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SEASONAL VARIATIONS OF NITRATE REDUCTASE, GLUTAMATE DEHYDROGENASE AND THE SOLUBLE NITROGENOUS COMPOUNDS DURING RICE GROWTH[1,2]

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Abstract

Both [the nitrate reductase and glutamate dehydrogenase in leaves of Taichung 65 and Ai-chaw-wu-chien showed two higher levels of activities during their growth in regardless of crop seasons. The first peak occurred at the beginning of tillering, and the second peak at the flowering stage except the glutamate dehydrogenase of the first crop season which was at the booting stage. In the first crop season, the highest activity of nitrate reductase in leaves was at the flowering stage, and the highest activity of glutamate dehydrogenase in leaves at the beginning of tillering. At the beginning of tillering, the nitrate reductase activity in roots was higher than that in leaves, but the glutamate dehydrogenase activity in roots was lower than that in leaves. In the second crop season, the nitrate reductase activity in leaves at tillering had the same high level as at flowering, however, the glutamate dehydrogenase activity had the highest level at flowering. Both the nitrate reductase and glutamate dehydrogenase activities in roots were very low. The leaves had a higher level of free amino acids content at the flowering stage in regardless of crop seasons. The leaves of Taichung 65 showed two higher levels of ammonium nitrogen content during its growth in regardless of crop seasons; however, the leaves of Ai-chaw-wu-chien showed a higher level of ammonium nitrogen content at flowering of the first crop season only. The amide nitrogen content in leaves of the first crop season maintained a higher level from tillering to flowering, but its content in leaves of the second crop season had a lower level at tillering and increased to a higher level at flowering. The amide nitrogen in roots of the first crop season was higher than that of the second crop season at the tillering stage. The leaves had a higher level of nitrate-nitrite nitrogen at the booting stage in regardless of crop seasons.

Introduction

It is well known that nitrogen is an essential nutrient element for plant growth, and also an important factor to improve the grain yield of rice plants.

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Rice plants can absorb either the ammonium or the nitrate as nitrogen source, and Ishizuka (1976) pointed out that more ammonium nitrogen can be absorbed at the early growth stage. Moreover, in a submerged paddy soil, ammonium ion is more stable than nitrate ion which is readily denitrified by anaerobic bacteria to lead a loss of nitrogen from the soil. Therefore, ammonium nitrogen has been preferentially used as a fertilizer for rice plants. Muhammad and Kumazawa (1974a, 1974b) considered that the assimilation of ammonium or nitrate nitrogen does not follow the same route. Ammonium nitrogen is assimilated mainly in roots and nitrate nitrogen is assimilated mainly in leaves of rice seedlings. Yoneyama and Kumazawa (1974, 1975) also considered that the assimilation and metabolism of ammonium and nitrate nitrogen are not as the same pattern in rice plants. It is generally considered that in higher plants ammonium nitrogen is incorporated into α-ketoglutarate to form glutamate catalized by glutamate dehydrogenase, however, nitrate nitrogen is reduced to ammonia through the catalization of nitrate and nitrite reductases. Marwaha *et al.* (1976) pointed out that the activity of glutamate dehydrogenase in roots of rice seedlings is higher than that in leaves, and the reverse is true for the activity of nitrate reductase. However, little is known about the variations of these enzyme activities throughout the growth period of rice plants.

Rice is a major crop in Taiwan, and it is harvested twice a year. However, the grain yield in the second crop season is always about 25% lower than that in the first crop season. Wu *et al.* (1975) pointed out that the main factor on the causes of low yielding in the second crop season are due to the decrease of tiller number, panicle number and fertility rate. Huang (1977) considered that the higher temperature in the early growth stage of the second crop season is the main factor causing the reduction of tillering capacity. Because the growth patterns are different between the two crop seasons due to the difference of wheather conditions, therefore, the nutrient absorption, translocation, metabolism and the effect of utilization may also be different between the two crop seasons. This paper presents the variations of nitrate reductase and glutamate dehydrogenase activities in leaves and roots during rice growth. Furthermore, the content of soluble nitrogenous compounds such as free amino acids, ammonium nitrogen, amide nitrogen and nitrate-nitrite nitrogen in leaves and roots are also surveyed.

Materials and Methods

Cultivation of rice plants

This experiment was conducted at the farm of the Institute of Botany,

Academia Sinica, located in Nankang, Taipei, Taiwan. Two rice varieties of Taichung 65 (Japonica type) and Ai-chaw-wu-chien (Indica type) were employed. Rice seedling at four leaves stage were transplanted to paddy field with 5 to 7 seedlings as one hill. The second crop season began in the middle of August and ended in the middle of Decenber of 1977, while the first crop season began in the middle of March and ended in the middle of July of 1978. A split-plot design was adopted, and each treatment was repeated four times in randomization with a planting density of 20×17 cm (29 hills/m^2). The fertilizers with a basal dressing of N. P. K was supplied on the surface within 4 cm. The supplying of fertilizers for top dressing are summerized in Table 1. In which, ammonium sulfate was used as ammonium nitrogen and calcium nitrate as nitrate nitrogen. The drying treatment in the middle growth stage was at leaf-age index between 69–92. During this stage, water supply was stopped until the sulface of paddy soil cracked to 1–2 cm. The time of fertilizing treatment for top dressing in the later growth stage of the two crop seasons

Table 1. *Treatment of fertilizing for cultivation of rice plants*

Basal dressing of fertilizing		$N_2(NH_4^+\text{-}N)$: P_2O_5: K_2O = 30 : 50 : 25 (kg/ha)	
Treatment of fertilizing for top dressing		t_1	t_2
Top dressing in the early growth stage (leaf-age inbex defore 69)	1st crop season	1. 15 days after transplanting NH_4^+-N: 15 kg/ha 2. 30 days after transplanting NH_4^+-N: 20 kg/ha	Same as t_1
	2nd crop season	1. 10 days after transplanting NH_4^+-N: 15 kg/ha 2. 20 days after transplanting NH_4^+-N: 20 kg/ha	Same as t_1
Top dressing in the later growth stage (leaf-age index after 92)		1. Meiosis stage at leaf-age index 92 NH_4^+-N: 20 Kg/ha K_2O: 15 kg/ha 2. Heading stage NH_4^+-N: 15 kg/ha K_2O: 10 kg/ha	1. Meiosis stage at leaf-age index 92 NO_3^--N: 20 kg/ha K_2O: 15 kg/ha 2. Heading stage NO_3^--N: 15 kg/ha K_2O: 10 kg/ha

were depend upon the leaf-age index of rice plants. In the first crop season, the twice top dressing for t_1 and t_2 were applied on 59 and 84 days after transplanting, respectively. In the second crop season, the twice top dressing for t_1 and t_2 were on 53 and 66 days after transplanting, respectively.

Sampling and treatment of samples

The variations of nitrate reductase and glutamate dehydrogenase activities as well as the content of free amino acids, ammonium nitrogen, amide nitrogen and nitrate-nitrite nitrogen in leaves and roots were analyzed in four replications at every two-or three-week intervals throughout the growth period of rice plants. Every sampling was made between 10 to 12 A.M.. The sampling method was that the whole hill of rice plant was scooped out from the paddy soil and four hills were taken as one sample. Leaves 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 plastic bag and kept in cold room below 5°C for the determination of enzyme activities. Another part of the sample (10 grams) were soaked in 40 ml of 80% ethanol for the determination of free amino acids, ammonium nitrogen, amide nitrogen and nitrate-nitrite nitrogen.

Assay of nitrate reductase

The *in vivo* assay for the nitrate reductase activity was based on that of Jaworski (1971) and modified with vacuum infiltration according to the method of Harper (1972). 0.5 gram of sample was weighed and transferred into a test tube (2.5 × 15 cm) standing in a ice bath. Ten milliliters of a pre-cold incubation medium consisting of 0.1 M phosphate buffer (pH 7.5), 0.02 M KNO_3, 5% n-propanol and two drops of chloramphenicol (0.5 mg/ml) was added into the tube. A stainless steel wire screen was placed into the tube to hold the sample below the sulface of the assay medium. The sample was then evacuated for two minutes in a vacuum desicator. Air was reintroduced rapidly and the procedure repeated. The tube was then wrapped in aluminum foil and was incubated in a water bath at 25°C with gentle shaking for desired length of time. The nitrite released into the medium was determined at zero time and at varoius intervals thereafter by treating 0.8 ml aliquots with 0.6 ml each of 1% sulfanilamide in 3 M hyrochloric acid and 0.02% of N-1-naphthyl-ethylenediamine. After 20 minutes, the solution was diluted with 3 ml of water and the absorbance measured at 540 nm. This reaction had been carried out in duplicate for each sample. The nitrate reductase activity was expressed as nmole NO_2^- produced per gram fresh weight of sample per hour.

Assay of glutamate dehydrogenase

The glutamate dehydrogenase in leaves and roots was extracted according to the procedure of Pšenáková (1976). Ten grams of the chilled sample was homogenized with 100 ml of 0.066 M Tris-HC l buffer (pH 8.0) containing of 5 $\times 10^{-5}$ M cysteine and 0.1% Triton X-100 in an ice bath using a Polytron Lab Homogenizer at 10,000 rpm for 5 minutes. The homogenate was squeezed through four layers of cheesecloth. The extracts was collected and clarified by centrifugation for 20 minutes at 10,000 × g. The glutamate dehydrogenase activity in the extracts was determined by measuring the initial rate of NADH oxidation at 30°C in a Gilford 250 spectrophotometer at 340 nm based on the procedure of Kanamori *et al.* (1972). The assay system consisted of the following components: 1.0 ml of 0.2 M Tris-HCl buffer (pH 8.0), 0.3 ml of 0.1 M α-ketoglutarate (adjusted to pH 7.0), 0.3 ml of 1.0 M NH_4Cl, 0.2 ml of 1.0 mM NADH and appropriate amount of the extracts. The final volume was made up to 3.0 ml by adding distilled water. The reaction was started by the addition of NADH, and α-ketoglutarate was omited in the blank test. One unit of glutamate dehydrogenase activity was defined as the amount of enzyme causing a change of 0.01 absorbance at 340 nm per minute. The glutamate dehydrogenase activity in the sample is calculated as units per gram fresh weight of sample per hour.

Analysis of free amino acids, ammonium nitrogen, amide nitrogen and nitrate-nitrite nitrogen

Soluble nitrogenous constituents in leaves and roots was extracted as follows: Ten grams of sample which was soaked in 40 ml of 80% ethanol as described before was homogenized with 100 ml of 70% ethanol for 5 minutes using a Polytron Lab Homogenizer at 10,000 rpm, then filtered through a sintered-glass funnel. The residues was washed three times with 50 ml portion of 70% ethanol. The extracts and washings were combined and concentrated at temperature bellow 40°C in a rotary evaporator, then adjusted to 50 ml with water. The precipitates was removed by centrifugation at 12,000 × g for 15 minutes.

Total free amino acids in the extracts was determined as follows: A Dowex 50-X 8 column (1.5 × 4 cm, H^+, 200–400 mesh) was prepared and saturated with 0.2 M citrate buffer (pH 2.2). An appropriate volume of the extracts was passed through the column allowing the free amino acids to be absorbed on the resin, and the column washed with 50 ml of water. The amino acids was eluted from the column with 25 ml of 2 N NaOH, and the column washed with 50 ml of water. The eluates and washings were combined, neutralized with 6 N HCl and adjusted to pH 5.0 with 0.1 M sodium citrate. The total free amino acids was determined according to the method of Moore and Stein (1954)

with L-leucine as standard. The free amino acids content in the sample was caulculated as μmole per gram fresh weight of sample.

The ammonium, amide and nitrate-nitrite nitrogen in the extracts were analyzed according to the method of Varner *et al.* (1953) with a semimicro-Kjeldahl distillation apparatus. Ten milliliters of the extracts was introduced into the sample chamber, followed with several drops of iso-amyl alcohol and 5 ml of borate buffer (saturated solution of sodium tetraborate, adjusted to pH 10 with NaOH). A receiver, containing 10 ml of 4% boric acid with several drops of a mixed indicator (one part of 0.2% methyl red in ethanol and five parts of 0.2% bromocresol green in ethanol) was put under the condenser. A small water bath at 50° to 55°C was brought up around the sample chamber. A hot air current (50° to 55°C) was introduced into the distillation apparatus by passing through the steam generator. In 15 minutes, all of the ammonia from ammonium salts was collected in the receiver. The receiver was removed and the content was titrated with 0.02 N HCl. A new receiver containing boric acid and mixed indicator was replaced under the condenser, 15 ml of 40% NaOH solution was introduced into the sample chamber, and steam distillation at 100°C started. After 10 minutes, all of the ammonia from amides was collected in the receiver. The receiver was removed and titrated with 0.02 N HCl. Then 2 ml of 15% tartaric acid and 0.5 ml of 25% cupric sulfate were introduced into the sample chamber and heated at 100°C for 5 minutes by passing steam through the distillation apparatus. Another new receiver containing boric acid and mixed indicator was replaced under the condenser. Then 5 ml of 20% ferrous sulfate was introduced into the sample chamber, and the nitrate plus nitrite nitrogen was reduced to ammonia under such conditions. The steam distillation at 100°C continued for 10 minutes, all of the ammonia from nitrate and nitrate was distilled off and collected in the receiver and titrated with 0.02 N HCl. The percent nitrogen was calculated according to the formura from A.O.A.C. (Horwitz, 1960): N% = (ml HCl − ml blank) × normality × 14.003 × 100/mg sample. Furthermore, the ammonium, amide and nitrate-nitrite nitrogen content in samples were calculated as mg N per gram fresh weight of sample.

Results

Variations of nitrate reductase activity during rice growth

The nitrate reductase activity in intact leaves and roots of Taichung 65 and Ai-chaw-wu-chien were assayed throughout their growth period. The variations of nitrate reductase activities are shown in Fig 1. The nitrate reductase activity in leaves showed a higher level at the beginning of tillering,

and decreased to a lower level at the booting stage, then increased to the highest level at the flowering stage. And at the tillering stage, the nitrate reductase activity of the first crop season was lower than that of the second

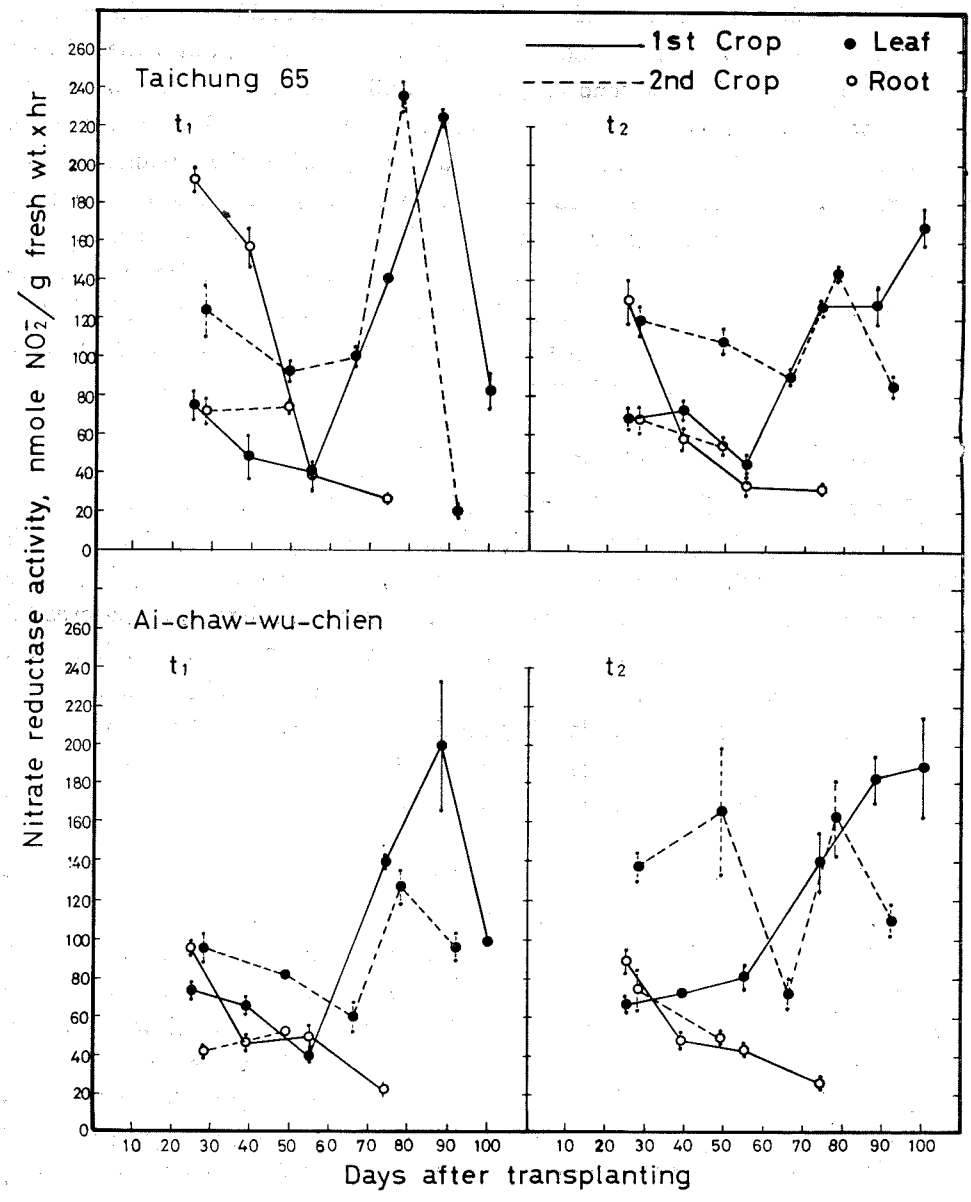

Fig. 1. Nitrate reductase activities in leaves and roots of Taichung 65 and Ai-chaw-wu-chien during growth. t_1: Ammonium sulfate was used as top dressing both in the early and later growth stages. t_2: Ammonium sulfate was used as top dressing in the early growth stage, while calcium nitrate was used in the later growth stage.

crop season. When ammonium nitrogen was used as the top dressing in the later growth stage (t_1), the nitrate reductase activity decreased to a lower level after flowering both in the first and second crop seasons. When the nitrate nitrogen was used as the top dressing in the later growth stage (t_2), the nitrate reductase activity of the first crop season could maintain a very high level from flowering through ripening until harvesting, but, the nitrate reductase activity of the second crop season still decreased to a lower level after flowering. The nitrate reductase activity in roots had a higher level at the beginning of tillering, and decreased quickly with the advancement of rice growth. However, the nitrate reductase activity in roots of the first crop season was much higher than that of the second crop season at the beginning of tillering. Furthermore, in the first crop season, the nitrate reductase activity in roots was higher than that in leaves at the beginning of tillering; but, in the second crop season, the nitrate reductase activity in roots was much lower than that in leaves at the beginning of tillering.

Variations of glutamate dehydrogenase activity during rice growth

The variations of NADH-dependent glutamate dehydrogenase activities in leaves and roots of Taichung 65 and Ai-chaw-wu-chien during their growth are shown in Fig 2. In the first crop season, the glutamate dehydrogenase activity in leaves had a higher level at the beginning of tillering, and decreased to a lower level at the end of tillering, then increased to another higher level again at the booting stage, and the activity decreased gradually to a very low level from flowering through ripening until harvesting. In the second crop season, the glutamate dehydrogenase activity in leaves also had a higher level at the beginning of tillering, but decreased to a lower level at the booting stage, then increased rapidly to reach the highest level at flowering, afterwords, the activity decreased to a lower level at the ripening stage until harvesting. In the first crop season, the glutamate dehydrogenase activity at the beginning of tillering almost had the same high level as at the booting stage; however, in the second crop season, the glutamate dehydrogenase activity at the beginning of tillering was much lower than that at flowering. On the other hand, the glutamate dehydrogenase activity in roots had a higher level at the beginning of tillering and decreased gradually with the advancement of rice growth. And the glutamate dehydrogenase activity in roots of the first crop season was slightly higher than that of the second crop season.

Changes of free amino acids content during rice growth

The changes of free amino acids content in leaves and roots of Taichung 65 and Ai-chaw-wu-chien during their growth are shown in Fig 3. The free

amino acids content in leaves showed a higher level at the flowering stage both in the first and second crop seasons. In Taichung 65, the free amino acids content at the flowering stage of the first crop season was much higher than that of the second crop season; but, in Ai-chaw-wu-chien, the free amino

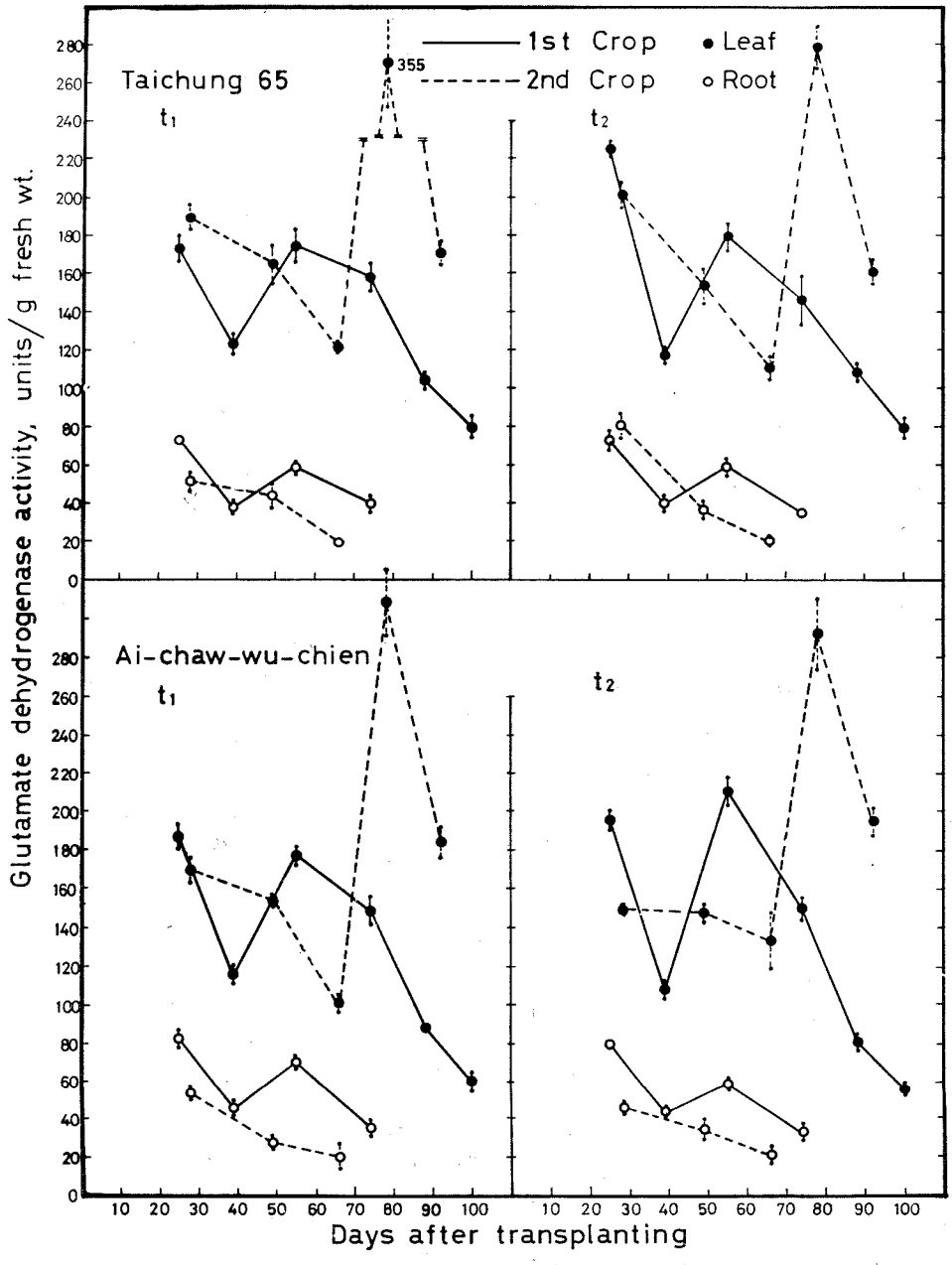

Fig. 2. Glutamate dehydrogenase activities in leaves and roots of Taichung 65 and Ai-chaw-wu-chien during growth.

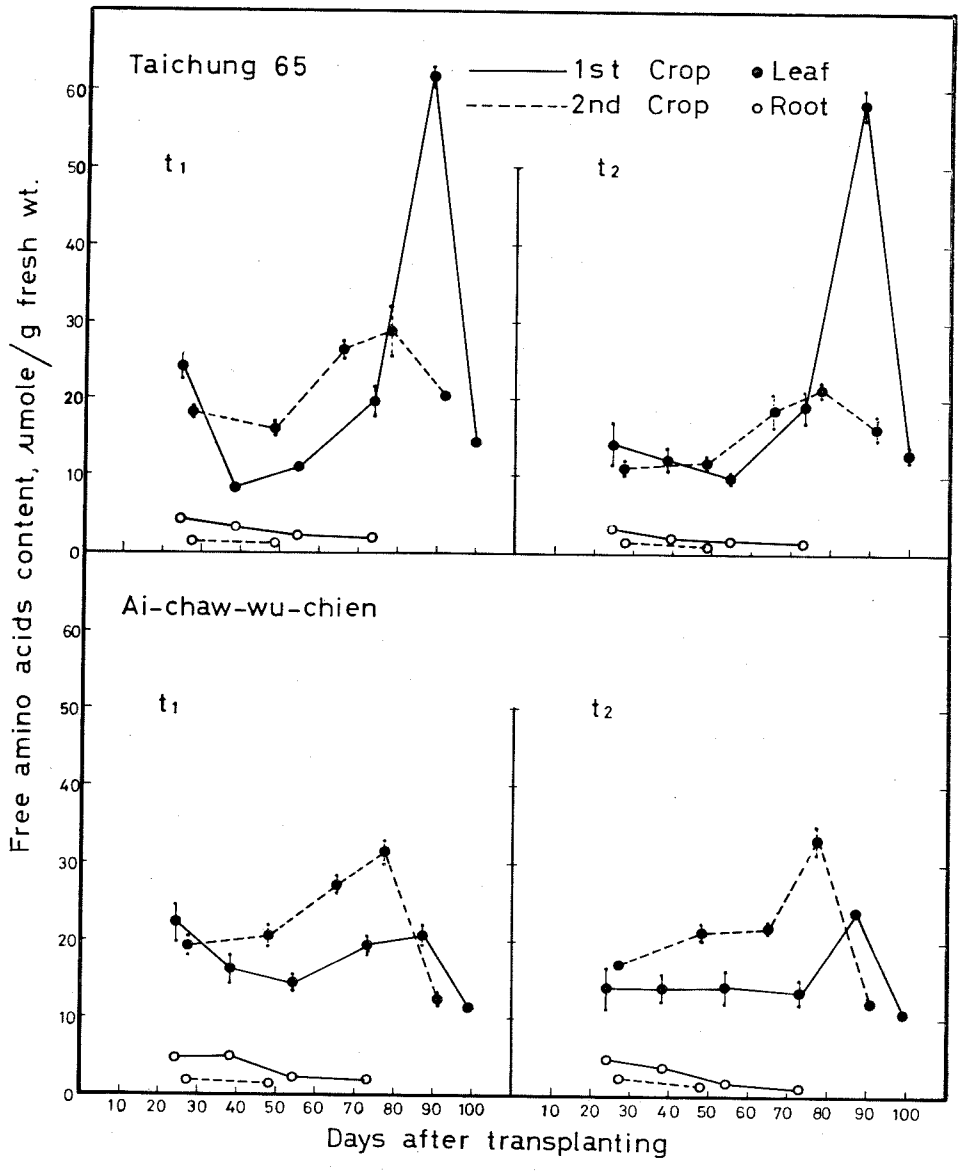

Fig. 3. Free amino acids content in leaves and roots of Taichung 65 and Ai-chaw-wu-chien during growth.

acids content at the flowering stage of the first crop season was slightly lower than that of the second crop season. The free amino acids content in roots exhibited a slightly higher level at the beginning of tillering of the first crop season, and its content in roots of the second crop season was very low.

Changes of ammonium, amide and nitrate-nitrite nitrogen content during rice growth

The changes of ammonium, amide and nitrate-nitrite nitrogen content in leaves and roots of Taichung 65 and Ai-chaw-wu-chien were analyzed during their growth.

The changes of ammonium nitrogen content in leaves are shown in Fig 4. In Taichung 65, the ammonium nitrogen content in the first crop season showed higher levels at the beginning of tillering and at the booting stage; however, in the second crop season, the ammonium nitrogen content had higher levels at the beginning of tillering and at the flowering stage. In Ai-chaw-wu-chien, the ammonium nitrogen content in the first crop season had a higher level at the flowering stage; however, in the second crop season, there was no significant change in the ammonium nitrogen content during its growth. In roots, only a small amount of ammonium nitrogen could be detected.

The changes of amide nitrogen content in leaves and roots are shown in Fig 5.

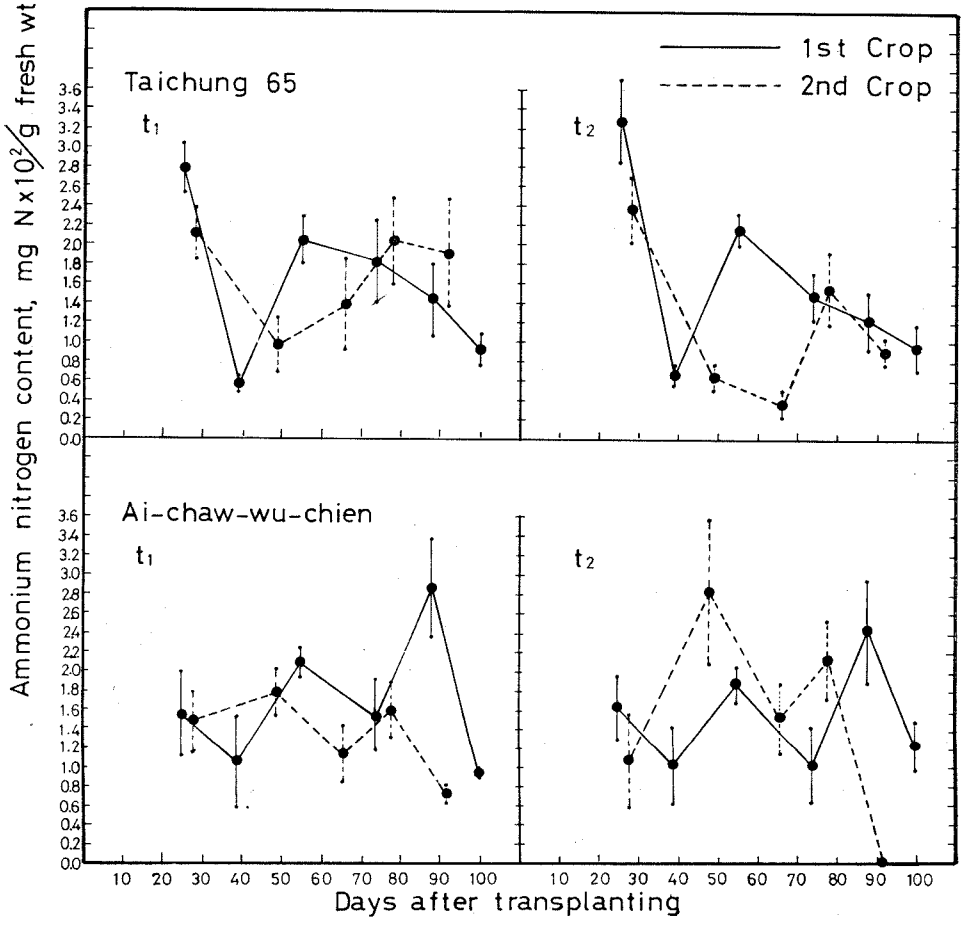

Fig. 4. Ammonium nitrogen content in leaves of Taichung 65 and Ai-chaw-wu-chien during growth.

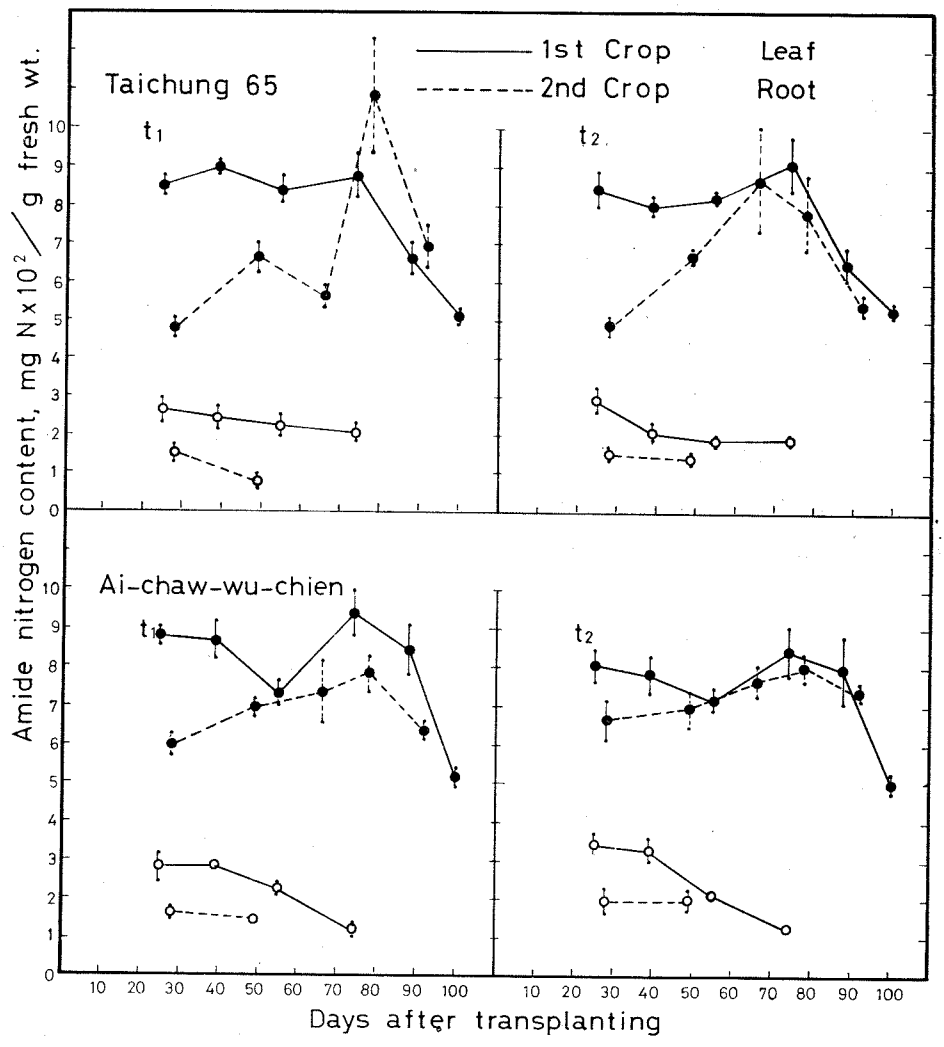

Fig. 5. Amide nitrogen content in leaves and roots of Taichung 65 and Ai-chaw-wu-chien during growth.

The amide nitrogen content in leaves of the first crop season maintained a higher level from tillering through the booting stage until flowering, however, it decreased to a lower level at ripening. On the contrary, the amide nitrogen content in leaves of the second crop season had a lower level from tillering through the booting stage, however, it increased gradually with the advancement of rice growth and reached the highest level at the flowering stage, then decreased to a lower level at ripening. The amide nitrogen content in roots of the first crop season was higher than that of the second crop season in the early growth stage.

The changes of nitrate-nitrite nitrogen content in leaves are shown in Fig 6.

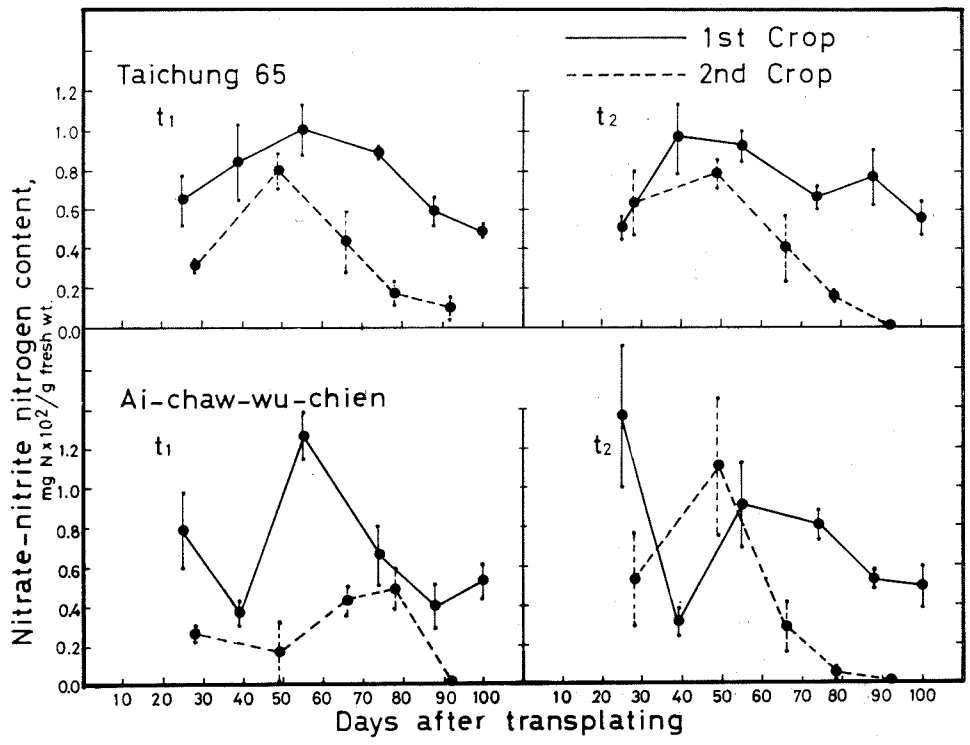

Fig. 6. Nitrate-nitrite nitrogen content in leaves of Taichung 65 and Ai-chaw-wu-chien during growth.

The nitrate-nitrite nitrogen content had a higher level at the booting stage both in the first and second crop seasons. However, the nitrate-nitrite nitrogen content in the first crop season was much higher than that in the second crop season at the ripening stage. The nitrate-nitrite nitrogen content in roots was too low to be detected.

Discussion

It has been generally accepted that a considerable part of inoganic nitrogen absorbed by plant roots can be incorporated into organic compounds even before entering the shoots (Pate and Wallace, 1964). Rice plants can absorb either the ammonium or the nitrate nitrogen as nitrogen source. One of an important route for assimilation of ammonium nitrogen in higher plants has been established to be the reductive amination of α-ketoglutarate catalyzed by the glutamate dehydrogenase. However, the assimilation of nitrate nitrogen in higher plants requires its prior reduction to ammonia by the nitrate and nitrite reductases, followed, by the direct incorporation of the ammonia into glutamate to yield glutamine catalyzed by the glutamine synthetase (Lea and Miflin, 1974; Lewis, 1975).

Our results indicated that the nitrate reductase in leaves showed two higher levels of activity during rice growth in regardless of crop seasons and rice varieties. The first peak occurred at the beginning of tillering and the second peak at the flowering stage. Judging from the time of sampling, it was found that the appearance of nitrate reductase peak seemed to be closely related to the time of top dressing. When nitrate nitrogen was used as the top dressing in the later growth stage, the nitrate reductase activity of the first crop season could maintain a higher level from flowering through ripening until harvesting. The higher nitrate reductase activity might be due to the better growth conditions in the later growth stage of the first crop season, and it is likely that the nitrate reductase was induced by the higher nitrate nitrogen effectively.

The glutamate dehyrogenase in leaves also showed two higher levels of activity during rice growth in regardless of different nitrogen sources for top dressing and rice varieties. The first peak occurred at the beginning of tillering both in the first and second crop seasons, however, the second peak at the booting stage in the first crop season and at the flowering stage in the second crop season. It clearly indicated that the physiological changes of rice plants in two crop seasons did not follow the same pattern during their growth.

In the first crop season, the nitrate reductase activity in leaves had the highest level at the flowering stage, and glutamate dehydrogenase activity in leaves at the beginning of tillering almost had the same high level as at the booting stage. The nitrate reductase activity in roots was much higher than that in leaves at the beginning of tillering, but, the glutamate dehydrogenase activity in roots was much lower than that in leaves at the beginning of tillering. In the second crop season, the nitrate reductase activity in leaves at the beginning of tillering almost had the same high level as at flowering, however, the glutamate dehydrogenase activity had the highest level at flowering. And both the nitrate reductase and glutamate dehydrogenase activities in roots were much lower than that in leaves at the beginning of tillering. The above observations indicated that the primary assimilation of nitrogen into rice plants in two crop seasons also did not follow the same pattern during their growth. In the first crop season, the nitrate reductase played a more important role in roots while the glutamate dehydrogenase played a more important role in leaves at the beginning of tillering, however, the nitrate reductase played a more important role in leaves at the flowering stage. In the second crop season, the nitrate reductase played a more important role in leaves at the beginning of tillering, however, the glutamate dehydrogenase played a more important role in leaves at the flowering stage.

The higher level of nitrate reductase and glutamate dehydrogenase activities in roots at the beginning of tillering of the first crop season indicated that roots also played an important role for primary assimilation of nitrogen in the early growth stage of the first crop season. However, the low level of nitrate reductase and glutamate dehydrogenase in roots of the second crop season indicated that the primary assimilation of nitrogen in roots was insignificant in the second crop season.

Free amino acids are the precursors of storage proteins in rice grains. Hence, a higher level of free amino acids in leaves would contribute to a faster and greater accumulation of proteins in grains. Cagampang *et al.* (1971) pointed out that the higher effeciency in translocation of free amino acids into the developing grains was related to the higher level of free amino acids in the corresponding sap entering the grain. Perez *et al.* (1973) also mentioned that grains with higher percentage of protein had higher concentration of free amino acids in the developing grains. Physiologists believe that the free amino acids of rice grains are derived mainly from the breakdown of proteins already present in the vegetative tissues of the plant at the flowering stage (Murayama, 1965). Figure 3 indicates that the free amino acids content in leaves had a higher level at the flowering stage both in the first and second crop seasons. The increase of free amino acids content in leaves at the flowering stage might be due to the breakdown of the storage proteins rendering a effective translocation of amino acids into the developing grains. The presence of certain amounts of free amino acids in roots at the tillering stage suggested that the assimilation and metabolism of nitrogen could also be proceeded to a significant extent in roots in the early growth stage.

The ammonium nitrogen content in leaves of Taichung 65 had two higher levels during its growth both in the first and second crop seasons. It is almost consistant with the variations of glutamate dehydrogenase. In Ai-chaw-wu-chien, the ammonium nitrogen content in leaves had a higher level at the flowering stage of the first crop season only, and did not have significant changes in the second crop season. The results also indicated that the changes of ammonium nitrogen content in leaves was influenced by crop seasons and rice varieties in regardless of different nitrogen sources for top dressing. The very low level of ammonium nitrogen content in roots might indicate that the absorbed ammonium nitrogen in roots was quickly translocated into leaves or assimilated into glutamate and glutamine directly in roots.

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 thorugh the glutamate synthase system (Miflin and Lea, 1976), and asparagine is mainly as the storage form of nitrogen in rice plants (Marwaha *et al.*, 1976). Figure 5 showed that the amide nitrogen content in leaves of the first crop season was higher than that of the second crop season at the tillering and booting stages. It might indicate that the ability of ammonia assimilation in leaves of the first crop season was stronger than that of the second crop season at the tillering and booting stages. And the lower level of amide nitrogen content in leaves of the first crop season at the ripening stage might indicate that the translocation of the storage nitrogen into the developing grains of the first crop season was more effective than that of the second crop season. The amide nitrogen content in roots of the first crop season was higher than that of the second crop season at the tillering stage. It might indicate that the ability of nitrogen assimilation in roots of the first crop season was also stronger than that of the second crop season at the tillering stage.

There were two possible ways to accumulate the nitrate-nitrite nitrogen in leaves. The first way was that the presence of nitrate nitrogen in paddy soil was absorbed by roots and translocated into leaves directly, and the second way was that the nitrate and nitrite were produced due to the oxidation reaction of ammonia in leaves (Muhammad and Kumazawa, 1974b). Therefore, the results in Figure 6 might indicate that the oxidizing ability of leaves of the first crop season was stronger than that of the second crop season.

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水稻生長期間硝酸鹽還原酶麥氨酸脫氫酶與可溶性含氮物質之季節性變異

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水稻於生長過程中，無論爲一期作或二期作，其硝酸鹽還原酶（nitrate reductase）與麥氨酸脫氫酶（glutamate dehydrogenase）都有兩次達到較高活性之時期；第一次在分蘖初期，第二次在開花期，可是麥氨酸脫氫酶在第一期作時，其第二次較高活性之時期在孕穗期。於一期作時，葉片中硝酸鹽還原酶之活性以開花期爲最高，而麥氨酸脫氫酶之活性，則以分蘖初期爲最高。在分蘖初期，根部之硝酸鹽還原酶，其活性較葉片中者爲高；而根部之麥氨酸脫氫酶，其活性則較葉片中者爲低。於二期作時葉片中硝酸鹽還原酶之活性，無論於分蘖期或開花期都很高，且無顯著差異；而麥氨酸脫氫酶之活性，則以開花期爲最高。根部之硝酸鹽還原酶與麥氨酸脫氫酶之活性都很低。葉片中游離氨基酸之含量，無論爲一期作或二期作均於開花期較高。於臺中65號葉片中之氨態氮，無論爲一期作或二期作均有兩次較高含量之時期；而矮脚烏尖葉片中之氨態氮祇有在一期作之開花期爲較高。葉片中之醯胺態氮，於一期作時，自分蘖期到開花期均能保持很高的含量；而於二期作時，葉片中醯胺態氮之含量於分蘖期較低然後漸漸增加，直到開花期達到最高含量。根部之醯胺態氮之含量，則以一期作時爲高。葉片中硝酸和亞硝酸態氮之含量，無論爲一期作或二期作，均於孕穗期較高。