韓草誌(J. Korean Grassl. Sci.) 20(4) : 285~294, 2000

Effects of Inoculation of Mycorrhiza and Rhizobium on N, P utilization and Vegetative Growth in Alfalfa/Perennial Ryegrass Intercropping

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Mycorrhiza 및 Rhizobium 접종이 알팔파 - 페레니얼 라이그라스 혼파에 의한 질소와 인의 이용성 및 성장에 미치는 영향

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Abstract

To investigate the effect of Mycorrhiza (*Glomus intradics*) and Rhizobium inoculation on the N, P utilization and growth response of Alfalfa (*Medicago sativa* L.) and Perennial ryegrass (*Lolium perenne* L.) in mixed sward, four treatments (non-inoculation, Control; Mycorrhiza inoculation, M; Rhizobium inoculation, R and Mycorrhiza and Rhizobium inoculation, M+R) were applied. The associated analyses were carried out on the early vegetative growth stage (DAS 56, 56 days after seeding) and on the early flowering stage (DAS 126). The decreased rate of total N and P content in soil, with advancing plant growth, was relatively higher in the M and M + R treatment than control. The content of available phosphorus in soil at DAS 126 increased by about 34 and 38 % in M and M+R treatment compared to control (189.2 mg P_2O_5/kg DM), while non-significant changes was observed in R treatment. Total N uptake and P uptake in the control at DAS 126 were 44.71 and 3.52 mg/plant in mixed sward, respectively. About 71, 98 and 197 % of increases in total N uptake and 70, 72 and 111 % of increases in total P uptake were estimated in M, R and M+R treatment. Comparing to control, total dry matter yield significantly increased by 27, 33 and 53 %, and crude protein yield also by 78, 83 and 204 %, respectively, in M, R and M+R treatment. The present data indicated that mycorrhiza or/and rhizobium inoculation improved N, P utilization of both alfalfa and perennial ryegrass plants, and consequently increased total yield (especially by dual inoculation, M+R).

(**Key words** : Alfalfa, Perennial ryegrass, Mycorrhiza, Rhizobium, N and P Utilization, Growth, Yield)

"이 논문은 한국과학재단에서 지원한 '99국산연구기기활용 연구과제에 의하여 수행되었음"

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Ⅰ. INTRODUCTION

Mountainous soils in Korea are acidic and infertile. These properties are closely associated with the deficiency of available P and N, resulting from phosphate fixation with metal cations and low level of organic matter. Therefore, the amendment of these soils are needed to develop for grassland establishment.

Studies of microbial inoculation into host plant have been made to enhance nutrient utilization (Nadian, 1997; Toro et al., 1998). Vesicular-arbuscular mycorrhizal (VAM) fungal infection improve nutrient utilization and growth of plants, particularly when plants are under environmental stress or in the soils of low available P (Harley and Smith, 1983). A number of *in vitro* experiments has shown that many soil microorganisms are able to solubilize phosphate ions from sparingly soluble inorganic or organic P compounds (Kucey et al., 1989). However, the effectiveness of this process in soil is unclear because of difficulties related to the translocation of phosphate ions to the root surface, if there is any solubilization. The microbiologically solubilized phosphate would be taken up by a mycorrhizal system, thereby developing synergistic microbial interations (Barea et al., 1997). The phosphate pool must be restored in any agricultural system, because plant-available P is scare in soil. Thus the use of rock phosphate has been proposed for sustainability purpose. The problem with this inexpensive, sparingly soluble form of P is its low effectiveness (Khasawneh and Doll, 1978). To improve its agronomic performance integrated approaches involving mycorrhiza-bacteria interaction were proposed (Barea et al., 1983).

Rhizobium inoculation leads symbiotic dinitrogen fixation. Recent physiological works investigated the phosphorus effects on nodule formation and N_2 fixation activity. Francis et al. (1986) suggested that phosphorus increased N_2 fixation activity. Sa and Israrel (1991) reported that decreased specific nitrogenase activity in nodules of P-deficient soybean plants was associated with decreased energy status (i.e. Adenosine 5-Phosphate concentration and energy charge). Israel (1993) concluded that phosphorus increased symbiotic dinitrogen fixation primarily by stimulating host plant growth rather than by exerting specific effects on either rhizobium growth and survival or on nodule development and function. These latter observations imply specific involvement of phosphorus in symbiotic dinitrogen fixation.

Mycorrhizal colonization influences the transfer of nutrients between root systems of the associated plants (Chiariello et al., 1982; Francis et al., 1986). The occurrence of VAM-mediated interplant transfer of C (Francis and Read, 1984), N (Van Kessel et al., 1985) and P (Ritz and Newman, 1984) has been determined by isotopic technique. Bethlenfalvay et al. (1991) reported that when maize was grown with nodulated soybean, maize N content increased by 22 %, but P content declined by 16 %.

In previous study on alfalfa single culture, we observed that mycorrhiza or/and rhizobium inoculation improved N, P utilization, and consequently increased the yield on the infertile-mountainous soils. The purpose of this study was to investigate the effect of mycorrhiza and/or rhizobium inoculation on the N, P utilization and on the respective growth response of alfalfa and perennial ryegrass under a mixed culture.

Ⅱ. MATRIALS AND METHODS

1. Plant materials

Sterilized seeds of Alfalfa (*Medicago sativa* L.) and Perennial ryegrass (*Lolium perenne* L.) were germinated on wet filter paper. 1 % (w/w) of rock phosphate and 3 % (w/w) of organic matter were added into the soils and then sterilized. Chemical characteristics of the soils utilized for this experiment are shown in Table 1. Germinated seedlings were transplanted to 3L pot [2 plants (Alfalfa) and 3 plants (Perennial ryegrass) per pot]. To inoculate mycorrhiza, about 1.7 % (w/w) of the soils abundantly infected with vesicular-arbuscular mycorrhiza (*Glomus intradics*) were mixed around the seedling transplanted. Rhizobium separated from the nodules of wild alfalfa cultivar was incubated on

Table 1. Chemical characteristics of soil utilized for the experiment

pH (1:5 H_2O)	TN (%)	OM (%)	P_2O_5 (ppm)	Exchangeable cation (Cmol/kg)				CEC (Cmol/kg)
				K	Ca	Mg	Na	
5.4	0.11	1.93	21.8	0.6	3.5	1.9	0.2	9.6

the wright medium (Kim et al., 1992) at 30℃ for 3 days. One mL of incubated rhizobium (8.67×10^9 /ml) was injected into the soils around individual seedling.

Fourty pots were allocated to four treatments [non-inoculation (C), mycorrhiza inoculation (M), rhizobium inoculation (R), mycorrhiza and rhizobium dual inoculation (M+R)] and two harvests [early vegetative growth (DAS 56), early flowering growth (DAS 126)] with 5 replications. A completed randomized block design was applied. The data obtained from 4 microbial inoculations and 2 harvest times were analyzed by using paired t-tests. Means were expressed ± standard error.

2. Chemical analysis

Total N was determined by ammonia micro-diffusion on Conway dish as described by Kim and Kim (1996). The collected NH_4Cl reacted with Nesslers ammonium color reagent. The optic density (OD) was read at 410 nm.

For total P determination, appropriate samples were placed on a electronic furnace at 550℃ for 3hrs, and digested on the mixture of 5 mL of $HClO_4$ and 5 mL of H_2O_2 on a hot plate. The aliquot was filtered after solubilizing with 5 mL of 1 N HCl, and adjusted to final volume of 50 mL. The prepared sample solution reacted with P-color reagent and incubated at 30℃ for 30 min. The OD was read at 470 nm.

3. Acetylene reduction activity

Acetylene (C_2H_2)-dependent ethylene (C_2H_4) production by nodulated roots was determined to measure the activity of N_2 fixation (Hardy at al., 1968). After removal of shoots, nodulated roots were gently collected free of soil. Roots were placed in 100 mL of sealed flasks. 10 mL of air were withdrawn and replaced with an equal volume of C_2H_2, and incubated at 30℃ for 60 min. The ethylene produced was measured using gas chromatography (Varian STAR 3400 CX, USA) equipped with a PLOT fused silica capillary column (CHROMPACK). The injector temperature was at 100℃ and the carrier gas was N_2 at a flow rate of 200 ml/min at 30 psi.

4. Determination of mycorrizal colonization

The mycorrhizal colonization of roots was assayed by modified method of Brundrett et al (1984). The roots of alfalfa and perennial ryegrass were gently collected free of soil and rinsed with distilled water. Roots were immerged in FAA solution (the mixture of formalin/acetic acid/ethanol, 13/5/200, v/v/v) for 24 hrs (Sass, 1958). After washing and rinsing with distilled water several times, the mycorrhizal roots were placed into 20 ml vials and filled with 10 % KOH, and then incubated at 90℃ for 60 min. Mycorrhizal roots were washed with water and dyed with stain solution (400ml 85% lactic acid, 1.2g Chlorazole black E, 400ml glycerine, 400ml distilled water) at 50℃ overnight. The root samples were rinsed with water until clear and put into destain solution (50% glycerol) overnight. Mycorrhizal colonization was visualized using on a microscope (Olympus model BX 50) with 200 × magnification. Hypae and vesicular in plants were counted. Total mycorrhizal colonization rate (%) was expressed as the percentage of the number of colonized root against total number of examined roots as described by Read et al. (1976).

Ⅲ. RESULTS AND DISCUSSION

1. Mycorrhizal colonization and soil fertility

All micrographs of vesicular and hyphae colonized with the roots of non-inoculated (control) and inoculated alfalfa and perennial ryegrass plants in mixed sward are presented in Fig. 1. Mycorrhizal colonization rate, vesicular and hyphae percentage at DAS 126 are shown in Table 2. The percentage of hyphae colonization was much higher than that of vesicular [(16.4 and 25.3 % of hyphae; 3.0 and 5.2 % of vesicular in alfalfa plants) (43.4 and 57.0 % of hyphae; 4.7 and 4.7 % of vesicular in M and M+R inoculated perennial ryegrass plants)]. From these results it is suggested that in alfalfa and perennial ryegrass roots mycorrhiza were colonized mainly as the forms of hyphae. Mycorrhizal colonization rate of M and M+R treatment was about 19.3 and 30.5 % in alfalfa, about 48.1 and 61.7 % in perennial ryegrass, respectively. These results showed that vesicular and hyphae were colonized more use in rhizosphere of perennial ryegrass than alfalfa plants in mixed sward by mycorrhizal inoculation at the infertile soils. Also, the colonization rate of mycorrhiza was higher in the

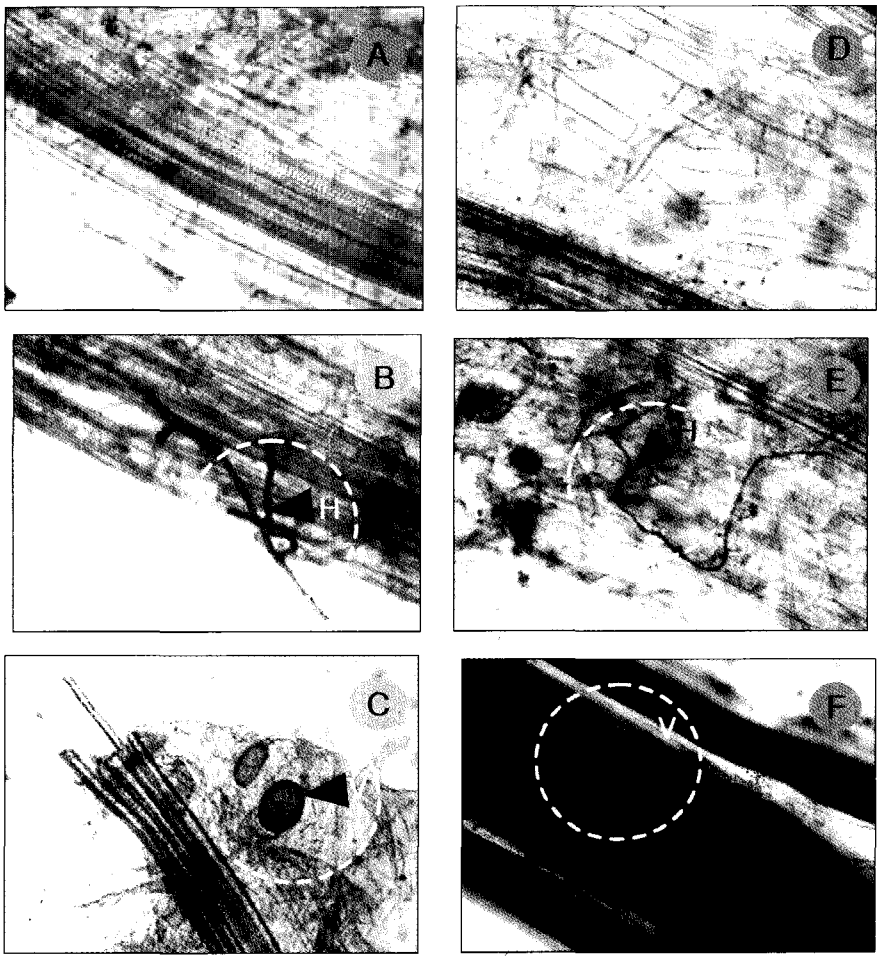

Fig. 1. All micrographs are oriented with the root of alfalfa (*Medicago sativa* L.) [A, Control; B, Hyphae (H); C, Vesicular (V)] and perennial ryegrass (*Lolium perenne* L.) [D, Control; E, Hyphae (H); F, Vesicular (V)]. Magnification ca. 200 x.

perennial ryegrass plants than in alfalfa plants. Mycorrhizal colonization was closely associated with root mass of host plant in agreement with the result of Wallander and Nylund (1992). High mycorrhizal colonization was widely reported in grass and legume mixed sward such as *Glycine max* L. and *Zea mays* L. (Bethlenfalvay et al., 1991), *Brachiaria arrecta* and *Sorghum vulgare* (Ibijbijen et al., 1996).

Alfalfa nodulation and relative activity of N_2 fixation as affected by rhizobium inoculation are shown in Table 3. Fresh weight of nodules in control and M treatment (non-rhizobium inoculation) was 107 and 127 mg/pot. The nodulation in R and R+M treatment (rhizobium inoculation) was about 3.3-fold higher than that in non-rhizobium inculated plants. Relative activity of N_2 fixation well reflected the nodule mass, showing 3.2-fold and 4.1-fold higher activity in the R and M+R plants compared to the control plants (non-inoculated). When compared with rhizobium single inoculated plants (R), dual inoculation with mycorrhiza and rhizobium (M+R) enhanced significantly the fresh weight of nodules and the relative activity of N_2 fixation. This result was well agreed with the finding of Toro et al. (1998). Azcón and El-Atrash (1987) reported that mycorrhiza strongly affected the biological N_2 fixation by legume *Rhizobium* symbiosis.

Total contents of nitrogen and phosphorus in soil at 56 [early vegetative stage (DAS 56)] and 126 days after seeding [early flowering stage (DAS 126)] are shown in Table 4. At the start of experiment, total N in soil was 3.30±0.21 g/pot. At DAS 56 total N contents of soil were significantly decreased in all treatment compared to initial content. The decreased rate until this growth stage was relatively higher in the R (39 %) and M+R (42 %) treatments than control (35 %) and M (33 %) treatment. Total N contents in soil at DAS 126 were 2.31, 2.05, 2.61

Table 2. Mycorrhizal colonization on the alfalfa and perennial ryegrass roots at the early flowering stage

Microbial inoculation	Alfalfa			Perennial ryegrass		
	Vesicular	Hyphae	Colonization rate	Vesicular	Hyphae	Colonization rate
	..... (%)			..... (%)		
Non-inoculation (Control)	0.0	2.6	2.6	0.0	2.0	2.0
Mycorrhiza (M)	3.0	16.4	19.3	4.7	43.4	48.1
Rhizobium (R)	0.0	0.0	0.0	0.0	1.5	2.8
Mycorrhiza + Rhizobium (M+R)	5.2	25.3	30.5	4.7	57.0	61.7

Table 3. Fresh weight of nodules and relative activity of acetylene reduction as affected by microbial inoculation at the early flowering stage. Relative acetylene reduction activity was expressed as percentage against the control plants. The absolute activity of acetylene reduction in the control plants was 15.2 μmol.C_2H_2/g FW/hour. Each value is mean±s.e. for n=5

Microbial inoculation	Nodule F.W (mg/pot)	Relative activity
Non-inoculation (Control)	107± 8	100
Mycorrhiza (M)	127±11	119
Rhizobium (R)	346±21	323
Mycorrhiza + Rhizobium (M+R)	428±36	400

Table 4. Total content of nitrogen and phosphorus in soil at the early vegetative stage (56 days after seeding ; DAS 56) and the early flowering stage (126 days after seeding ; DAS 126). Each value is mean±s.e. for n=3

Microbiol inoculation	Day after seeding			
	DAS 56	DAS 126	DAS 56	DAS 126
	····· Nitrogen (g/pot) ·····		····· Phosphorus (g/pot) ·····	
Non-inoculation (Control)	2.16±0.13	2.31±0.11	1.78±0.09	1.44±0.10
Mycorrhiza (M)	2.01±0.13	2.05±0.20	1.59±0.07	1.07±0.08
Rhizobium (R)	2.22±0.17	2.61±0.08	1.79±0.11	1.38±0.11
Mycorrhiza + Rhizobium (M+R)	1.90±0.05	1.96±0.10	1.66±0.06	1.17±0.04

cf : total contents of N and P in soil at the start of experimental were 3.3 and 2.07 g/pot, respectively.

Table 5. Concentration of available phosphorus in soil (mg P_2O_5/kg DM) as affected by microbial treatment at the early vegetative stage (56 days after seeding ; DAS 56) and the early flowering stage (126 days after seeding ; DAS 126). Each value is mean±s.e. for n=3

Microbial inoculation	DAS 56	DAS 126
Non-inoculation (Control)	165.4±13.2	189.2±19.2
Mycorrhiza (M)	239.3±11.4	254.2±11.3
Rhizobium (R)	178.8±16.4	178.8±13.6
Mycorrhiza + Rhizobium (M+R)	257.8±21.0	261.3±20.4

and 1.96 g/pot in the control, M, R and M+R treatment, respectively. The decreased rate until this growth stage was the highest in the M+R (41 %) treatment. These indicated that nitrogen utilization was improved by dual inculation with mycorrhiza + rhizobium rather than mycorrhiza or rhizobium single inoculation.

At the start of experiment, total P contents in soil was 2.07±0.12 g/pot. At DAS 56, total P content in soil was significantly decreased in all treatments. The decreased rate of total P during this period was relatively higher in the M and M+R treatment. The decreased rate until the early flowering stage (DAS 126) was also significantly higher in the M, R and M+R treatment than control. The data obtained in this experiment suggested that mycorrhizal inoculation enhanced of phosphorus utilization. The content of available phosphorus in soil (Table 5) well consist with this suggestion. The content of available phosphorus in control soil was the lowest throughout entire experimental period. Comparing to the control soils (165.4 mg P_2O_5/kg DM) at DAS 56, available phosphorus in soils was not significantly changed in the R treatment, but M and M+R inoculation increased about 45 % and 56 %, respectively. At DAS 126, the available P content was also significantly higher in the M and M+R treatment (about 34 % and 38 % of increase) than control (189.2 mg P_2O_5/kg DM). These results suggested that mycorrhiza infection leaded to increase P availability from P substances having low solubility and mobility as like rock phosphate, which was applied in this experiment. Azcón and El-Atrash (1997) reported that P concentration increased with the amount of plant-available P or mycorrhizal inoculum in the soil.

2. N, P uptake

Total uptake of nitrogen and phosphorus as affected by microbial inoculation during experimental period are shown in Fig. 2. At DAS 56, total N uptake in control (non-inoculation) was the lowest at 14.95 mg/plant [(4.63 mg/plant by alfalfa) (10.32 mg/plant by perennial ryegrass)]. It significantly increased by 51 %, 51 % and 94 % in the M, R and M+R amended soils, respectively. At DAS 126, total N uptake in control soils was also the lowest at 44.71 mg/plant [(7.44 mg/plant by alfalfa) (37.27 mg/plant by perennial ryegrass)]. It significantly increased by 71 %, 98 % and 197 % in the M, R and M+R amended soils, respectively. It was noteworthy that the individual N uptake by perennial ryegrass was relatively higher in rhizobium inoculated soils (R) and that of alfalfa in mycorrhiza inoculated soils (M) (especially in dual R+M inoculation). These well agreed with the data of soil nitrogen analysis (Table 4), which showed the improvement of nitrogen utilization in the plants inoculated with mycorrhiza (in perennial ryegrass) and mycorrhiza + rhizobium (in alfalfa). Ibijbijen et al. (1996) reported that AM fungal inoculation of both *Brachiaria arrecta* and *Sorghum vulgare* increased N accumulation by between 17 and 46%. When compared to the N uptake of M or R single inoculation, an additional increase by dual (M+R) inoculation was clearly observed in alfalfa, while non-significant increase in perennial ryegrass. This indicated that legumes much usefully benefited the effectiveness of mycorrhizal inoculation on N utilization rather than grasses. The large increase of N uptake into the intercrop system by the alfalfa in R+M dual inoculated soils might be closely related to high availability of P in soils (Table 5) and high uptake of P (Fig. 2). Voss and Schrader (1984) reported that the effectiveness of N uptake in the intercrop system by the legume component has been associated with the P availability. Although N transport from N-rich legume to grass plants has been reported in the several literature (Haynes, 1980; Heichel and Barnes, 1984), similar evidence could not be determined by this study.

The absolute value for P uptake was much lower than the N uptake throughout experimental period. At DAS 56, total P uptake in the control plants was the lowest at 0.39 mg/plant [(0.06 mg/plant by alfalfa) (0.33 mg/plant by perennial ryegrass)]. It slightly increased by 0.48, 0.52 and 0.82 mg/plant in the M, R and M+R treatment. Total P uptake at DAS 126 in the control plant was also the lowest at 3.52 mg/plant [(0.92 mg/plant in alfalfa) (2.6 mg/plant in perennial ryegrass)]. It was significantly increased by 70 %, 72 % and 111 % in the M, R and M+R treatment. P uptake of perennial ryegrass at DAS 126 was 2.6, 4.0, 3.9 and 4.3 mg/plant in control, M, R and M+R treatment, respectively. Compared to control soils, P uptake of alfalfa in M or R treatment significantly increased with same rate (about 2.1 and 2.4 fold higher than control), An additional increase of P uptake (3.4 fold higher than control) was observed in dual M+R inoculated soils. These results indicated that P uptake was similarly responded to mycorrhiza or rhizobium inocuation in both legume and grass plants, and that the increase of P uptake by dual (M+R) inoculation more significant in alfalfa than in perennial ryegrass. These are well consistent with the data of N uptake

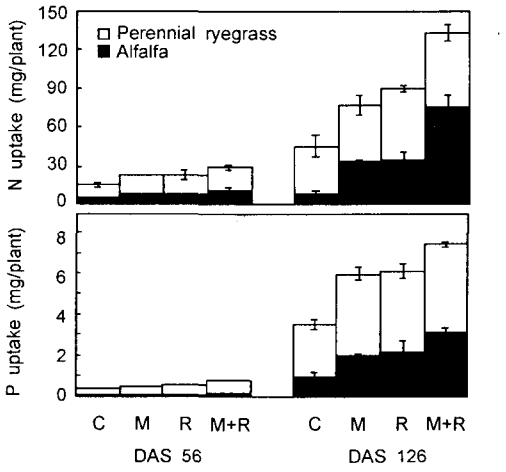

Fig. 2. The effects of microbial inoculation on the total uptake of nitrogen and phosphorus during the early vegetative stage (DAS 56) and the early flowering stage (DAS 126). Each value is mean±s.e. for n=5.

(Fig. 2). The similar results on the increase of P uptake by mycorrhiza fungus inoculation were reported in legumes ; *Trifolium repens* L. (Nadian et al., 1997) and *Medicago sativa* L. (Toro et al., 1998). In addition, the remarkable increases of N and P uptake in dual (M+R) inoculation were likely to be associated with the improvement of P utilization by mycohhriza (Table 5) and enhancement of Rhizobium activity for N2 fixation by increased P supply (Robson et al., 1981; Israel, 1993).

3. Growth characteristics and productivity

Growth characteristics of alfalfa and perennial ryegrass plants as affected by microbial inoculation are shown in Table 6. Plant height, root diameter, number of branch and number of tiller by microbial inoculation were not significant different. SPAD value (chlorophyll content measurement) in the control leaves was the lowest at 14.7 in alfalfa and 34.0 in perennial ryegrass. It significantly increased by 19.9, 16.7 and 21.5 % (in alfalfa), by 39.0, 42.2 and 38.2 % (in perennial ryegrass) in the M, R and M+R inoculated plant, respectively.

Dry matter and crude protein yield as affected by microbial inoculation are shown in Fig. 3. Total dry matter yield in the control was the lowest at 551 kg/10a [(35 kg/10a by alfalfa) (516 kg/10a by perennial ryegrass)]. It significantly increased by about 27 %, 33 % and 53 % in the M, R and M+R treatment, respectively, compared to the control plants. Total crude protein yield in the control was

Table 6. Growth characteristics as affect by microbial inoculation at the early flowering stage. Each value is mean±s.e. for n=15

Microbial inoculation	Alfalfa				Perennial ryegrass		
	Plant height (cm)	Root diameter (mm)	No. of Branch	SPAD value	Plant height (cm)	No. of tiller	SPAD value
Non-inoculation (Control)	28.6±4.2	3.4±0.3	2.4±0.4	14.7±0.3	14.6±2.3	37.4±5.8	34.0±5.2
Mycorrhiza (M)	37.2±6.8	4.9±0.5	4.8±0.9	19.9±5.5	15.9±1.2	39.7±6.7	39.0±4.3
Rhizobium (R)	31.3±5.9	3.7±0.7	3.4±1.0	16.7±0.7	18.0±1.4	35.8±2.5	42.2±5.5
Mycorrhiza + Rhizobium (M+R)	41.2±5.4	5.8±1.3	5.2±1.4	21.5±4.9	16.4±1.4	33.5±3.2	38.2±2.5

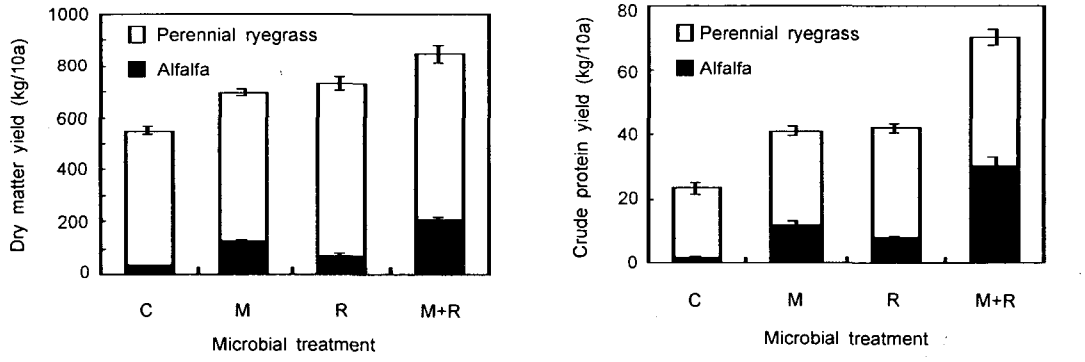

Fig. 3. Dry matter yield and crude protein as affected by microbial inoculation at the early flowering satge. Each value is mean±s.e. for n=5.

also the lowest at 23 kg/10a [(2 kg/10a by alfalfa) (21 kg/10a by perennial ryegrass)]. About 78 %, 83 % and 204 % of increase in crude protein yield was observed in the M, R and M+R treatment when compared to control. It was noteworthy that the relative high effectiveness of mycorrhiza inoculation to alfalfa yield and rhizobium inocultion to perennial ryegrass yield were observed. An additional increase of yield in dual (M+R) inoculation was also significative. Relatively low effectiveness of mycorrhiza in perennial ryegrass was well agreed with the results of Hall (1978) who reported that poor response to vesicular-arbuscular mycorrhiza by *Lolium perenne* L. was associated with *Trifolium repens* L. to intra-specific competition for soil P. Barea et al. (1989) reported that N2 fixation activity was apparently impaired as a result of competition with ryegrass. Our overall results clearly showed that mycorrhiza or/and rhizobium inoculation improved N, P utilization, and consequently increased the yield of alfalfa and perennial ryegrass under intercrop system.

Ⅳ. ACKNOWLEDGMENTS

The authors thank Dr. B. G. Shon and S. M. Park of Lab. of Soil Sci., University of Sunchun for their technical support for mycorrhizal colonization determination.

Ⅴ. 적 요

Mycorrhiza 및 *Rhizobium* 접종이 알팔파와 페레니얼 라이그라스 혼파 조건에서 질소와 인의 이용성 및 성장에 미치는 영향을 조사하고자, 4가지 미생물 처리 [비접종구 (대조구), Mycorrhiza 접종구 (M), Rhizobium 접종구 (R) 그리고 Mycorrhiza+Rhizobium 동시접종구 (M+R)]를 한 후 초기영양생장기(DAS 56)과 개화초기(DAS 126)에 화학적 성분, 성장 및 생산성을 각각 비교하였다. 토양내 총 질소와 인의 함량은 식물이 성장함에 따라 유의적으로 감소하였다. 그 감소 비율은 Mycorrhiza와 Mycorrhiza+Rhizobium 동시접종구에서 대조구보다 상대적으로 높았다. 개화초기(DAS 126)에 토양내 유효인산 농도는 대조구 (189.2 mg P_2O_5/kg DM)와 비교할 때 Mycorrhiza 접종구와 Mycorrhiza+ Rhizobium 동시접종구에서 각각 약 34와 38 % 증가하였으나, Rhizobium 접종구에서는 유의적인 차이가 없었다. 대조구와 비교할 때 질소 총흡수량은 Mycorrhiza 접종구, Rhizobium 접종구 및 mycorrhiza+Rhizobium 동시접종구에서 71, 98 및 197 % 증가하였고, 인의 총흡수량은 70, 72 및 111 %로 각각 증가하였다. 건물 수량과 조단백질 수량은 대조구와 비교할 때 Mycorrhiza 접종구, *Rhizobium* 접종구 및 *Mycorrhiza+Rhizobium* 동시접종구에서 각각 27, 33 및 53 %와 78, 83 및 204 %가 증가하였다. 이상의 결과들은 Mycorrhiza와 Rhizobium 접종은 알팔파 및 페레니얼 라이그라스에 의한 질소와 인의 이용성을 향상시키며, 건물 및 조단백질 수량의 유의적인 증가(특히 Mycorrhiza+Rhizobium 이중접종) 시킴을 잘 보여 주었다.

Ⅵ. REFERENCE

1. Azcón, R. and E. El-Atrash. 1997. Influence of arbuscular mycorrhizae and phosphorus fertilization on growth, nodulation and N_2 fixation (^{15}N) in *Medicago sativa* at four salinity levels. Biol. Fertil. Soils. 24:81-86.
2. Barea, J.M., C. Azcón-Aguilar and C. Azcón. 1997. Interactions between mycorrhizal fungi and rhizosphere microorganisms within the context of sustainable soil-plant systems. In, *Multitrophic Interactions in Terrestrial Systems*, Gange, A. C. and V. K. Brown, eds. Cambridge, Blackwell Science. pp 65-77.
3. Bethlenfalvay, G.J., M.G. Reyes-soils, S.B. Camel and R. Ferrera-Cerrato. 1991. Nutrient transfer between the root zones of soybean and maize plants connected by a common mycorrhizal mycelium. Physiol. Plant. 82:423-432.
4. Brundrett, M.C., Y. Piche and R.L. Peterson. 1984. A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can. J. Bot. 62:2128-2134.
5. Chiariello, N., J.C. Hickman and H.A. 1982. Endo-mycorrhizal role for interspecific transfer of phosphorus in a community of annual plants. Science. 217:941-943.
6. Francis, R. and D.J. Read, 1984. Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium. Nature. 307:53-56.

7. Hall, I.R. 1978. Effects of endomycorrhizas on the competitive ability of white clover. N. Z. J. Agri. Res. 21:509-515.
8. Hardy, R.W.F., R.D. Holsten, E.K. Jackson and R.C. Burns. 1968. The acetylene- ethylene assay for N_2-fixation : labotarory and field evalution. Plant Physiology. 43:1185-1207.
9. Harley, J.L. and S.E. Smith, 1983. Mycorrhizal symbiosis. London ; Academic press.
10. Haynes, R.J. 1980. Competitive aspects of the grass-legume association. Adv. Agron. 33:227-261.
11. Heichel, G.H. and D.K. 1984. Opportunities fro meeting crop nitrogen needs from symbiotic nitrogen fixation. In, *Organic farming : Current Technology and its Role in Sustainable Agriculture*, Bezdicek, D.F., J.F. Power, D.R. Keeney and M, J. Wright, eds. Am. Soc. Agron. Madison. pp 49-59.
12. Ibijbijen, J., S. Urquiaga, M. Ismail, B.J.R. Alves and R.M. Boddey. 1996. Effect of arbuscular mycorrhizas on uptake of nitrogen by Brachiaria arrecta and sorghum vulgare from soils labeled for several years with ^{15}N. New Phytol. 133:487-494.
13. Israel, D.W. 1993. Symbictic dinitrogen fixation and host-plant growth during development of and recovery from phosphorus deficiency. Physiol. Plant. 88:294-300.
14. Khasawneh, F.E. and E.C. Doll. 1978. The use of phosphate rock for direct application to soils. In, *Advance in Agronomy*, Brady, N.C. ed. Academic Press, New York, Vol. 30, pp 159-206.
15. Kim, T.H. and A. Ourry, J. Boucaud and G. Lemaire. 1992. Changes in source/sink relationship for nitrogen during regrowth of alfalfa (*Medicago sativa* L.) following removal of shoots. Aust. J. Plant Physiology. 18(6):593-602.
16. Kim, T.H. and B.H. Kim. 1996. Ammonia microdiffusion and colorimetric method for determining nitrogen in plant tissues. J. Korean Grassl. Sci. 16(4):253-259.
17. Kucey R.M.N., H.H. Janzen and M.E. Leggett. 1989. Microbiologically mediated increases in plant plant-available phosphorus. In, *Advance in Agronomy*, Brady, N. C. ed. Academic Press, New York, Vol. 42, pp 199-228.
18. Nadian, H., S.E. Smith, A.M. Alston and R.S. Murray. 1997. Effects of soil compacton on plant growth, phosphorus uptake and morphological characteristics of vesicular-arbuscular mycorrhizal colonization of *Trifolium subterraneum*. New Phytol. 135:303-311.
19. Read, D.J., H.K. Koucheki and J. Hodgson. 1976. Vesicular-arbuscular mycorrhiza in native vegetation system. New Phytol. 77:641-653.
20. Ritz, K. and E.I. Newman. 1984. Movement of 32P between intact grassland plants of the same age. Oikos. 43:138-142.
21. Robson, A.D., G.W. Ohara and L.K. Abbott. 1981. Involvement of phosphorus in nitrogen fixation by subterranean clover (*Trifolium subterraneum* L.). Aust. J. Plant Physiol. 8:427-436.
22. Sa, T.M. and D.W. Israel. 1991. Energy status and functioning of phosphorus-deficient soybean nodules. Plant Physiol. 97:928-935.
23. Sass, J.E. 1958. Botanical microtechnique. 3rd ed. Iowa State University Press. Ames. IA.
24. Toro, M., R. Azcon and J.M. Barea. 1998. The use of isotopic dilution techniques to evalutate the interactivity effects of *Rhizobium* genotype, mycorrhizal fungi, phosphate-solubilizing rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by *Medicago sativa*. New Phytol. 138:265-273.
25. Van Kessel, C., P.W. Singleton and H.J. Hoben. 1985. Enhanced N-transfer from soybean to maize by vesicular-arbuscular mycorrhizal (VAM) fungi. Plant Physiol. 79:562-563.
26. Voss, R.D. and W.D. Shrader. 1984. Rotation effects and legume sources of nitrogen for corn. In, *Organic farming : Current Technology and its Role in Sustainable Agriculture*, Bezdicek, D.F., J.F. Power, D.R. Keeney and M.J. Wright, eds. Am. Soc. Agron. Madison. pp 49- 59.
27. Wallander, H. and J.E. Nylund. 1992. Effects of excess nitrogen and phosphorus starvation on the extramarrical mycelium of ectomycorrhizas of *Pinus sylvestris* L. New Phytol. 120:495-503.