏尖根部之醯胺總量和天門冬醯胺之含量於分蘖期較高。葉片中游離態氨之含量，於一期作時於分蘖期和開花期爲較高；而於二期作時，則於分蘖期和成熟期爲較高。