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Effect of Mixed Amino Acids on Crop Growth

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1. Introduction

1.1 Nitrogen uptake and assimilation

Among the mineral nutrient elements, nitrogen is a kind of macronutrient. Most plant species are able to absorb and assimilate nitrate (NO_3^-), ammonium (NH_4^+), urea and amino acids as nitrogen sources. Most soils do not have sufficient N in available form to support desired production levels. Therefore, addition of N from fertilizer is typically needed to maximize crop yields. Many kinds of N fertilizers are used which contain varying forms of N such as NO_3^-–N, NH_4^+–N and urea. However, NO_3^- form of nitrogen is the predominant form of N absorbed by plants, regardless of the source of applied N (Breteler and Luczak, 1982). This preference is due to several autotrophic soil bacteria, which rapidly oxidize NH_4^+ to NO_2^-, and then to NO_3^- in warm, well–aerated soils. Even though NO_3^- is the most available form of N to plants, it can be more readily lost from the root zone because it is very mobile and easy to leach. This economically and environmentally undesirable process perpetuates a large amount of the uncertainty associated with N fertilizer management (Pessarakli, 2002).

In the soil solution, nitrate is carried towards the root by bulk flow and is absorbed into the epidermal and cortical symplasm. Within the root symplasm, nitrate has four fates: (1) reduced to nitrite by the cytoplasmic enzyme nitrate reductase; (2) efflux back across the plasma membrane to the apoplasm; (3) influx and stored in the vacuole; or (4) transported to the xylem for long–distance translocation to the leaves (Andrews, 1986; Ashley et al., 1975; Black et al., 2002; Cooper and Charkson, 1989). Translocated from the xylem, nitrate enters the leaf apoplasm to reach leaf mesophyll cells, where nitrate is again absorbed and either reduced to nitrite or stored in the vacuole.

Nitrate translocated from the roots through the xylem is absorbed by a mesophyll cell via one of the nitrate–proton symporters into the cytoplasm, reduced to nitrite by nitrate reductase (NR) in the cytoplasm, and then reduced to ammonium by nitrite reductase (NiR)

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in the chloroplast, which is then incorporated into amino acids by the glutamine synthetase−glutamine− 2−oxoglutarate amidotransferase (GS/GOGAT) enzyme system, giving rise to glutamine (Gln) and ultimately other amino acids and their metabolites (Fig. 1; Taiz and Zeiger, 2002). Therefore, NR, NiR and GS constitute the first three enzymes of the nitrate assimilatory pathway. The NR activity is the limiting step of NO_3^-–N conversion to amino acid synthesis (Campbell, 1999). In most plant species only a proportion of the absorbed nitrate is assimilated in the root, the remainder being transported upwards through the xylem for assimilation in the shoot where it is reduced and incorporated into amino acids (Forde, 2000).
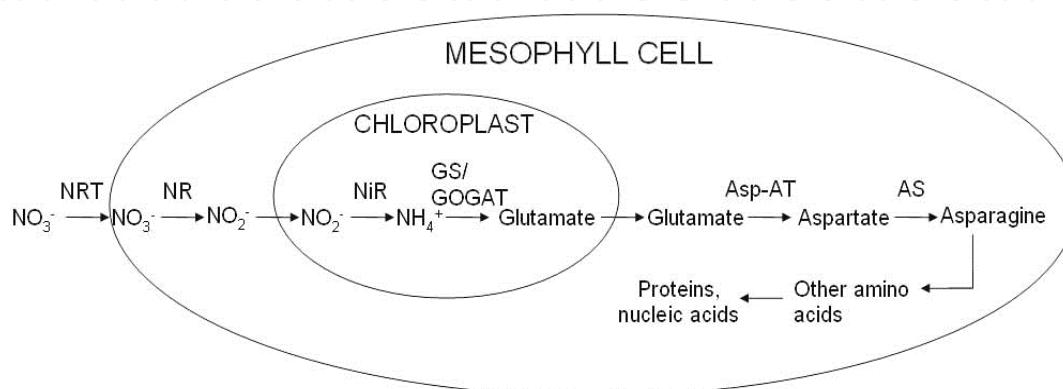
Fig. 1. The main process of nitrate assimilation.

1.2 Availability of amino acids

Traditional models of nutrient cycling assume that organic N matter must be decomposed by soil microorganisms to release inorganic N, before that N becomes available for plant uptake. But, there are growing evidences that plant can absorb organic N directly. Earlier studies of nutrient absorption demonstrated that higher plants could take up amino acids (Virtanen and Linkola, 1946). More recent studies of amino acid absorption have further focused on the characteristics of the carrier systems and other mechanistic aspects of the uptake process and a wide array of amino acid transporters has been identified in several different plants species (Frommer et al., 1993; Montamat et al., 1999; Neelam et al., 1999).

In the moist tundra of the arctic, inorganic N supplied to plants by mineralization is not sufficient to meet their requirement of N due to low temperatures and anoxic soils. But these soils have large stocks of water–extractable free amino acids (Atkin, 1996). The studies of nitrogen cycling in artic tundra have indicated that some non–mycorrhizal plant species, such as *Eriophorum vaginatum*, could absorb amino acids rapidly, accounting for at least 60% of total the nitrogen absorbed (Chapin et al., 1993). Ectomycorrhizal species have higher amino acid uptake than non–mycorrhizal species (Kielland, 1994). Amino acid uptake was the general ability found widely in plants from boreal forest (Näsholm et al., 1998; Persson and Näsholm, 2001).

1.3 Amino acids and nitrate uptake

Plants can store high levels of nitrate, or they can translocate it from tissue to tissue without deleterious effect. However, hazardous effects may occur when livestocks and humans

consume plant material with rich nitrate, they may suffer from methemoglobinemia or carcinoma by converting nitrate to nitrite of nitrosamines. Some countries limit the nitrate content in plant material sold for human consumption.

Several authors reported that free amino acids could down regulate nitrate uptake and nitrate content in plant. It was found that exogenously supplied amino acids and amides could decrease the uptake of nitrate by soybean (Muller and Touraine, 1992); wheat (Rodgers and Barneix, 1993); maize (Ivashikina and Sokolov, 1997; Padgett and Leonard, 1993, 1996; Sivasankar et al., 1997); barley (Aslam et al., 2001)（Table 1）. Plants appear to have multiple mechanisms for regulating nitrate uptake in addition to amino acids or N-status (Padgett and Leonard, 1993).

Work by Breteler and Arnozis (1985) determined that pretreatment of dwarf bean roots with many different individual amino acids inhibited nitrate uptake to varying degrees dependent upon prior exposure of the plants to nitrogen and the specific amino acid treatment. No significant effect of amino acids on nitrate transport was detected when both NO_3^- and amino acids were present in the bathing solution, and no correlation emerged between inhibition of nitrate uptake and inhibition of nitrate reductase relative to specific amino acids. A more detailed study, presented by Muller and Touraine (1992), demonstrated inhibition of uptake by 50% or greater by alanine, glutamine, asparagines, arginine, β–alanine and serine when soybean seedlings were pretreated for 18 h prior to exposure to NO_3^-. The mechanisms of inhibition by arginine and alanine appeared to differ, however. Arginine stopped NO_3^- uptake immediately upon introduction to the uptake solution, kinetically similar to NH_4^+ inhibition. The authors suggested that this may be the result of a non-metabolic response such as alteration of membrane potentials. Inhibition by alanine was slower to develop, suggesting a metabolic component to the regulation rather than a physical or chemical interference.

Plant materials	Amino acid treatment	NO_3^- supplied	NO_3^- uptake	Remarks
	(mM)		(%)	
Soybean	10	0.5	5−85	14 amino acids
	100	0.5	40−120	(Muller, 1992)
Wheat	1.0	0.3	50−105	3 days N starvation
	1.0	0.3	89−106	Non−starvation (Rodger, 1993)
Maize root	15	5.0	84	(Padgett, 1993)
Barley root	1.0	0.1	40−50	(Aslam, 2001)
	1.0	10	70	

Table 1. Effect of amino acid on NO_3^- uptake in several plants

The N status of the plants could also affect the inhibitory effect of amino acids on nitrate uptake. Rodger and Barneix (1993) had supplied amino acids exogenously to N starved or non−starved wheat seedlings. Exogenously supplied amino acids and amides had no effect on the wheat seedlings under well nourishment. However, some of the amino acids and amides supplied seedlings starved of N for 3 days inhibited up to 50% of the nitrate uptake rate.

Aslam et al. (2001) had conducted study on differential effect of amino acids (Glu, Asp, Gln and Asn) on nitrate uptake and reduction systems in barley roots. Similar results were observed i.e. 50–60% inhibition in the NO_3^- uptake when the roots were supplied with 0.1 mM NO_3^-. However, no inhibition occurred at 10 mM NO_3^-. In contrast, Kim (2002) had conducted study on effect of mixed amino acids on nitrate uptake in rice, pea, cucumber and red pepper. The result showed that the effect of mixed amino acids (MAA) on nitrate uptake in nutrient solution was unaffected in low MAA concentration and accelerated in high MAA concentration. The results indicated that external MAA could regulate nitrate uptake.

1.4 Amino acids and enzyme regulation

Nitrate reductase (NR) is a substrate inducible enzyme involved in the nitrate assimilation in higher plant, and the enzyme occupying a control point in the pathway of nitrate assimilation. Activity of the NR fluctuates widely in response to many environmental or physiological factors, such as the presence of NH_4^+ or amino acids in the growth medium. In studies of the possible regulation of NR activity by amino acids in higher plants, the results have often been conflicting. For example, Radin (1975, 1977) had shown that the reduction of nitrate to nitrite in cotton roots is inhibited by specific amino acids. On the other hand, Oaks (1977) had found using an *in vitro* assay those amino acids results in enhanced levels of NR and also cause only minor inhibitions in both intact and excised corn roots. Aslam et al. (2001) reported that the amino acids partially inhibited (35%) the induction of nitrate reductase activity (NRA) in barley roots supplied with 0.1 mM NO_3^-, but no inhibition occurred at 10 mM NO_3^-. He has concluded that the inhibition of induction of NRA by the amino acids is a result of the lack of substrate availability due to inhibition of the NO_3^- uptake system at low NO_3^- supply. It has been suggested that glutamate inhibited NRA in roots, but not in shoots (Ivashikina and Sokolov, 1997). This inhibition seems be dependent on plant materials, age of plants, growth conditions, nitrate concentration, amino acid kinds, amino acids concentration and other factors.

Effect of amino acids on the regulation of NR gene expression has been studied at the molecular level. Deng et al. (1991) reported that the addition of 5 mM glutamine to the nutrient solution of tobacco plants grown in 1 mM NO_3^- resulted in a pronounced inhibition of NR mRNA accumulation in the roots. Vincentz et al. (1993) showed, under low light conditions (limiting photo synthetic conditions), the supply of glutamine or glutamate led to a drop in the level of NR mRNA, while glutamine and glutamate were less efficient at decreasing NiR mRNA than NR mRNA levels. Li et al. (1995) also demonstrated that 5 mM glutamine added together with NO_3^- resulted in reduced levels of NR mRNA in both root and shoot of maize. Sivasankar et al. (1997) observed that Gln and asparagine (Asn) inhibited the induction of NR activity (NRA) in corn roots at an external supply of 250 μM and 5 mM NO_3^-. They concluded that inhibition was not the result of altered NO_3^- uptake, and tissue nitrate accumulation was reduced at 250 μM external nitrate in the presence of 1mM Asn, but not at 5mM Asn.

In the studies of the possible regulation of NR activity by multiple amino acids in higher plants, the conclusions are again contradictory. The inhibition on NR activity by glycine, asparagines, and glutamine could be partially or wholly prevented by the presence of other

amino acids during the induction (Radin, 1977). However when glutamine and asparagine were included along with the "corn amino acid mixture", the inhibition on the induction of NR in corn roots was more severe (Oaks et al., 1977). Chen and Gao (2002) have applied different mixture of glycine, isoleucine and proline replacing nitrate of solution partially (20%) to Chinese cabbage and lettuce in hydroponics. Amino acids enhanced the NR activity in Chinese cabbage, while it decreased in lettuce.

1.5 Influence on yield and N assimilation

L−tryptophan, considered as a physiological precursor of auxins in higher plants, was applied to soil to evaluate its influence on yield of several crops. Kucharski and Nowak (1994) found that L−tryptophan did not affect the yield of above ground part and roots of field bean. On the other hand, positive effects on corn and cabbage growth were reported (Sarwar and Frankenberger, 1994; Chen et al., 1997).

Amino acids were used to partially replace NO_3^- or foliar spray in many plants. In most case, the application of amino acids led to decreased nitrate content and increased total nitrogen content in lettuce, Chinese cabbage, onion, pakchoi or other leafy crops (Gunes et al., 1994, 1996; Chen and Gao, 2002; Wang et al., 2004). Some authors suggested that plants probably preferred amino acids as sources of reduced nitrogen, and nitrate uptake was inhibited by amino acids. In fact, there was little evidence or data to support the conclusions. It has not been distinguished that increased total nitrogen came of nitrate or amino acids.

1.6 Objectives

Regulation of induction of the NO_3^- uptake and reduction systems by nitrogen metabolites has been attributed to feed−back inhibition (Pal'ove−Balang, 2002). It was found that nitrate uptake rate follows a biphasic relationship with external nitrate concentration, suggesting the existence of at least two different uptake systems (Cerezo et al., 2000). At high external nitrate concentration (> 0.5 mM), a low affinity transport system (LATS), which shows linear kinetics, contributes significantly to the uptake rate and appears to be constitutively expressed and essentially unregulated. At low external concentrations (< 0.5 mM), two high affinity transport systems (HATS) operate, one of these being constitutive whereas the other is induced by nitrate. The HATS for nitrate uptake is sensitive to metabolic inhibitors and appears to be an active transport system (Daniel−Vedele et al., 1998).

Although the regulatory effect of amino acids on nitrate uptake and NR has been examined extensively, its effect on GS has not been examined in detail. Otherwise, a lot of amino acids were investigated about their regulation on nitrate uptake and assimilation, but very little information has been reported about effect of mixed amino acids (MAA).

In fact, there are two possible reasons for the increase of total N content in the plants: preference for amino acids as sources of reduced nitrogen and regulation of amino acids on inorganic nitrogen uptake and assimilation.

The solution experiments were carried out to investigate the regulation of the induction of NO_3^- uptake, NRA, NiRA and GSA in radish and red pepper by applying mixed amino

acids (MAA) under the conventional fertilization. These two plants were selected because radish is NO_3^- preferred crop and red pepper is NH_4^+ preferred crop. The amino acids used in this experiment were alanine (Ala), β–alanine (β–Ala), aspartic acid (Asp), asparagine (Asn), glutamic acid (Glu), glutamine (Gln) and glycine (Gly). These amino acids were selected for the reasons include: (1) their structural role in proteins, (2) significant effect on NO_3^- uptake which was found in many works, and (3) considerable amounts in plant phloem and xylem (Caputo and Barneix, 1997; Lohaus et al., 1997; Peeters and Van Laere, 1994; Winter et al., 1992).

In the frame of the studies on the effect of the mixed amino acids (MAA) on nitrate uptake and assimilation, the pot experiments were focused on the role of MAA in process of NO_3^- uptake and assimilation. In order to distinguish the origin of N in radish, ^{15}N labeled nitrate was used.

In order to develop an approach for more efficient N fertilizer use and to prevent environmental pollution due to nitrate leaching, the aim of the study presented here, is to investigate the effect of amino acid fertilizer (AAF) on nitrate removal in high nitrate soils.

2. Hydroponic experiment of radish

2.1 Materials and method

Seeds of Ilsan radish (*Raphanus sativus*) soaked for 6 h allowed to germinate on paper towels were soaked in water in the dark. After 5 days the seedlings were transferred to 50 mL plastic tubes containing 10 mL inorganic nutrient solution. The nutrient solution was renewed every day. The composition of the inorganic nutrient solution is given in Table 2. Iron (Fe-EDTA), boron (H_3BO_3), manganese ($MnCl_2 \cdot 4H_2O$), zinc ($ZnSO_4 \cdot 7H_2O$), copper ($CuSO_4 \cdot 5H_2O$) and molybdenum ($H_2MoO_4 \cdot H_2O$) were supplied to all treatments at rates of 40, 460, 90, 7.7, 3.2 and 0.1 μM, respectively. Seedlings were grown in a growth chamber maintained at 25°C, 70–80% relative humidity, with a 14 h light/10 h dark cycle and a light intensity of 300 μmol m^{-2}s^{-1}.

Chemicals	K$^+$	NO$_3^-$	Ca^{2+}	H$_2$PO$_4^-$	Mg^{2+}	SO$_4^{2-}$
KNO$_3$	1.25	1.25				
Ca(NO$_3$)$_2$		2.50	1.25			
KH$_2$PO$_4$	0.25			0.25		
MgSO$_4$					0.50	0.50
Total	1.50	3.75	1.25	0.25	0.50	0.50

Table 2. The main compositions of the nutrient solution for hydroponic experiment (mM)

The mixed amino acids (MAA) solution contained 7 equal concentrations of amino acids were as follows: alanine (Ala), β–alanine (β–Ala), aspartic acid (Asp), asparagine (Asn), glutamic acid (Glu), glutamine (Gln) and glycine (Gly). After 10 days, radish seedlings were placed in 10 ml inorganic nutrient solution containing 5.0 mM NO_3^- and 0, 0.3 or 3.0 mM MAA, as indicated in Table 3. The pH of the nutrient solutions were maintained between 6.0–6.1 by adding 1.0 M KOH appropriately. The nutrient solutions were renewed at 4, 8 and 16 h, respectively. The choice of the levels of MAA and renewed time of the nutrient solutions were according to the study of Kim (2002)

Treatments	K⁺	NO₃⁻	Ala	β–Ala	Asp	Asn	Glu	Gln	Gly
A0	5.25	5.0	—	—	—	—	—	—	—
A1	6.78	5.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3
A2	13.10	5.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

Table 3. The compositions of the treatment solutions for radish in hydroponic experiment (mM)

Plants were harvested 24 h after treatment and separated into roots and shoots for enzymes assay and N content analysis. Net NO_3^- uptake rates were determined by amount of NO_3^- disappeared from the initially treated solution.

2.2 Results and discussion

2.2.1 Effect on NO_3^- uptake

The MAA treatments showed different effect on nitrate uptake depending on the concentrations (Fig. 2). The NO_3^- uptake in treatment A1 was similar to that of A0 after 8 h exposure to NO_3^-. However, exposure for longer hours (16 or 24 h) to 0.3 mM MAA inhibited the NO_3^- uptake by 38% compared with A0. In contrast, the highest NO_3^- uptake was found in treatment A2 that showed 305% higher than A0.
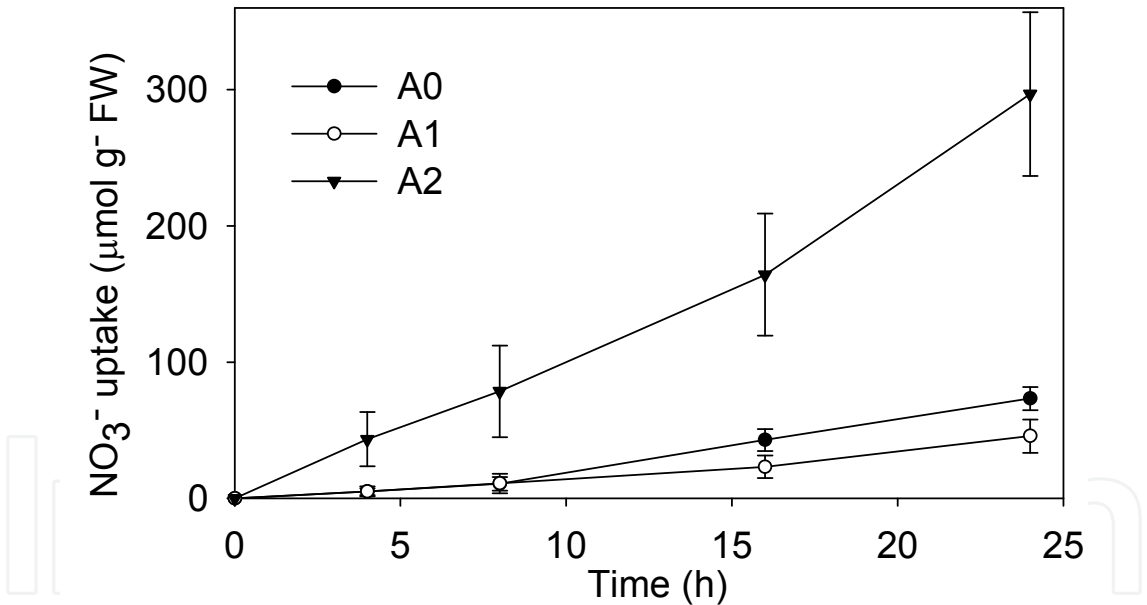

Fig. 2. Effect of mixed amino acids on the nitrate uptake in radish supplied with 5.0 mM NO_3^-. Values are means ± SD (n=5).

Several authors reported that free amino acids could down regulate NO_3^- uptake. It was found that exogenously supplied amino acids and amides could decrease the uptake of NO_3^- by soybean (Muller and Touraine, 1992); wheat (Rodgers and Barneix, 1993); maize (Ivashikian and Sokolov, 1997; Padgett and Leonard, 1996; Sivasankar et al., 1997); barley (Aslam et al., 2001). In this experiment, the effectiveness of the MAA treatments on NO_3^- uptake was similar to above references at low MAA treatment rate (0.3 mM MAA, Fig. 2). However, contrary result was found at high MAA treatment rate (3.0 mM MAA, Fig. 2), in

which NO_3^- uptake was 4–fold higher than the control. This result was similar to rice, pea, cucumber and red pepper, which were treated with 5.0 mM MAA (Kim, 2002).

The effect on nitrate uptake seems to respond to kinds and concentration of amino acids. Muller and Touraine (1992) had examined the effect of 14 different amino acids on nitrate uptake in soybean seedlings supplied with 0.5 mM nitrate. After 10 mM single amino acid pretreatment, about half of the tested amino acids had a substantial inhibitory effect on nitrate uptake, mainly Ala, Glu (almost 100% inhibition), Asn and Arg (about 80%), and Asp, βAla, Scr, and Gln (from 70% to 48%). However, when supplied at 100 mM amino acid to the tip−cut cotyledons, only eight of fourteen amino acids had inhibitory effect, and four amino acids had enhanced nitrate uptake.

2.2.2 Effect on NO_3^- and NO_2^- accumulation

The application of MAA increased the NO_3^- concentrations both in shoots and in roots regardless of application rates (Table 4), resulting in the highest concentration in A1 and the lowest concentration in A0. The high concentration of NO_3^- in A2 was attributed to the high NR activity (Fig. 3). Although A1 treatment showed the lowest uptake of NO_3^- (Fig. 2), the highest concentration of NO_3^- was found by the reason of that low NR activity in A1 (Fig. 3) led to a blocking of the reduction of NO_3^- to NO_2^-. With respect to the NO_2^- values (Table 4), in our experiments, the highest NO_2^- concentrations in both shoots and roots were found in the A2. In shoots, the lowest NO_2^- concentration was found in A1 and the lowest in A0 in roots.

Treatments	NO_3^- (μmol g^{-1})		NO_2^- (nmol g^{-1})	
	Shoot	**Root**	**Shoot**	**Root**
A0	62.47 ± 4.06 a	16.30 ±1.88 b	6.76 ± 0.62 b	11.43 ± 1.67 c
A1	67.73 ± 7.49 a	22.99 ±2.23 a	3.77 ± 0.34 c	17.14 ± 2.10 b
A2	63.37 ± 3.58 a	17.41 ±1.92 b	29.70 ± 2.78 a	30.39 ± 4.13 a

Data are means ± SD (n=5). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05).

Table 4. Effect of mixed amino acids on NO_3^- and NO_2^- concentration in fresh weight of radish at 24 h after treatment

Although many authors agree that amino acid can negatively regulate nitrate content in higher plants (Chen and Gao, 2002; Gunes et al., 1994, 1996; Wang et al., 2004), the results in the present experiment do not support this interpretation. Both in shoots and in roots, the MAA used in this study led to little increase of NO_3^- concentrations (Table 4). The contradiction may reside in treatment method and treatment period of amino acids. It was demonstrated in other studies that amino acid pretreatment decreased NO_3^- accumulation slightly, but Gln and Asn increased the NO_3^- concentration in barley roots when they were used together with nitrate (Aslam et al., 2001). The reason of difference between this experiment and others is that NO_3^- content of shoots includes portion of NO_3^- in xylem sap. Concentrations of NO_3^- in xylem sap can be quite high, especially in plants that transport most of the NO_3^- taken up to the shoot for reduction (e.g., maize 10.5 mM, Oaks, 1986; barley 27 to 34 mM, Lews et al., 1982).

As interim product of NO_3^- assimilation procedure, the concentration of NO_2^- depended on the reduction rate of nitrate and nitrite. The highest concentration of NO_2^- found in A2 (Table 4) was due to high NR activity (Fig. 3), and the lowest concentration of NO_2^- in shoots in A1 (Table 4) was due to low NR activity (Fig. 3) too. However, low NiR activity (Fig. 4) led to a blocking of the reduction of NO_2^- to NH_4^+ in roots in A1, so that concentration of NO_2^- showed higher than A0 (Table 4).

2.2.3 Effect on NRA, NiRA and GSA

For NO_3^- assimilation, NO_3^- is reduced to NO_2^- by catalysis of NR. In this experiment, low concentration and high concentration of MAA treatments led to different effects on NR activity (Fig. 3). Both in the shoots and in the roots NR activities were inhibited slightly in A1. Significant increases of NR activities were found in A2 treatment, with 75% in shoots and 340% in roots respectively, relative to A0.
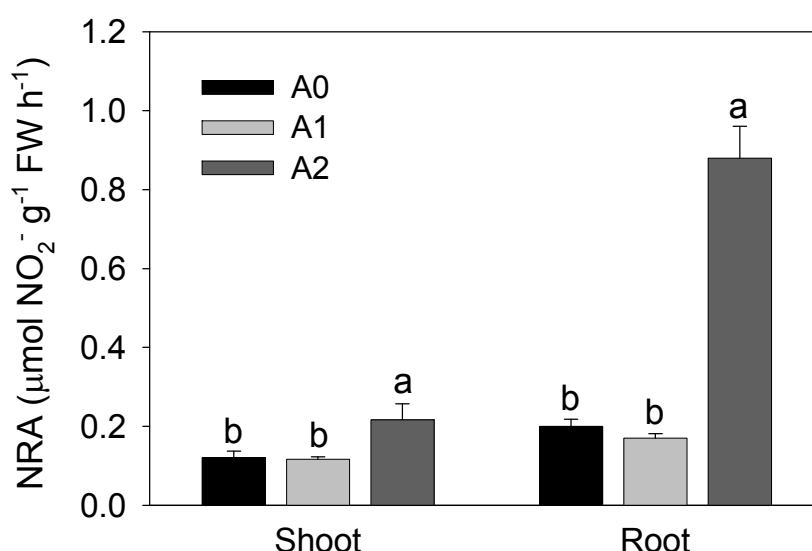

Fig. 3. Effect of mixed amino acids on nitrate reductase activity in radish at 24 h after treatment. Values are means ± SD (n=5).

There are contradictory results for the possible regulation of NR activity by amino acids for higher plants. For example, Radin (1975, 1977) has shown that the reduction of NO_3^- to NO_2^- in cotton roots is inhibited by specific amino acids. On the other hand, Oaks et al. (1979) have found that amino acids inhibited minor levels of NR in both intact and excised corn roots using an *in vitro* assay. Aslam *et al.*, (2001) reported that the amino acids partially inhibited the increase of NR activity in barley roots where most NO_3^- uptake was facilitated via high affinity transport system (HATS) but had little effect where low affinity transport system (LATS) is operative. It has been suggested that glutamate inhibited NR activity in roots, but no inhibition in shoots (Ivashikian and Sokolov, 1997). Sivasankar et al. (1997) observed that Gln and asparagine (Asn) inhibited the induction of NR activity in corn roots at both 250 μM and 5 mM of external NO_3^- supply. They concluded that inhibition was not the result of altered NO_3^- uptake, and tissue nitrate accumulation was reduced at 250 μM external nitrate in the presence of 1 mM Asn, but not at 5 mM Asn.

In the studies of the possible regulation of NR activity by multiple amino acids in higher plants, the conclusions are also contradictory. The inhibition on NR activity by glycine, asparagines, and glutamine could be partially or wholly prevented by the presence of other amino acids during the induction (Radin, 1977). However when glutamine and asparagines were included along with the "corn amino acid mixture", the inhibition on the induction of NR in corn roots was more severe (Oaks et al., 1979). Chen and Gao (2002) have applied different mixture of glycine, isoleucine and proline to Chinese cabbage and lettuce in hydroponic experiment. They found the amino acids treatment enhanced NR activity in Chinese cabbage, while decreasing it slightly in lettuce.

In this experiment, at 5.0 mM NO_3^- which is facilitated by LATS, the presence of 0.3 mM MAA partially inhibited NR activity, as observed in other works, whereas the 3.0 mM MAA increased the NR activity more than 4 times (Fig. 3). In addition, the very high NO_2^- content was found in A2 (Table 4). These results suggest that high concentration MAA can increase NO_3^- uptake by enhancing NR activity in radish, especially in roots.

The next step in NO_3^- assimilation is the conversion of NO_2^- to NH_4^+ by the action of NiR. Both enzymes, NR and NiR, are induced by the same factors, and therefore the response of NiR to the MAA treatments resembled that of NR in roots, but was a little different with that of the NR in shoots (Fig. 3 and Fig. 4). NiR activities in shoots and roots in A1 were inhibited by 17% and 52% respectively in relation to A0. In A2, NiR activity was inhibited by 15% in shoots and enhanced 8 times in roots. In the present study, the decrease of NiR activity in shoots in A2 might be attributed to the low concentration of amino acids in shoots, too. The increase of NiR activity in roots in A2 was due to the same reason with NR.
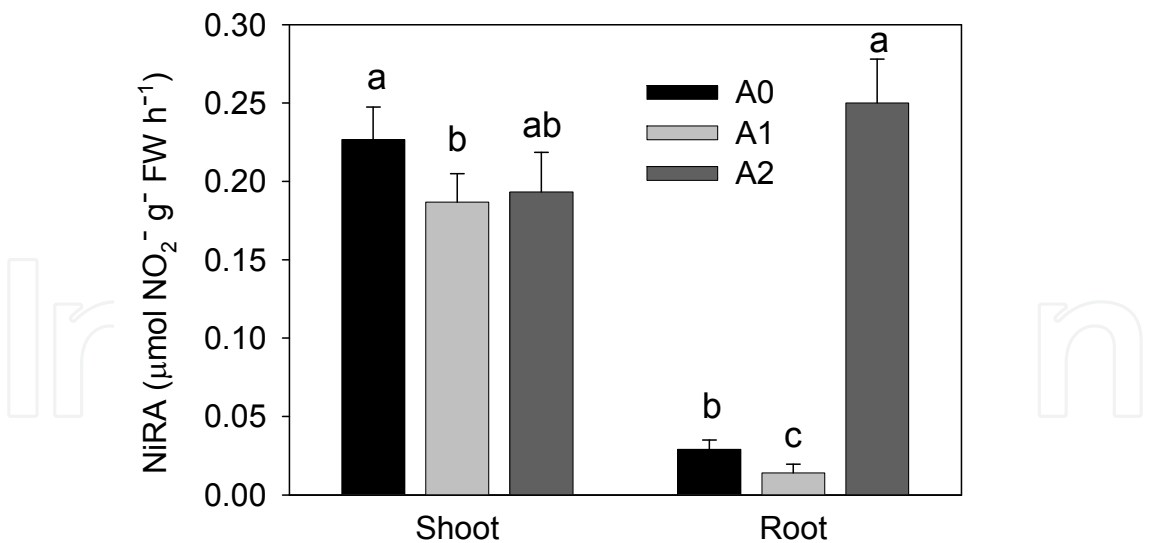

Fig. 4. Effect of mixed amino acids on nitrite reductase activity in radish at 24 h after treatment. Values are means ± SD (n=5).

The principal NH_4^+ pathway is the glutamine synthetase (GS)/glutamate synthase (GOGAT) cycle. The behavior of GS activities in shoots was not affected by MAA treatments (Fig. 5). However differences were found in roots between treatments, showing 22% inhibition in A1 and 17% increase in A2 in relation to A0.

The NH_4^+ originating in the plant from NO_3^- reduction is incorporated into an organic form primarily by the enzyme GS. In the present experiment, GS activity was inhibited by 0.3 mM MAA treatment in radish roots, whereas 3.0 mM of MAA treatment enhanced the activity (Fig. 5). It is also striking that effect of MAA on NO_3^- assimilation in the roots was higher than in the shoots, presumably NO_3^- was more available and the MAA content was higher in the roots.

The results of the present experiment clearly indicated that NO_3^- uptake and NO_3^- assimilation was regulated by MAA in radish, especially at high concentration of MAA treatment. In conclusion, the application of high MAA rates (principally A2) could be the direct cause of increased activities of the three enzymes (NR, NiR and GS) of the NO_3^- assimilatory pathway and the NO_3^- uptake was enhanced when supplied with LATS range of NO_3^-.
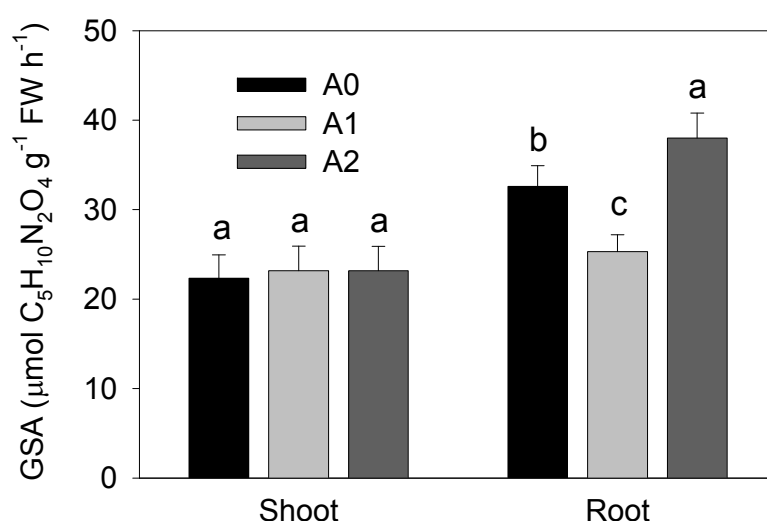

Fig. 5. Effect of mixed amino acids on glutamine synthetase activity in radish at 24 h after treatment. Values are means ± SD (n=5).

3. Hydroponic experiment of red pepper

3.1 Materials and methods

Seeds of Chongok red pepper (*Capsicum annuum*) were sown in February 2005. The seedlings were grown in individual pots filled with commercialized artificial soil in an experimental greenhouse for 35 days and then transferred to 50 mL plastic tubes containing 20 mL inorganic nutrient solution. The nutrient solution was renewed every day. The composition of the inorganic nutrient solution and the cultural condition were the same with hydroponic experiment of radish.

The mixed amino acids (MAA) solution was the same with that used in hydroponic experiment of radish which contained 7 equal concentrations of amino acids. At 7 days after transferring, red pepper seedlings were placed in inorganic nutrient solution containing 1.0 mM NO_3^- and 0, 0.3 or 3.0 mM MAA, as indicated in Table 5. The pH of the nutrient solutions were maintained between 6.0–6.1 by adding 1.0 M KOH appropriately. The nutrient solutions were renewed at 4, 8, and 16 h, respectively.

Treatments	K$^+$	NO$_3^-$	Ala	β–Ala	Asp	Asn	Glu	Gln	Gly
A0	10.25	10.0	—	—	—	—	—	—	—
A1	11.78	10.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3
A2	18.10	10.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

Table 5. The compositions of the treatment solutions for red pepper in hydroponic experiment (mM)

Plants were harvested 24 h after treatment and separated into roots and leaves for enzymes assay and N content analysis. Net NO$_3^-$ uptake rates were determined by amount of NO$_3^-$ disappeared from the initially treated solution.

3.2 Results and discussion

3.2.1 Effect on NO$_3^-$ uptake

The MAA treatments showed different effect on nitrate uptake depending on the concentrations (Fig. 6). Application of MAA at both 0.3 mM and 3.0 mM concentrations increased NO$_3^-$ uptake in red pepper ($P < 0.001$) and the highest NO$_3^-$ uptake was found in treatment A2 showing 7 fold increases over A0.
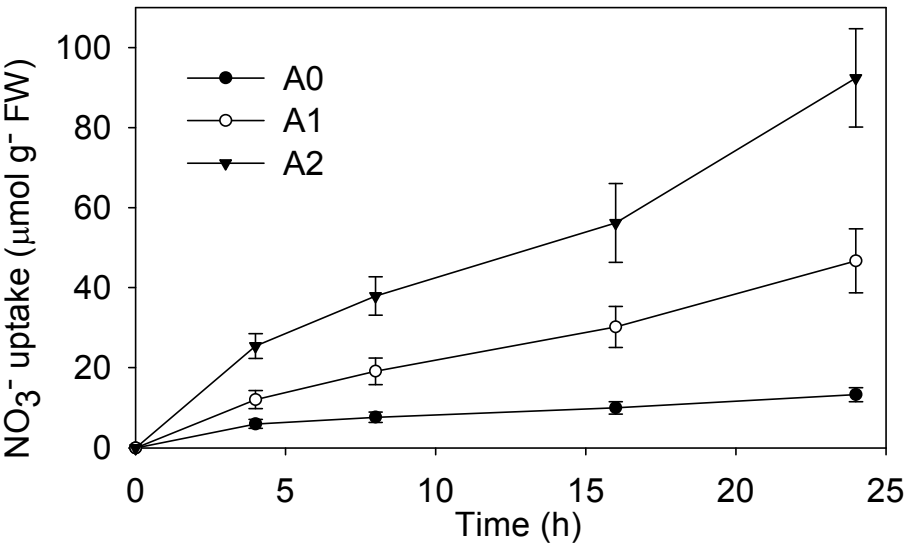

Fig. 6. Effect of mixed amino acids on the nitrate uptake in red pepper supplied with 10.0 mM NO$_3^-$. Values are means ± SD (n=5).

3.2.2 Effect on NO$_3^-$ and NO$_2^-$ accumulation

The highest NO$_3^-$ concentration both in the roots and leaves were found in treatment A0 (Table 6), with respect to the lowest NO$_3^-$ content found in treatment A1 in the leaves ($P < 0.05$) and A2 in the roots ($P < 0.01$). With respect to the NO$_2^-$ values (Table 6), in this experiment, the highest NO$_2^-$ concentrations in roots were found in the A2 and the lowest in A0 ($P < 0.001$). In leaves, the lowest NO$_2^-$ concentration was found in A2 and the lowest in A1 ($P > 0.05$).

Treatments	NO_3^-		NO_2^-	
	Leaf	**Root**	**Leaf**	**Root**
A0	9.12±0.58 a	6.20±0.23 a	0.036±0.003 b	0.596±0.032 c
A1	7.54±0.34 b	3.99±0.36 b	0.046±0.006 a	1.164±0.046 b
A2	8.31±0.43 ab	2.66±0.19 c	0.024±0.004 c	2.371±0.085 a

Values are means ± SD (n=5). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05).

Table 6. Effect of mixed amino acids on NO_3^- and NO_2^- concentration in fresh weight of red pepper at 24 h after treatment ($\mu mol\ g^{-1}$)

3.2.3 Effect on NRA, NiRA and GSA

For NO_3^- assimilation, NO_3^- is reduced to NO_2^- by catalysis of NR. In this experiment, MAA treatments led to different effects on NR activity in leaves and in roots (Fig. 7). In the roots, treatment A1 and treatment A2 showed increases of 35% and 212% respectively in relation to A0 ($P < 0.01$). In contrast, NR activities were inhibited slightly in leaves by MAA treatments, showing 8.2% in A1 and 10.5% in A2, respectively ($P > 0.05$).

The response of NiR to the MAA treatments resembled that of NR in roots, but was different with that of the NR in leaves (Fig. 8). NiR activities in leaves and roots in A1 were increased by 18% and 60% respectively in relation to A0 (leaves: $P < 0.05$; roots: $P < 0.01$). In A2, NiR activities were the same with A0 in leaves and enhanced 138% in roots (leaves: $P > 0.05$; roots: $P < 0.01$).
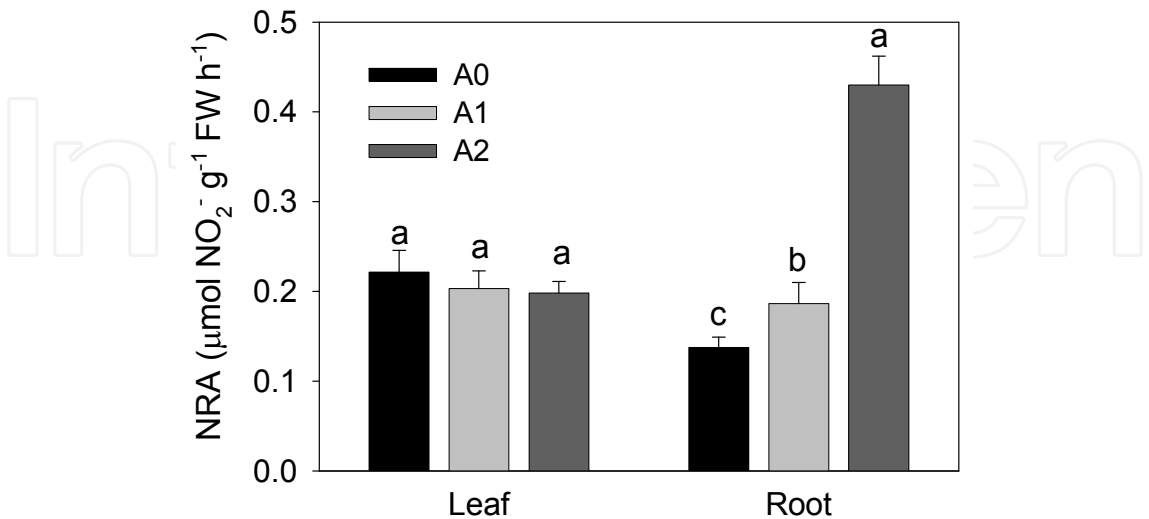

Fig. 7. Effect of mixed amino acids on nitrate reductase activity in red pepper at 24 h after treatment. Values are means ± SD (n=5).
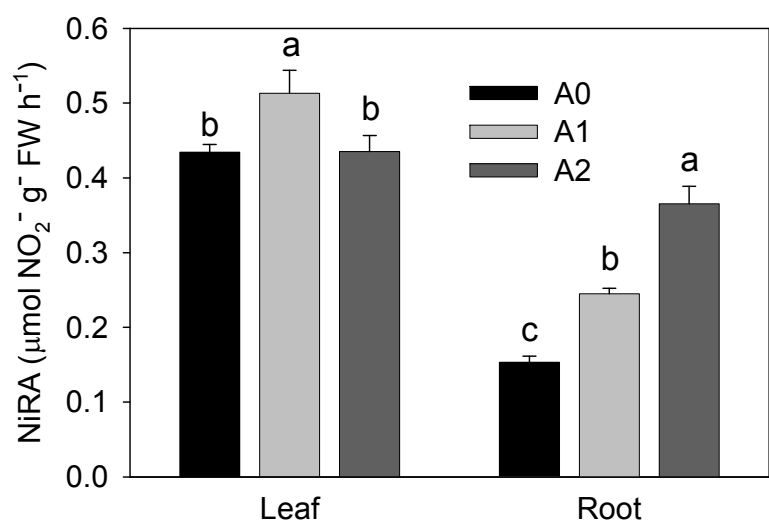
Fig. 8. Effect of mixed amino acids on nitrite reductase activity in red pepper at 24 h after treatment. Values are means ± SD (n=5).

The principal NH_4^+ pathway is the glutamine synthetase (GS)/glutamate synthase (GOGAT) cycle. The behavior of GS activities in leaves was increased by 16% in A1 but not affected in A2 (Fig. 9; $P > 0.05$). However, slight inhibitions were found in roots, showing 7% in A1 and 17% in A2 in relation to A0 ($P < 0.05$).
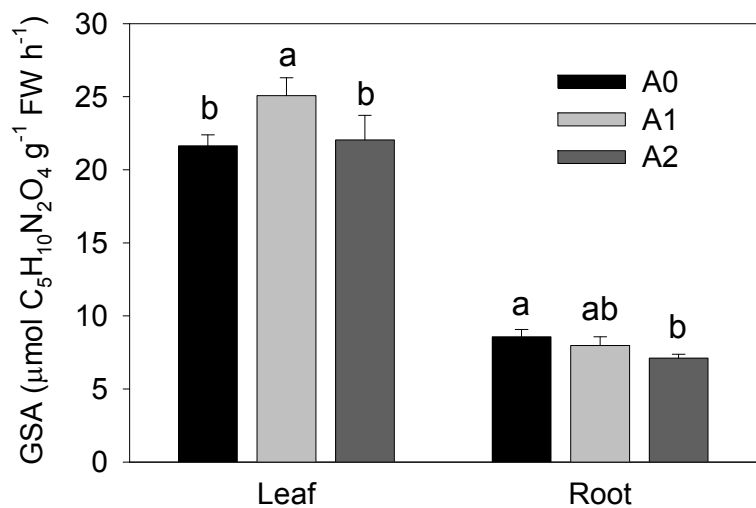

Fig. 9. Effect of mixed amino acids on glutamine synthetase activity in red pepper at 24 h after treatment. Values are means ± SD (n=5).

The first step in nitrate assimilation is the reduction of NO_3^- to NO_2^- by NR, the main and most limiting step, in addition to being the most prone to regulation (Sivasankar et al., 1997; Ruiz et al., 1999). The next step in NO_3^- assimilation is the conversion of the NO_2^- to NH_4^+ by the action of NiR. Both enzymes, NR and NiR, are induced by the same factors (Oaks, 1994). In our experiment, at 10 mM NO_3^- which is facilitated by LATS, the presence of MAA could increase the activities of NR and NiR in roots (Fig. 7 and Fig. 8). In addition, the very high NO_2^- content was found in MAA treatments in roots (Table 6). These results suggest that MAA can increase NO_3^- uptake by enhancing NR activity in roots of red pepper. It is

also striking that effect of MAA on NO_3^- assimilation in the roots was higher than in the leaves, presumably NO_3^- was more available and the MAA content was higher in the roots.

Ammonium assimilation in higher plants was long thought to begin with the synthesis of glutamate by glutamate dehydrogenase (GDH). It is now believed that the major pathway of NH_4^+ assimilation is the GS-GOGAT pathway, and GDH generally acts in a deaminating direction (Milflin and Habash, 2001). However, a role in NH_4^+ detoxification would explain the increase in GDH expression under conditions that provoke high tissue NH_4^+ levels (Lancien et al., 2000).

Two possible effect ways of amino acids on N assimilation process had been suggested: direct effect on mRNA of NR (Deng et al., 1991; Li et al., 1995; Vincentz et al., 1993) and feed–back inhibition on NO_3^- reduction systems (King et al., 1993; Ivashikian and Sokolov, 1997; Sivasankar et al., 1997). The hypothesis is that these two effect ways can collectively influence N assimilation in higher plant. This might probably be the main reason for differential effects on NO_3^- uptake observed in different studies. In the present experiment, GS activity was inhibited slightly by MAA treatments in roots, whereas irregular results were obtained in leaves (Fig. 9).

3.2.4 Effect on amino acids and proteins accumulation

With respect to the main products of NO_3^- assimilation, amino acids and proteins (Table 7), the plants treated with MAA did not show increase in these compounds as being supposed apart from amino acids in roots ($P < 0.05$). In contrast, the concentration of proteins in the roots ($P < 0.05$) and leaves ($P > 0.05$) decreased with the MAA rate. Amino acids in leaves ($P > 0.05$) showed the same tendency too.

Amino acids are the building blocks for proteins and also the products of their hydrolysis (Barneix and Causin, 1996). In the present experiment, amino acids concentrations (Table 7) were higher in the roots than in leaves. This is normal since the N assimilation occurs primarily in the roots than in the leaves. In roots, proteins concentrations (Table 7) were decreased by MAA treatment due to the possibility that amino acids content had effect on protein breakdown.

Treatments	Amino acids		Proteins	
	Leaf	Root	Leaf	Root
A0	0.93±0.03 a	2.35±0.12 b	5.03±0.27 a	1.92±0.12 a
A1	0.78±0.06 b	2.81±0.16 a	4.53±0.18 b	1.90±0.10 a
A2	0.67±0.03 b	3.05±0.08 a	4.35±0.24 b	1.45±0.11 b

Data are means ± SD (n=5). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05).

Table 7. Effect of mixed amino acids on level of amino acids and proteins in fresh weight of red pepper at 24 h after treatment (mg g^{-1})

In conclusion, the results of the present experiment clearly indicated that NO_3^- uptake and NO_3^- assimilation were regulated by MAA in red pepper. The application of MAA rates could be the direct cause of increased activities of the enzymes (NR and NiR) of the NO_3^- assimilatory pathway and the NO_3^- uptake was enhanced when supplied with

LATS range of NO_3^-. In addition, NO_3^- uptake by red pepper in unit weight plant was less than that of radish due to the different preference on N form between these two plants.

4. Pot experiment of radish with high NO_3^- soil

4.1 Materials and methods

Commercialized artificial soil (pH, 5.2; EC, 1240 mS m^{-1}; NO_3^-–N, 280 mg Kg[-1]; available P_2O_5, 1020 mg Kg[-1]) was mixed with ^{15}N labeled potassium nitrate (10 atom % [15]N) and incubated at room temperature for 14 days at 60% of their maximum water–holding capacity. Finally, the high nitrate soil (pH, 5.0; EC, 3230 mS m^{-1}; NO_3^-–N, 1906 mg Kg[-1]; available P_2O_5, 1060 mg Kg[-1]) was obtained and used for this experiment. Seeds of radish were sown into 100 mL pots filled with the incubated soil and grown in a glasshouse.

The mixed amino acids (MAA) solution contained equal concentrations of amino acids viz., alanine (Ala), β–alanine (β–Ala), aspartic acid (Asp), asparagines (Asn), glutamic acid (Glu), glutamine (Gln) and glycine (Gly). From 17 or 24 days after sowing, seedlings of radish were sprayed with 0.2 or 0.5 mM MAA solution for 2 or 4 times, as indicated in Table 8. The pH of the MAA solutions was maintained between 6.0–6.1 by adding 1.0 M KOH appropriately.

Treatments	Composition of treated solutions (mM)								Applied time
	K+	Ala	β–Ala	Asp	Asn	Glu	Gln	Gly	DAS
A0*	—	—	—	—	—	—	—	—	—
A1	0.78	0.2	0.2	0.2	0.2	0.2	0.2	0.2	17, 20, 24, 27
A2	2.10	0.5	0.5	0.5	0.5	0.5	0.5	0.5	17, 20, 24, 27
A3	0.78	0.2	0.2	0.2	0.2	0.2	0.2	0.2	24, 27

* Same amount of distilled water sprayed

Table 8. Composition of the treated solutions and application times for radish in pot experiment

Fresh leaves were collected at 28 days after sowing to determine the NO_3^- content and enzyme activities and at 30 days after sowing to determine the NO_3^-, amino acids and protein contents. Plant shoots were harvested at 30 days after sowing to determine crop yield and N assimilation. After harvest the soils were collected for chemical analysis.

4.2 Results and discussion

4.2.1 Effect of MAA on enzyme activities

Nitrate reductase is the first enzyme involved in the metabolic route of NO_3^- assimilation in higher plants. Significant differences were found in the NR activity between the treatments ($P < 0.01$) (Fig. 10). The highest activity was attained with A2, showing an increase of 30% compared with the activity attained with A0. Treatment A1 and A3 were less effective in increasing the activity of NR than A2, with increase of 21% and 7%, respectively.
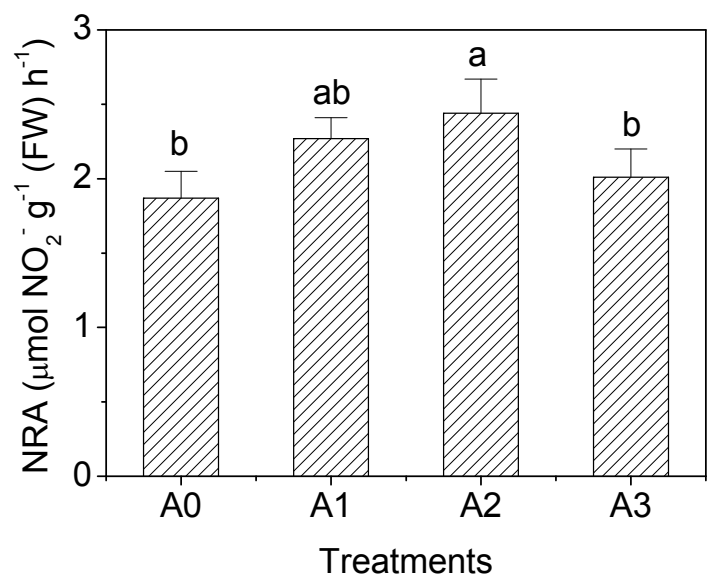
Fig. 10. Effect of mixed amino acids on nitrate reductase activity of radish leaves 28 day after sowing in pot experiment with high NO_3^- soil. Values are means ± SD (n=4).

The next step in NO_3^- assimilation is the conversion of the NO_2^- to NH_4^+ by the action of NiR. The MAA treatments showed different effects on NiR activity depending on the applied concentrations and times of MAA (Fig. 11). The highest activity of NiR was found in treatment A2, showing an increase of 7% compared with A0 ($P < 0.1$). However, the activity of NiR showed a decrease of 11% in A1 ($P < 0.05$).
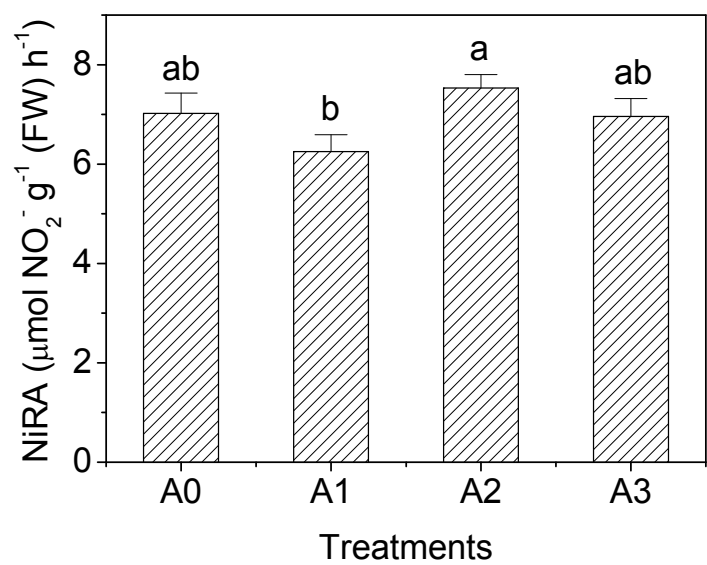

Fig. 11. Effect of mixed amino acids on nitrite reductase activity of radish leaves 28 day after sowing in pot experiment with high NO_3^- soil. Values are means ± SD (n=4).
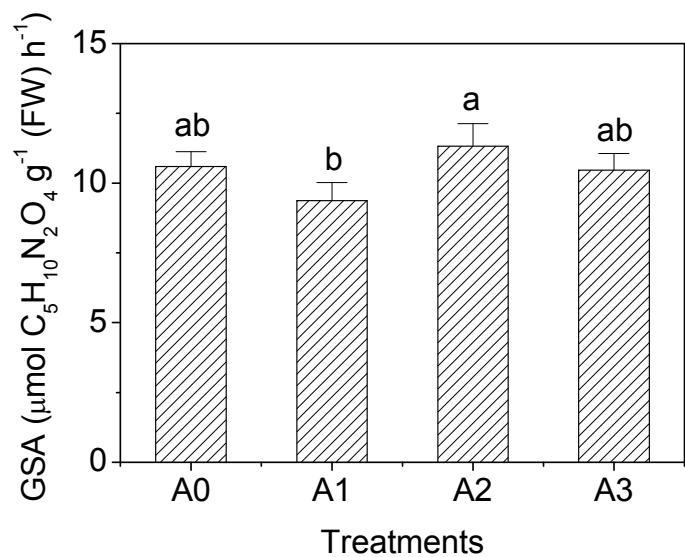
Fig. 12. Effect of mixed amino acids on glutamine synthetase activity of radish leaves 28 day after sowing in pot experiment with high NO_3^- soil. Values are means ± SD (n=4).

The response of GS to MAA treatments is showed in Fig. 12. The greatest activity was observed in treatment A2, with an increase of 7% over the reference treatment ($P > 0.1$). On the contrary, the least activity of GS was found in A1, with a 12% decrease compared with A0 ($P < 0.05$).

The results of activities of enzymes are similar to those of other research, which indicated that treatment of MAA (Section 3.1) and amino acid fertilizer (Section 3.7) could enhance activity of NR in radish when supplied with high rate NO_3^-. In the present experiment, the treatments of MAA led to different rates of increase in NR activity and also affect NiR and GS activities depending on applied rates. Higher activities of three enzymes were found in A2 for the reason that the positive effect on NR was stronger than the feed–back inhibition. However, decrease of NiR and GS was observed in A1 due to the feed–back inhibition on NO_3^- reduction systems which affected GS first.

4.2.2 Effect of MAA on N contents

The data in Table 9 showed that N contents of the plants were affected by using MAA. The NO_3^- content of radish was decreased by 24–38% by applying MAA ($P < 0.001$) compared with the reference treatment.

Treatments	Amino acids	Proteins	NO_3^-	Total N
	(mg g^{-1} FW)		(mg g^{-1} DW)	
A0	2.91 ± 0.10 a	9.05 ± 0.58 a	5.79 ± 0.59 a	44.9 ± 1.9 a
A1	2.93 ± 0.07 a	9.57 ± 0.46 a	3.85 ± 0.44 bc	38.4 ± 1.3 b
A2	3.03 ± 0.07 a	9.77 ± 0.54 a	3.57 ± 0.45 c	41.9 ± 1.8 ab
A3	2.99 ± 0.09 a	9.19 ± 0.69 a	4.38 ± 0.18 b	40.2 ± 1.7 ab

Data are means ± SD (n=4). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05).

Table 9. Effect of mixed amino acids on nitrogen contents of radish leaves 30 day after sowing in pot experiment with high NO_3^- soil

With respect to the main products of NO_3^- assimilation, amino acids and proteins (Table 9), the plants treated with MAA showed a little increase of these compounds ($P > 0.05$) and the highest contents were found in A2.

The total N content of the plants was affected significantly by using MAA ($P < 0.01$). Treatments of A1, A2 and A3 showed to decrease the total N content to 14%, 7% and 10% compared with the control, respectively.

The result of NO_3^- content agrees with the interpretation that amino acid can negatively regulate nitrate content in higher plants (Chen and Gao, 2002; Gunes et al., 1994, 1996; Wang et al., 2004). In the present experiment, surged value of NO_3^- content was also found at 24 h after MAA treating (the data were not shown). This was probably due to the different response of individual plant to the complex mechanism of MAA in NO_3^- assimilation process in short period. However, 3 days after MAA application, regular result of NO_3^- content in shoots of radish was found.

The predominance of amino acids and proteins were attributed to high activities of main enzymes of NO_3^- assimilation and the direct uptake of amino acids from MAA.

The result of total N content was opposite from that of field experiment in which total N content was increased by applying amino acid fertilizer. These contradictory results were due to different stage of amino acids treatment. Possibly, young plants may lack a complete functional system for NO_3^- uptake and assimilation (Pessarakli, 2002). Wang et al. (2004) reported that application of amino acids in autumn could increase total N in pakchoi but no significant effect was observed when treated in summer.

4.2.3 Effect of MAA on radish yield and N utilization

The plant production in terms of fresh weight was found to be significantly higher ($P < 0.05$) in treatment A1 and A2, with increases of 13% and 12% compared with the control, respectively (Table 10). The response of production in dry weight to MAA treatments was more sensitive than that of fresh weight (Table 10), with significant influences in MAA application ($P < 0.01$). The highest yield in dry weight was found in A2, with an increase of 44% in relation to A0. The results of N utilization (Table 10) were similar to dry yield described above, again registering the highest value in A2, with an increase of 34% compared with A0 ($P < 0.01$). Furthermore, significant effects were also observed in A1 and A3, with increase of 27% and 13% respectively, relative to A0 ($P < 0.01$).

Treatments	Fresh weight	Dry weight	N utilization
	(g/plant)		(mg/plant)
A0	13.32 ± 0.71 b	0.86 ± 0.10 b	37.60 ± 2.87 c
A1	14.99 ± 1.01 a	1.22 ± 0.13 a	47.60 ± 4.11 ab
A2	14.86 ± 0.57 a	1.23 ± 0.11 a	50.72 ± 2.53 a
A3	13.01 ± 0.71 b	1.06 ± 0.07 ab	42.43 ± 3.67 bc

Data are means ± SD (n=4). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05).

Table 10. Effect of mixed amino acids on radish yield and nitrogen utilization 30 day after sowing in pot experiment with high NO_3^- soil

For responses of growth, the application of MAA showed enhanced effects obviously. These results are in agreement with those observed by Chen et al. (1997), who reported that application of amino acids led to positive effects on Chinese cabbage growth. Among the treatments of MAA, the growth responses were increased by increasing the application rate of MAA. The increases of yield were due to the positive adjusting of MAA on growth of plants, thus contributing to the increases of N utilization (Table 10) even though the total N content was decreased in MAA treatments (Table 9).

4.2.4 Recovered fertilizer nitrogen

It has become evident that amino acids are a principal source of nitrogen for certain plants, such as mycorrihizal, heathland species (Read, 1993), non–mycorrihizal plants from arctic and alpine ecosystems (Chapin et al., 1993; Kielland, 1994) and boreal forest plants (Näsholm et al., 1998; Persson and Näsholm, 2001). These systems are similar in that N mineralization rates are heavily constrained by climate, and plant N demands cannot be met through the uptake of inorganic ions (Raab et al., 1999). Based on these researches, the amino acids were used to partially replace NO_3^- in hydroponic experiment or spray to leaves in many plants. In most case, the application of amino acids led to the decrease of nitrate content and total nitrogen content in lettuce, Chinese cabbage, onion, pakchoi or other leafy crops (Chen and Gao, 2002; Gunes et al., 1994, 1996; Wang et al., 2004). It had been suggested that plants probably preferred amino acids as sources of reduced nitrogen, and nitrate uptake was inhibited by amino acids.

In this study, the high NdfF was found in MAA treatments (Table 11), indicating that applied MAA did not act as a source of nitrogen for plants. On the contrary, plants had taken up more NO_3^-–N from soil due to the regulation of MAA on NO_3^- uptake and assimilation. The results for the possible regulation of NO_3^- uptake and assimilation by amino acids for higher plants are contradictory. Many authors agreed that amino acids can down regulate the NO_3^- uptake and assimilation in higher plants (Aslam et al., 2001; Ivashikian and Sokolov, 1997; Oaks et al., 1979; Radin, 1975, 1977; Sivasankar et al., 1997). But Aslam et al. (2001) reported that inhibition did not occur when the concentration of NO_3^- in the external solutions had been increased to 10 mM. This result is consistent with the other research, which indicated that radish treated with mixed amino acids containing 5.0 mM NO_3^- in growth medium show significantly increased the NO_3^- uptake. In this experiment, the positive effect on NO_3^- uptake by applying MAA was due to very high NO_3^- content in soil (1906 mg Kg^{-1}).

Treatments	NdfF (%)	QNdfF (mg/plant)	NdfFRec (%)
A0	65.9 ± 1.5 b	24.8 ± 0.7 d	33.0 ± 1.3 d
A1	68.6 ± 2.2 ab	32.7 ± 1.1 b	43.6 ± 2.0 b
A2	71.6 ± 0.9 a	36.3 ± 0.8 a	48.4 ± 1.9 a
A3	67.2 ± 2.1 ab	28.5 ± 1.0 c	38.1 ± 2.1 a

NdfF; the percentage of N derived from fertilizer, QNdfF ; the quantity of N derived from fertilizer, NdfFRec ; the fertilizer-N recovery

Data are means ± SD (n=4). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05).

Table 11. Nitrogen derived from fertilizer in the radish shoots

4.2.5 Effect of MAA on chemical properties of soil

The chemical properties of soil at the end of experiment are showed in Table 12. The planting of radish affected these chemical properties of soil clearly. However, there were no differences in pH of soil among treatments planted with radish. On the other hand, either planting treatment or MAA treatment showed effect on nitrate in soil. Compared with the non planting treatment, the treatments of planting showed a decrease of 65~81% and 35~47% of nitrate and available P at 30 days after sowing, respectively. The different rates of decrease were due to the different growth rates led by MAA treatment.

Treatments	pH (1:5)	EC* (mS m^{-1})	Available P$_2$O$_5$ (mg Kg^{-1})	NO$_3^-$–N (mg Kg^{-1})
NP	5.0	1985	599.7	1008.6
A0	5.7	981	390.6	339.4
A1	5.7	902	344.5	298.5
A2	5.7	674	316.5	192.0
A3	5.7	894	356.0	355.0

* The soil used in these experiments was commercialized artificial soil with lower soil density (about 0.4 g cm^{-3}) and higher water–holding capacity. Since determination of soil chemical properties is based on dry weight, the determined values of EC and NO$_3^-$–N are quite high relative to ordinary soil. However, these are not very higher in soil solution.

Table 12. Chemical properties of soil at the end of pot experiment for radish with high NO$_3^-$ soil

In conclusion, the results of the present experiment suggest that application of MAA can affect activities of three enzymes of N assimilation (NR, NiR and GS). However, the exact reason for this observation is unknown and further investigation is necessary. Furthermore, the application of MAA can enhance growth, N utilization, and concentrations of proteins and amino acids, and reduce the NO$_3^-$ content in plant shoots. Considerable increase of N uptake from soil was indicated by the increased ^{15}N recovery by applying MAA compared with the control. These results suggest that the main role of MAA on nitrate uptake and assimilation might be relation with the regulation of NO$_3^-$ uptake and assimilation, but not as sources of reduced nitrogen.

5. Pot experiment of radish with low NO$_3^-$ soil

5.1 Materials and methods

Commercialized artificial soil (pH, 5.2; EC, 1240 mS m^{-1}; NO$_3^-$–N, 280 mg Kg^{-1}; available P$_2$O$_5$, 1020 mg Kg^{-1}) was used for this experiment. Plant culture and MAA treatment were the same with that of pot experiment of radish with high NO$_3^-$ soil. The sampling and analysis of plant and soil also were according to procedures adopted for radish with high NO$_3^-$ soil.

5.2 Results and discussion

5.2.1 Effect of MAA on enzyme activities

Significant differences were found in the NR activity among the treatments (P < 0.01) (Fig. 13). The NR activity was inhibited by foliar application of MAA in this experiment, contrary to that of radish in which was planted in high nitrate soil. The lowest activity was attained with A2, showing a decrease of 28% compared with the activity attained in treatment A0. Treatment A1 and A3 were less effective in decreasing the activity of NR than A2, with decreases of 8% and 17%, respectively.
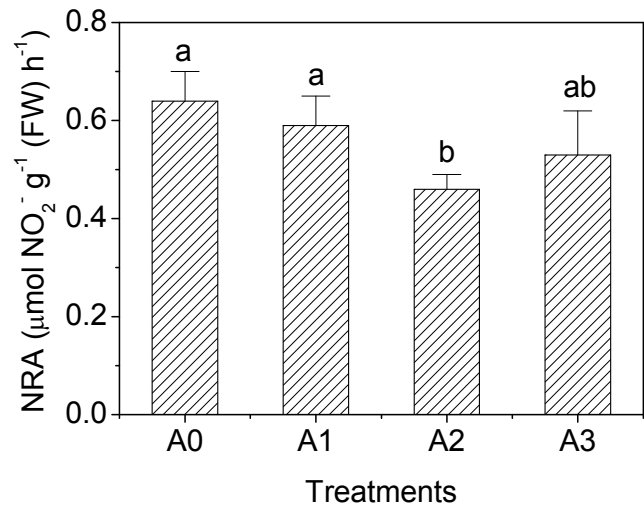
Fig. 13. Effect of mixed amino acids on nitrate reductase activity of radish leaves 28 day after sowing in pot experiment with low NO_3^- soil. Values are means ± SD (n=4).

The response of activity of NiR to the MAA application resembled that of NR (Fig. 14). The lowest activity of NiR was found in treatment A2, showing 40% decrease compared with the control treatment (P < 0.001). Treatment A1 and A3 also showed 23% and 32% decrease in relation to A0, respectively.
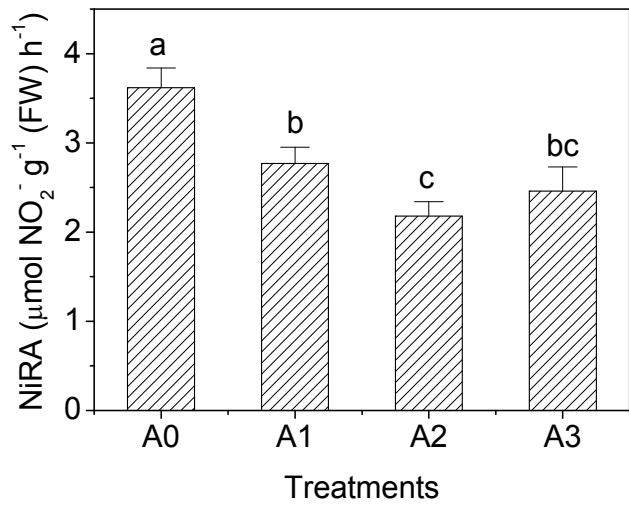

Fig. 14. Effect of mixed amino acids on nitrite reductase activity of radish leaves 28 day after sowing in pot experiment with low NO_3^- soil. Values are means ± SD (n=4).
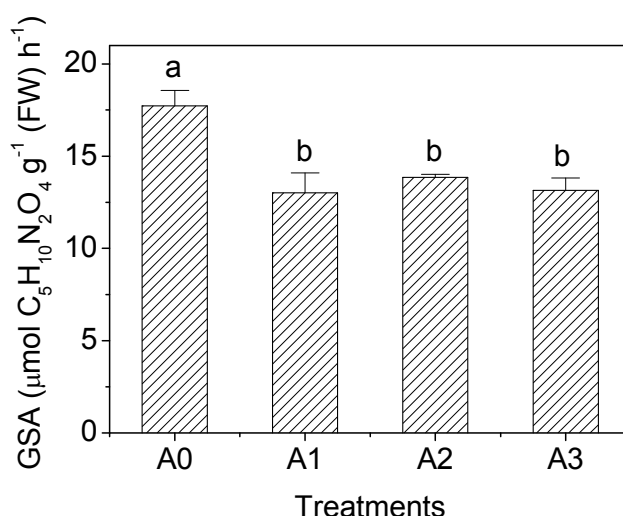
Fig. 15. Effect of mixed amino acids on glutamine synthetase activity of radish leaves 28 day after sowing in pot experiment with low NO_3^- soil. Values are means ± SD (n=4).

With respect to enzyme activity of GS (Fig. 15), the application of MAA led to significant decrease in the activity in leaves of radish in this experiment, the lowest activity being recorded in treatment A1, with a decrease of 27% in relation to the highest activity, found in the reference treatment A0 ($P > 0.01$). Treatment A2 and A3 also showed decreases of 22% and 26%, respectively.

Like some of the N transporters, NR is induced by its own substrate, NO_3^-, and this induction is fast, occurring within several minutes, and requires very low concentrations (< 10 μM) (Craw ford, 1995; Sueyoshi et al., 1995). NO_3^- is the primary factor, although other factors also influence the regulation of NO_3^- reduction and assimilation, including the end–products of assimilation such as amino acids. NiR and NR are similarly transcriptionally regulated for the reason of that NiR is strongly induced by the same factor, NO_3^-, probably to prevent the accumulation of toxic NO_2^- (Wang et al., 2000). The activities of NR and NiR were much lower than that of radish which was planted in high NO_3^- soil due to the poor NO_3^- in the soil used in the present experiment (Table 15).

In the present experiment, the activities of three enzymes decreased when treated with MAA. These results are in agreement with other researches which indicated that downstream N assimilation products such as amino acids can feed back to regulate NO_3^- uptake and reduction (Deng et al., 1991; Sivasanker et al., 1997; Vincentz et al., 1993). However, the effects of MAA on activities of enzymes are opposite to other experiments of ours, which indicated that treatment of MAA and amino acid fertilizer could enhance activity of NR in radish when supplied with high rate of NO_3^-. The contradictory results are due to the different NO_3^- levels of the soils.

5.2.2 Effect of MAA on N contents

The data in Table 13 showed that N contents of the plants were not affected significantly by using MAA. The highest concentrations of all N forms were observed in treatment A2 ($P > 0.05$). These results differed from radish which was planted in high NO_3^- soil. The different

effects of MAA on N contents of radish in high NO_3^- soil and low NO_3^- soil are in agreement with the supposition that amino acids have different effect on NO_3^- uptake and assimilation.

Treatments	Amino acids	Proteins	NO_3^-	Total N
	(mg g⁻¹ FW)		(mg g⁻¹ DW)	
A0	1.89 ± 0.07 a	7.35 ± 0.40 a	1.30 ± 0.06 a	20.5 ± 1.5 a
A1	1.87 ± 0.11 a	7.60 ± 0.37 a	1.32 ± 0.16 a	20.3 ± 2.4 a
A2	1.77 ± 0.12 a	7.92 ± 0.46 a	1.35 ± 0.08 a	21.0 ± 0.7 a
A3	1.97 ± 0.12 a	7.06 ± 0.50 a	1.34 ± 0.08 a	20.0 ± 0.8 a

Data are means ± SD (n=4). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05).

Table 13. Effect of mixed amino acids on nitrogen contents of radish leaves 30 day after sowing in pot experiment with low NO_3^- soil

5.2.3 Effect of MAA on radish yield and N utilization

The plant production in fresh weight was found to be higher ($P < 0.01$) in treatment of A2, with an increase of 9% compared with the control treatment (Table 14). The response of production in dry weight to MAA treatments was not as sensitive as that in fresh weight (Table 14), only with slight influences. The results of N utilization (Table 14) were similar to dry yield, registering the highest value in A1, with an increase of 15% compared with A0 ($P < 0.01$).

Treatments	Fresh weight	Dry weight	N utilization
	(g/plant)		(mg/plant)
A0	8.38 ± 0.40 b	0.84 ± 0.05 a	17.06 ± 0.85 b
A1	8.57 ± 0.34 b	0.88 ± 0.09 a	19.71 ± 0.58 a
A2	9.83 ± 0.32 a	0.89 ± 0.05 a	18.52 ± 0.51 ab
A3	8.44 ± 0.26 b	0.85 ± 0.03 a	18.88 ± 0.42 a

Data are means ± SD (n=4). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05).

Table 14. Effect of mixed amino acids on radish yield and nitrogen utilization 30 day after sowing in pot experiment with low NO_3^- soil

5.2.4 Effect of MAA on chemical properties of soil

The chemical properties of soil at the end of experiment were showed in Table 15. The planting of radish affected these chemical properties of soil clearly. However, there were no differences in pH of soil among treatments planted with radish. On the other hand, either planting treatment or MAA treatment showed effect on soil nitrate reduction. Compared with the non planting treatment, the treatments of planting showed decrease of 86~88% for nitrate and decrease of 56~70% for available P at 30 days after sowing, respectively. The different rates of decrease were due to the different growth rates resulted from by MAA treatment. And the EC decreased accordingly.

Treatments	pH (1:5)	EC (mS m^{-1})	Available P$_2$O$_5$	NO$_3^-$–N
			(mg Kg^{-1})	
NP	5.4	1485	878	214.7
A0	5.7	482	384	68.2
A1	5.7	515	303	62.2
A2	5.7	349	264	70.3
A3	5.7	503	277	68.6

Table 15. Chemical properties of soil at the end of pot experiment for radish with low NO$_3^-$ soil

The commercialized artificial soil used in this experiment was with lower soil density (about 0.4 g cm^{-3}) and higher water–holding capacity. Although the NO$_3^-$ contents of 62.2~70.3 mg Kg^{-1} are not low in ordinary soil, available NO$_3^-$ for plants is very poor in soil solution in this experiment. This might be the probable reason, that effects of MAA on N assimilation in the present experiment were different from that of radish which was planted in high NO$_3^-$ soil. Whether in our experiments or in other researches, different effects of amino acids on NO$_3^-$ reduction and assimilation were observed (Aslam et al., 2001).

In conclusion, the results of the present experiment suggest that application of MAA can decrease activities of three enzymes of N assimilation (NR, NiR and GS). However, except N utilization, the application of MAA did not have significant effects on growth, and concentrations of proteins, amino acids, total N and NO$_3^-$ content in plant shoots. The difference in the results were found in both the present experiment and pot experiment which radish was planted in high NO$_3^-$ soil may be due to different levels of NO$_3^-$ content in soil solution. The hypothesis that effect of amino acids on NO$_3^-$ uptake, reduction and assimilation depends on concentration of NO$_3^-$ was justified.

6. Field experiment of radish

6.1 Materials and methods

The study was conducted in summer of 2005 at the experimental farm of the Chungnam National University, Daejeon, Korea. The average chemical properties of the soil of the field are described in Table 16. The fertilizer mixture was uniformly broadcasted onto the soil surface and incorporated before ridging. The seeds of radish were sown at the end of May 2005 and arranged in a completely randomized block design, with three replications. The plots were 5 m × 2 m consisting of 2 rows.

At 15 and 22 days after sowing, AAF was applied 2 times to plots by spraying to leaves after diluting 500, 1000 and 2000 times by water, respectively. The main chemical contents of the AAF and application quantities are shown in Table 17.

Soils	pH (1:5)	EC (mS m^{-1})	Organic matter (g Kg^{-1})	Available P$_2$O$_5$ (mg Kg^{-1})	Total N (g Kg^{-1})	NO$_3^-$–N (mg Kg^{-1})
Before fertilization	6.0	122	15.6	170	0.81	80.2
After fertilization	6.0	191	15.8	279	0.87	191.2

Table 16. Chemical properties of soils used in field experiment of radish

Fresh leaves were collected at 23 days after sowing to determine the NO_3^-, amino acids and protein contents and enzyme activities. The plots were harvested at 35 days after sowing to determine crop yield and N assimilation. The topsoil samples (0–20 cm) were collected at 25 and 35 days after sowing for chemical analysis.

In order to compare the different AAF treatments for their N uptake, net N uptake was estimated by balancing N utilization and N input by applying AAF thus:

$$N_N = N_U - N_{AAF}. \tag{1}$$

where N_N is the net N uptake by plant; N_U is the total N utilization at harvest; N_{AAF} is N input by applying AAF.

It was assumed that N would have been either taken up by the plants or lost from the soil–plant system. In our experiment, leaching was the main way of N loss. Furthermore, N loss attributable to soil erosion and runoff was considered for our site with 2~5% slope. Since these losses may be influenced by protecting of the plants from the rain, the vegetation cover was observed at 25 and 35 days after sowing.

Classification (%)		Treatments				
		NP*	A0	A1	A2	A3
		(mg m⁻²)				
AAF application		—	—	750	1500	3000
Essential amino acid	2.22	—	—	16.7	33.3	66.6
Total amino acid	5.14	—	—	38.6	77.2	154.4
Total–N	3.80	—	—	28.5	57.0	114.0
Soluble P	3.12	—	—	23.4	46.8	93.6
Soluble K	4.97	—	—	37.3	74.6	149.2
Soluble B	0.13	—	—	0.98	1.95	3.90

* NP: No-planting

Table 17. Amino acid fertilizer applied to radish in the field experiment

6.2 Results and discussion

6.2.1 Effect of AAF on enzyme activities

Nitrate reductase is the first enzyme involved in the metabolic route of NO_3^- assimilation in higher plants. Significant differences were found in the NR activity between the treatments ($P < 0.01$) (Fig. 16). The highest activity was obtained with A1, showing an increase of 16% in relation to the activity obtained with A0. A2 was less effective in increasing the activity of NR than A1; whereas no increase of NRA occurred in A3, even treated with fourfold AAF than A1.

The next step in NO_3^- assimilation is the conversion of the NO_2^- to NH_4^+ by the action of NiR. The AAF treatments showed different effect on NiR activity depending on the applied rate of AAF (Fig. 17). The highest activity of NiR was found in treatment A1, showing an increase of 4% compared with A0 ($P < 0.05$). However, the activities of NiR were inhibited by 12 and 13% in A2 and A3, respectively.
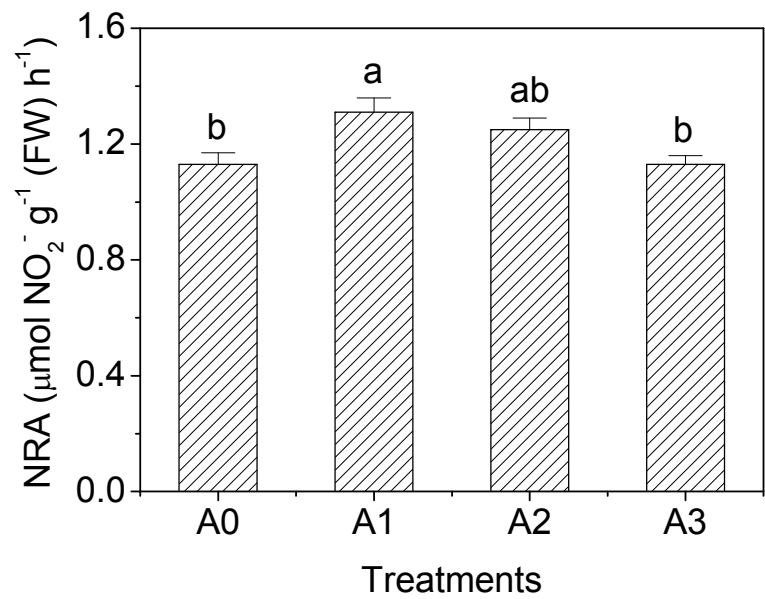
Fig. 16. Effect of amino acid fertilizer on nitrate reductase activity in leaves of radish 23 day after sowing. Values are means ± SD (n=3).
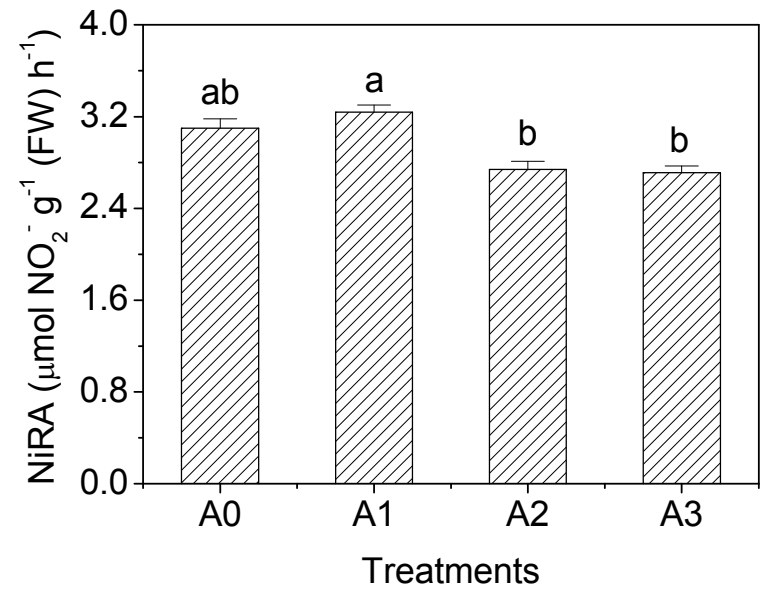

Fig. 17. Effect of amino acid fertilizer on nitrite reductase activity in leaves of radish 23 day after sowing. Values are means ± SD (n=3).

The reversible amination of 2–oxoglutarate to glutamic acid via GDH has long been considered as a major route of NH_4^+ assimilation (Srivastava and Singh, 1987). However the discovery of the enzyme GS–GOGAT system altered this point of view, and the incorporation of NH_4^+ to glutamine via GS and subsequently into glutamic acid by GOGAT is now widely accepted as the main route of NH_4^+ assimilation (Oaks, 1994). The response of GS (Fig. 18) to AAF treatments was similar to that of the NiR (Fig. 17). The greatest activity was reached in treatment A1, with an increase of 20% over the reference treatment ($P <$ 0.001). On the contrary, the activity of GS was the lowest in A3, with a decline of 11% compared with A0 ($P <$ 0.05).
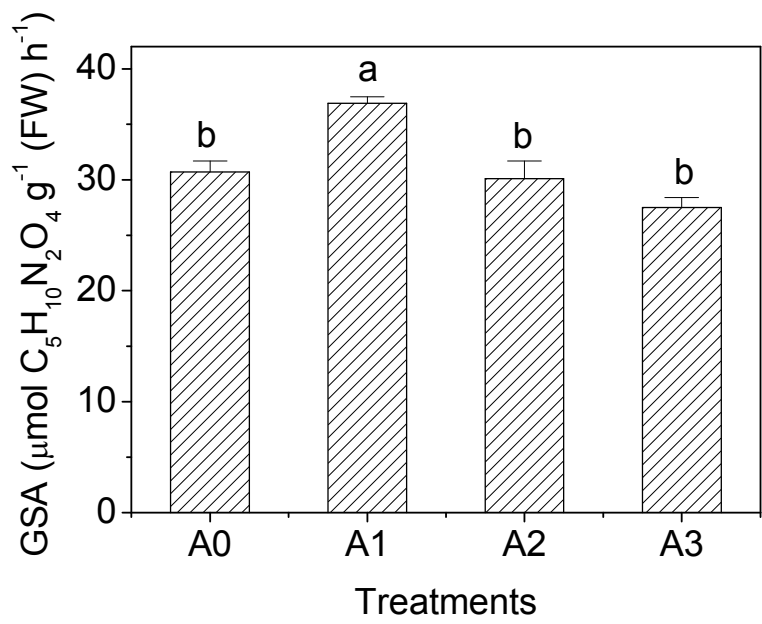
Fig. 18. Effect of amino acid fertilizer on glutamine synthetase activity in leaves of radish 23 day after sowing. Values are means ± SD (n=3).

The reduction of NO_3^- to NO_2^- by NR, is the main and most limiting step, in addition to being the most prone to regulation (Sivasankar et al., 1997; Ruiz et al., 1999). The synthesis of this enzyme is induced by nitrate (Oaks, 1994), but although its activity is known to be repressed by ambient ammonium, there are evidences that this enzyme can be regulated by certain amino acids. The results for the possible regulation of NR activity by amino acids for higher plants are contradictory. Many authors agree with that amino acids can inhibit the activity of NR in higher plants (Radin, 1975, 1977; Oaks et al., 1979; Ivashikian and Sokolov, 1997; Sivasankar et al., 1997; Aslam et al., 2001). But Aslam et al. (2001) reported that inhibition did not occur when the concentration of NO_3^- in the external solutions had been increased to 10 mM. This result is consistent with the other research, which indicates that radish treated with mixed amino acids containing 5.0 mM NO_3^- in growth medium showed significant increase of NR activity (Liu et al., 2005). The effect of amino acids on NR activity seems to be depended on plant materials, age of plants, growth conditions, nitrate concentration, kinds of amino acids, amino acids concentration and other factors. In this experiment, the positive effect on NR activity by applying AAF was due to high NO_3^- content in soil.

In the present experiment, the treatments of AAF led to different levels of increase of NR activity and inhibition on GS activity depending on applied rates. The high activities of three enzymes were found in A1 due to the positive effect of AAF on process of NO_3^- assimilation. However, inhibition on NiR and GS was observed in A2 and A3 for the reason that high rates of AAF application had high feed–back inhibition on NO_3^- reduction systems which affected GS first. This is probably the main reason why different effects on the enzymes were observed in this study.

6.2.2 Effect of AAF on biomass and utilization of N and P

The plant biomass production in fresh weight was found to be significantly higher ($P < 0.01$) in the AAF treatments (mean biomass in fresh weight of A1, A2 and A3 are 5.056, 4.738 and 4.653 Kg m^{-2}, respectively) compared with the control (mean biomass in fresh weight is 4.026 Kg m^{-2}) (Table 18). Among AAF treatments, the treatment with low concentration of AAF (A1) had a higher ($P > 0.05$) biomass production than the treatment with high concentration of AAF (A3). The response of biomass production in dry weight to AAF treatments resembled that in fresh weight (Table 28), with significant influence by applying AAF ($P < 0.01$). The highest biomass production in dry weight was found in A1, with an increase of 17% in relation to A0.

Treatments	Fresh weigh	Dry weight	N utilization	P utilization
A0	4026 ± 227 c	345.7 ± 14.2 c	9.33 ± 0.87 c	2.35 ± 0.09 b
A1	5056 ± 213 a	404.4 ± 11.6 a	14.48 ± 0.89 a	2.87 ± 0.11 a
A2	4738 ± 183 ab	394.8 ± 12.1 ab	13.04 ± 0.53 ab	2.68 ± 0.12 a
A3	4653 ± 189 b	382.0 ± 14.5 b	12.83 ± 0.67 b	2.64 ± 0.07 a

Values are means ± SD (n=3). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05)

Table 18. Effect of amino acid fertilizer on radish yield and utilization of nitrogen and phosphorus 35 day after sowing (g m^{-2})

The result of N utilization (Table 18) was similar to biomass production as described above, again registering the highest value in A1 (14.48 ± 0.89 g m^{-2}), with an increase of 55% compared with A0 (9.33 ± 0.87 g m^{-2}) ($P < 0.01$). Furthermore, significant effects were observed in A2 and A3 too, with increases of 40% and 37% respectively, in relation to A0 ($P < 0.01$). Even though P content was not influenced by the application of AAF (Table 20), P utilization increased in AAF treatments due to the increase of biomass production (Table 18).

The observed result of vegetation cover and calculated values of net N uptake are showed in Table 19. The treatments of AAF showed higher vegetation cover than the control. Besides the N input by applying of AAF, the treatments of AAF showed significant increase of 36~55%net N uptake compared with the control. Gunes et al. (1996) suggested that plants probably preferred amino acids as sources of reduced nitrogen, but they did not distinguish origin of the N contents in the plants. In our experiment, the increase of N uptake is about 200 times (Table 19) more than N supplied by applying AAF, indicating application of AAF could enhance the ability of uptake and assimilation of inorganic N by plants.

Treatments	Vegetation cover (%)		Net N uptake (g m^{-2})
	25 DAS	35 DAS	35 DAS
A0	63 ± 3 c	91 ± 2 b	9.33 ± 0.87 b
A1	85 ± 5 a	100 ± 0 a	14.45 ± 0.89 a
A2	79 ± 6 ab	100 ± 0 a	12.98 ± 0.53 a
A3	76 ± 3 b	100 ± 0 a	12.72 ± 0.67 a

Values are means ± SD (n=3). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05)

Table 19. Net nitrogen uptake and vegetation cover of radish

These results are in agreement with those observed by Chen et al. (1997), who reported that application of amino acids led to positive effects on cabbage growth. However, among the treatments of AAF, the growth responses were decreased by increasing the application rate of AAF. This may probably be related to the feed–back inhibition of high rate application of amino acids.

6.2.3 Effect of AAF on contents of N and P

The data in Table 20 showed that N contents of the plants were affected by using amino acid fertilizer. The NO_3^- content of radish was decreased by application of AAF ($P < 0.05$) compared with the reference treatment. Among the treatments, A1 gave the best result in reducing the nitrate to 1.16 mg g^{-1} (FW), with a decrease of 24% in relation to the highest NO_3^- content found in A0. This result agrees with the interpretation that amino acid can negatively regulate nitrate content in higher plants (Gunes et al., 1994, 1996; Chen and Gao, 2002; Wang et al., 2004). But this interpretation was not supported in all cases. It was observed that the mixed amino acids increased NO_3^- content slightly in radish when the plants growing in nutrient solution. The contradiction may reside in amino acids treatment method. It was demonstrated in other studies that amino acid pretreatment decreased NO_3^- accumulation slightly, but Gln and Asn led to NO_3^- concentration increase in barley roots when they were used together with nitrate (Aslam et al., 2001).

With respect to the main products of NO_3^- assimilation, amino acids and proteins (Table 20), the plants in treatment A1 gave the highest contents of these compounds ($P < 0.01$). In the A1 treatment, high activities of main enzymes of NO_3^- assimilation could explain the predominance of these nitrogenous compounds in radish. Under treatments of A2 and A3, the increases of amino acids and proteins derived from the direct uptake of amino acids from AAF.

Treatments	NO_3^-	Amino acids	Proteins	Total-N	Total-P
	(mg g^{-1} FW)			(mg g^{-1} DW)	
A0	1.53 ± 0.11 a	1.29 ± 0.02 b	1.28 ± 0.08 c	27.2 ± 1.6 c	6.8 ± 0.3 a
A1	1.16 ± 0.17 b	1.38 ± 0.02 a	1.98 ± 0.16 a	35.9 ± 1.6 a	7.1 ± 0.4 a
A2	1.32 ± 0.20 ab	1.33 ± 0.02 ab	1.74 ± 0.06 b	33.0 ± 0.9 ab	6.8 ± 0.4 a
A3	1.48 ± 0.08 a	1.32 ± 0.04 ab	1.70 ± 0.09 b	31.2 ± 1.2 bc	6.9 ± 0.5 a

Values are means ± SD (n=3). Analysis of variance (ANOVA) was employed followed by Duncan's new multi range test. Values with similar superscripts are not significantly different (P>0.05)

Table 20. Effect of amino acid fertilizer on contents of nitrogen and phosphorus in radish 23 day after sowing

The total N content of the plants was also affected significantly by the use of AAF ($P < 0.01$). Treatments of A1, A2 and A3 showed to increase the total N to 32%, 21% and 15% relative to the control, respectively. These increases were due to the positive adjusting of AAF on uptake and assimilation of N, and attributing to the increases of N utilization and net N uptake. The P content of radish was not affected significantly by the application of AAF (Table 20).

6.2.4 Effect of AAF on chemical properties of soil

The chemical properties of soil in middle growth period and at the end of experiment were showed in Table 21 and Table 22. The planting of radish affected total N of soil clearly, except at 35 days after sowing, with a fall of 10% compared with non planting treatment. However, there were no differences in total N of soil among treatments planted with radish. On the other hand, either planting treatment or AAF treatment showed effect on nitrate in soil.

Treatments	pH (1:5)	EC (mS m^{-1})	Organic matter (g Kg^{-1})	Available P$_2$O$_5$ (mg Kg^{-1})	Total N (g Kg^{-1})	NO$_3^-$–N (mg Kg^{-1})
NP	6.3	81	15.4	267	0.70	75.3
A0	6.3	57	15.2	297	0.67	52.5
A1	6.4	55	15.6	285	0.67	55.7
A2	6.5	65	15.1	310	0.67	58.9
A3	6.4	54	15.3	305	0.66	60.0

Table 21. Chemical properties of soil in the middle of growth period (25 day after sowing) for radish

In the soil of non planting, nitrate was decreased by leaching and runoff by rain. Compared with the non planting treatment, the treatments of planting showed 20~30% decrease at 25 days after sowing and 23~42% decrease at 35 days after sowing in the nitrate content of soil. Although with the lowest net N uptake, the lowest concentration of nitrate in soil was found in A0 treatment both at two sampling times. This was due to the fact that the vegetation covers of AAF treatments were higher than treatment of A0, and could effectively prevent nitrate of soil from leaching or runoff. The planting treatments showed lower values of EC than non planting treatment, but all were in the range of general soil. There were no significant differences among all treatments in pH and organic matter of soil. Moreover, very small differences were observed in available P due to different growth rate of the plants.

Treatments	pH (1:5)	EC (mS m^{-1})	Organic matter (g Kg^{-1})	Available P$_2$O$_5$ (mg Kg^{-1})	Total N (g Kg^{-1})	NO$_3^-$–N (mg Kg^{-1})
Before experiment	6.0	191	15.8	279	0.87	191.2
NP	6.4	99	15.9	308	0.70	94.3
A0	6.4	41	16.0	283	0.63	55.0
A1	6.4	42	15.8	263	0.63	65.9
A2	6.4	49	15.2	270	0.63	65.0
A3	6.4	45	15.3	272	0.63	73.2

Table 22. Chemical properties of soil at the end of field experiment (35 day after sowing) for radish

Treatments	NO_3^- removal (0~20cm) (g m^{-2})	Removal rate by plant	Removal rate by leaching
		(%)	
NP	25.2	–	100.0
A0	35.4	26.3	73.7
A1	32.6	44.4	55.6
A2	32.8	39.7	60.3
A3	30.7	41.8	58.2

Table 23. Effect of amino acid fertilizer on nitrate removal from the soil

The data of NO_3^- removal are showed in Table 23. Even though the highest NO_3^- removal was found in treatment A0, the most removed NO_3^- was leached (73.7%) and would lead to pollution for groundwater. The application of AAF can enhance NO_3^- removal rate by planting, and avoid N losses through leaching and runoff due to increases of N utilization (Table 18) and vegetation cover (Table 19).

In conclusion, the results of the present experiment suggest that application of amino acid fertilizer can affect activities of three enzymes of N assimilation (NR, NiR and GS) and increase the growth and N assimilation in radish. However, the exact reason for this observation is not known and requires further investigation. The planting of radish proves very effective for nitrate removal in soil by its fast growth and very high biomass production (345.7~404.4 g DW m^{-2}) and N utilization (9.33~14.48 g m^{-2}) in short time (only 35 days in our experiment). Furthermore, the application of amino acid fertilizer can enhance biomass production, N utilization, and concentrations of proteins and amino acids, and it can reduce N losses through leaching and runoff.

7. Conclusions

By conducting these experiments, several findings were obtained: (1) increase of NO_3^- uptake by application of MAA, (2) different effect of MAA dictated by N status, (3) efficient NO_3^- removal by application of AAF, and (4) true role of MAA in process of NO_3^- uptake and assimilation.

Both for radish and red pepper, the application of MAA led to significant increase of NO_3^- uptake and activities of the three enzymes (NR, NiR and GS) of the NO_3^- assimilatory pathway in solution experiment. These results are different from other researches which inhibition was observed in most case. This difference was caused by two main reasons: (1) that effect of MAA was different to single amino acid, and (2) comparative high level NO_3^- was supplied in these experiments.

In pot experiments, responses to applied MAA were affected by plant species and NO_3^- level in soil. For radish, application of MAA led to increases of activities of three enzymes, growth, N utilization, and concentrations of proteins, and decrease of NO_3^- content in plant shoots, when the plants were planted in high NO_3^- soil. However, in the case that radish was planted in low NO_3^- soil, activities of the enzymes were decreased by using MAA, and growth, and concentrations of proteins, amino acids, total N and NO_3^- content were not affected. These phenomena indicate that the effect of MAA is dependent on NO_3^- level.

With respect to red pepper which was planted in high NO_3^- soil, foliar MAA sprays increased activities of the three enzymes, while reduced NO_3^- content, concentrations of proteins and amino acids, total N and N utilization. Partially different results were found in red pepper which was planted in low NO_3^- soil, including decreased activities of NiR and GS and increased of NO_3^- content in plant shoots by the application of MAA. The reason for these differences is the same to that of radish.

In field experiment of radish, the foliar sprays of AAF increased NO_3^- removal rate by planting, and avoid N losses through leaching and runoff due to increases of N utilization and vegetation cover. In addition, the application of AAF enhanced activities of three enzymes, biomass production, and concentrations of proteins and amino acids, reduced NO_3^- content in plant shoots. Similarly, for red pepper, the use of AAF led to increase of N utilization. However, decrease of total N content in red pepper plants was found in AAF treatments.

These results of ^{15}N labeled experiments and field experiments suggest that the main role of amino acids on nitrate uptake and assimilation might be relation with the regulation of NO_3^- uptake and assimilation, but not as sources of reduced nitrogen. In pot experiments, it was indicated that the N utilization of plants was depended on soil NO_3^- uptake which was regulated by application of MAA. In field experiment of radish, the increase of N utilization is about 200 times more than N supplied by applying AAF, indicating application of AAF could enhance the ability of uptake and assimilation of inorganic N by plants.

Finally, the effect of amino acids on NO_3^- uptake and assimilation was also influenced by stage of plant growth. For leaf radish, response of enzymes activity and yield was not affected by the stage of growth, while N accumulation (total N content) was more sensitive to applied amino acids in vegetative stage than that of young stage. With regard to red pepper, effects of amino acids on enzymes activity and N content in different growth stage were quite similar, while growth (dry biomass) showed to be increased significantly in vegetative stage.

A better understanding of effect of amino acid on process of NO_3^- uptake and assimilation will undoubtedly help in developing an approach to improve the management of fertilizer nitrogen and to prevent N loss through leaching or runoff. In the further study, more detailed researches should be carried out to investigate the precise manner by which MAA influences NO_3^- uptake and assimilation. The researches will focus on the effect of MAA on NR gene expression and relation between GDH and GS.

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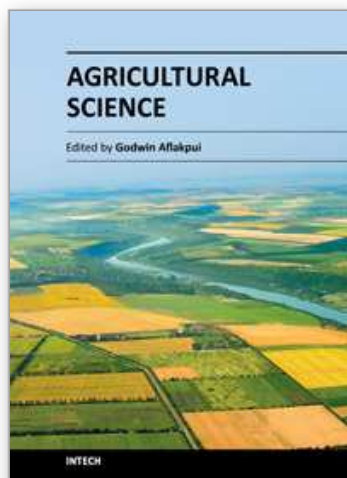
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