The beneficial effect of dual inoculation of vesicular-arbuscular mycorrhizae + rhizobium on growth of white clover

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Summary

Investigation on the effect of phosphorus on vesicular-arbuscular mycorrhizal infection, and dual inoculation of vesicular-arbuscular mycorrhizae + rhizobium on growth of white clover under field microplots and pot experiments was conducted on fluvo-aquic soils of semi-arid region in north China. The results showed that 60 kg P_2O_5 ha^{-1} in form of superphosphate was the most favorable phosphorus level for vesicular-arbuscular mycorrhizal infection; mycorrhizal infection, nodulation, dry weight of shoots and roots, total uptake of nitrogen, phosphorus and other elements, the final yields and recovery of phosphorus of white clover were significantly increased by vesicular-arbuscular mycorrhizal inoculation and dual inoculation with vesicular-arbuscular mycorrhizal fungi and rhizobium. The highest response of inoculation was obtained by adding fertilizer phosphorus at the level of 60 kg P_2O_5 ha^{-1} in form of superphosphate.

Résumé

Une étude sur l'effet du phosphore sur la pénétration mycorrhizienne vésiculo-arbusculaire et de l'inoculation combinée de mycorrhizae *vésiculo-arbusculaire plus* rhizobium *sur la croissance du trèfle blanc en petites parcelles au champ et en pots de végétation a été menée sur sols "fluvo-aquic"* de la région semi-aride du nord de la Chine. Les résultats ont montré que 60 kg de P_2O_5.ha^{-1} sous forme de super était le taux de P le plus efficace pour la pénétration mycorrhizienne vésiculo-arbusculaire; la pénétration mycorrhizienne, la modulation, le poids sec des tiges et racines, la quantité optimale d'N absorbé, le P et les autres éléments, les rendements finaux et la récupération du P du trèfle blanc ont été augmenté significativement par inoculation mycorrhizienne vésiculo-arbusculaire, par inoculation de mycélium mycorrhizien VA et du Rhizobium. La réponse la plus forte à l'inoculation fut obtenue après addition d'engrais phosphaté à la dose de 60 kg P_2O_5.ha^{-1} sous forme de superphosphate*

* *Soils taxonomy. U.S.A.*

Introduction

Fluvo-aquic soil covers a large area of semi-arid region of north China which is low in productivity due to the deficiency of available nutrients. White clover is newly introduced into this region as one kind of green manure and also for the exploitation of fishery and husbandry. It was indicated that white clover was high in mycorrhizal dependency (7).

Inoculation with vesicular-arbuscular mycorrhizae (VAM) strongly increased nutrient uptake and plant growth (1, 6, 11). Most experiments demonstrated that VAM stimulated phosphorus uptake of leguminous plants and greatly increased nodulation and nitrogen fixation (1, 2).

The purpose of the present project is to investigate the beneficial effect of dual inoculation of VA mycorrhizal fungi and rhizobium on yield and quality of white clover.

Materials and methods

1. The soil used were fluvo-aquic soil. The basic soil properties were listed in Table 1.
2. Inoculum composed of mycorrhizal sudangrass roots and adhering soils infected by indigenous *Glomus* spp. was mixed and placed as a thin layer (15 g fresh wt. pot^{-1}) 7 cm below soil surface.
3. Surface sterilized seeds of white clover (*Trifolium repens.*, L.) were inoculated with 1 ml pot^{-1} of dense suspension of *Rhizobium trifolii* (10^{-9} No ml^{-1}) at planting. A similar amount of sterilized inoculum was used as control.

4. Treatments:

The experiments were conducted in two parts:

Part A. Pot culture: The soil used was sandy fluvo-aquic soil. The level of phosphorus in form of superphosphate were:

TABLE 1

Basic properties of the soils used in experiments

Soil	Total P (P_2O_5 %)	Available P (P_2O_5 mg/100 g)	Total N (%)	OM (%)	Soil pH	mg/kg Mo	Co	Cu	B	Zn	Mn	Fe	g/100 g Ca	Mg	K
Sandy soil	0.096	1.33	0.029	0.45	8.1	1.9	4.69	8.1	82.5	39.4	259.8	1.45	1.41	0.767	0.13
Sandy loam	0.146	1.34	0.045	0.59	7.8	3.7	5.73	7.6	50.4	52.3	291.2	1.85	1.43	0.913	0.20

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0. 60. 180 kg P_2O_5 ha^{-1}. Four treatments were set; inoculation with mycorrhizal fungi (M), with Rhizobium (R), with Mycrorrhizal fungi + Rhizobium (M+R) and uninoculated control (CK). Seeds were sown in three holes per pot and thinned to six per hole after emergence. There were 5 replicates of each treatment harvested after 120 days from sowing.

Part B. Microplot experiment: The soil used was conducted on loamy fluvo-aquic soil with four treatments as in pot experiment. White clover was pre-inoculated (500 g inoculum in 2 m^2 per plot) and transplanted into 1x2 m^2 microplots after 40 days. 60 kg P_2O_5 ha^{-1} in form of superphosphate was applied with 4 replications arranged at random. There were four rows in each microplot and 14 holes for each row. Plant samples were collected at 80 and 240 days after transplantation, 20 plants and plants in 2 holes were randomly harvested respectively from each plot for measurement. Total yield of white clover was measured in spring and autumn in next year after transplantation.

5. Measurements

(1) VAM infection: roots were cleared and stained (10) and VAM infection was measured by the gridline intersect method (4).

(2) Nutrition uptake of plants; the total N.P content of plant were estimated by using the distillation methods and molybdate blue method respectively after digestion with H_2SO_4-H_2O_2. Other elements were determined by plasma emission spectrometer after digestion with HNO_3-$HClO_4$.

(3) Recovery of phosphorus: The recovery of phosphorus by plant was calculated according to Powell (12):

$$P\ recovery\ \% = \frac{P_r - P_o}{f} \times 100$$

where: P_r = total P uptake by plants at fertilizer rate r; P_o = total P uptake in unfertilized soil; f = rate of applied fertilizer.

Results and discussion

1. Effect of available phosphorus on VA mycorrhizal infection

Root infection of VAM fungi in fluvo-aquic soil was limited in most cases by phosphorus deficiency. The increase of P supply enhanced mycorrhizal infection of white clover as that of mungbeen (8). The highest percentage of root infection was found in treatment with the P level of 60 kg P_2O_5 ha^{-1} but it declined greatly with P level of 180 kg P_2O_5 ha^{-1} (Table 2). It appears to the same tendency as that obtained by Graham (5).

TABLE 2

Effect of P level on mycorrhizal infection (%) of white clover

Phosphorus level P_2O_5 kg/ha	Treatment	VA mycorrhizal infection (%)			
		70d (pot culture)	120d (pot culture)	80d (field experiment)	120d (field experiment)
0	CK	9.2B	17.0C		
	M	46.2A	27.0B		
	R	7.0B	18.2C		
	MR	38.8A	37.4A		
60	CK	4.4B	26.9B	18.75A	10.75A
	M	59.1A	44.9A	45.50B	33.25C
	R	10.0B	29.2B	21.60A	18.75B
	MR	68.6A	52.0A	48.75B	33.75C
180	CK	2.8C	18.4B		
	M	25.4A	29.0A		
	R	1.8C	21.0B		
	MR	14.8B	22.2B		

It is supposed that the mechanism for inhibition of VAM infection in higher phosphorus contents was associated with membrane-mediated decrease in root exudation. The rate of exudation is directly related to fundamental changes in root membrane permeability controlled by P. Subsequent mycorrhizal infection was highly correlated with initial differences in root exudation. Since the native VAM fungi in fluvo-aquic soil are low, VAM infections are consistently low without inoculation even after P fertilization. In treatments M and M+R, mycorrhizal infection increased markedly by the addition of available P especially under the P level of 60 kg P_2O_5 ha^{-1} as shown in Tab. 2. Table 2 also shows that in treatment of Rhizobium inoculation and in control the effect of P application on VAM infection was not as noticeable as M+R dual inoculation.

2. Effect of inoculation on nutrient uptake, nodulation and nitrogen fixation.

Leguminous plant is the tripartite symbiotic association of legumes, mycorrhizal fungi and rhizobia. Inoculation with VAM fungi increases VAM infection, nutrient uptake and plant growth in a phosphate-deficient soil, thus providing beneficial conditions for rhizobial infection. It is well known that effective nodulation and symbiotic N_2-fixation need a rather high P requirement. Mosse (9) reported that many strains of rhizobium in legumes require at least 0.1 per cent P in root tissues for nodulation. The results indicated that inoculation with M and M+R markedly increased VAM infection of plant roots, and strongly stimulated nodulation of white clover. It showed in Tables 2 and 3 that the number of nodules of mycorrhizal plants were much higher than that of non mycorrhizal controls. And, maximum nodulation was also occurred in treatment with the P level of 60 kg P_2O_5 per hectare. An insufficient or over addition of phosphorus just showed a limited effect on nodulation. In microplots with optimum P level (60 kg P_2O_5 ha^{-1}) effects of mycorrhizal inoculation on nodulation of white clover were similar to that in pot experiment.

TABLE 3

Effect of phosphorus application on nodule number of white clover

Phosphorus level Treatment P_2O_5 kg/ha		Nodule No. / plant		
		120d (pot culture)	80d (field experiment)	120d (field experiment)
0	CK	0B		
	M	4.6A		
	R	0.1B		
	MR	4.1A		
60	CK	0.3C	2.16A	16.45A
	M	29.6B	10.71B	27.70B
	R	9.5C	4.27A	19.10A
	MR	40.4A	10.00B	27.90B
180	CK	1.5C		
	M	13.0BC		
	R	24.2AB		
	MR	33.2A		

White clover responds particularly well to mycorrhizal infection which resulted in increasing nodulation and N_2-fixation and especially improving nutrient uptake. In our experiment, the results indicated that plants inoculated with either M or M+R

had considerably higher amount of total N and P absorbed than control plants, and than that was only inoculated with rhizobium in all levels of phosphorus applied. The difference between them was very significant under optimum P level. At the P levels of 60 kg and 180 kg P_2O_5 ha^{-1}, total phosphorus uptake of plant in both M and M+R treatments was 4.5-5.6 times and 5.4-5.7 times greater than control plants, and total N uptake was 12.5-10.9 times and 14-11.0 times greater than control plants respectively (Fig. 1). Inoculation with M and M+R also stimulated the uptake of other elements (Table 4). Similar results have been reported by Cooper and Tinker (3). It is indicated VA mycorrhizal inoculation and dual inoculation improve the uptake of N. P. and other elements which give support to nodulation and N_2-fixation.

3. Mycorrhyzal benefits to plant growth and reduction of fertilizer requirements

The effect of inoculation on plant growth due to the improvement of nutrient uptake and noduation is well demonstrated by the results mentioned above. The results of pot cultures (Fig. 2) showed inoculation with M and with dual inoculation of M+R significantly stimulated the growth of white clover. The mean dry weight of shoots and roots of M and M+R was significantly greater (P=0.01) than non-mycorrhizal plants and than in only rhizobium inoculated treatments. In all levels of P treatment, the increases of shoot dry weight was 380-744% by mycorrhizal inoculation, and 424-726% was increased by dual inoculation. White clover showed a large growth response to phosphorus. When no P was added, the increase of shoot dry weight in both M and M+R treatments was 380 and 422% respectively compared with uninoculation plants, but 657 and 726% over the corresponding control with the addition of 60 kg P_2O_5 ha^{-1} respectively. While further addition of P to 180 kg P_2O_5 ha^{-1}, dry weight of shoots was not increased. The mean root dry weight showed a similar trend with shoot dry weight in all levels of P treatments.

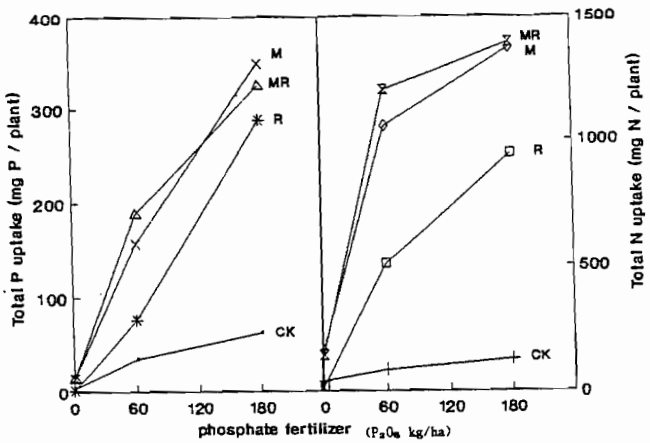

Fig. 1: Effect of phosphorus and inoculation on total P. N uptake (pot culture)

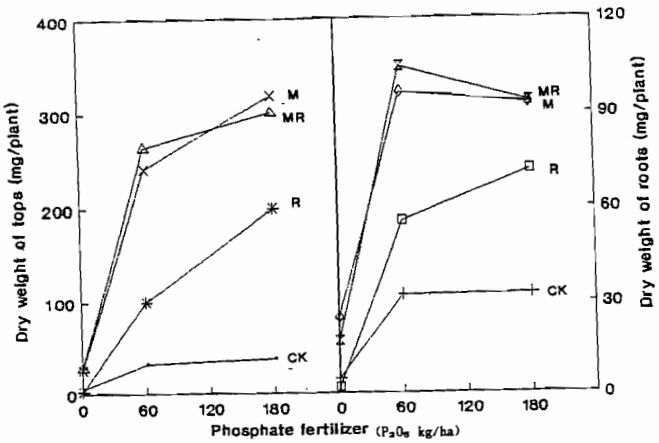

Fig.2: effect of phosphorus and inoculation on dry weight of shoots and roots (pot culture)

The results of field microplots also showed that inoculation with M or M+R had significant increase of dry weight of tops and roots at an optimum phosphorus application (60 kg P_2O_5 ha^{-1}) (Table 5), and it lasted to next year. The increase of total yield was similar to the dry weight raised in pot culture after inoculation (Fig. 3). The maximum yield (19.3 t ha^{-1}) was in the treatment of dual inoculation. About 46.8% of the shoot weight increased over the corresponding control. The increase of total yield was significantly different (P=0.01) between M and M+R as well.

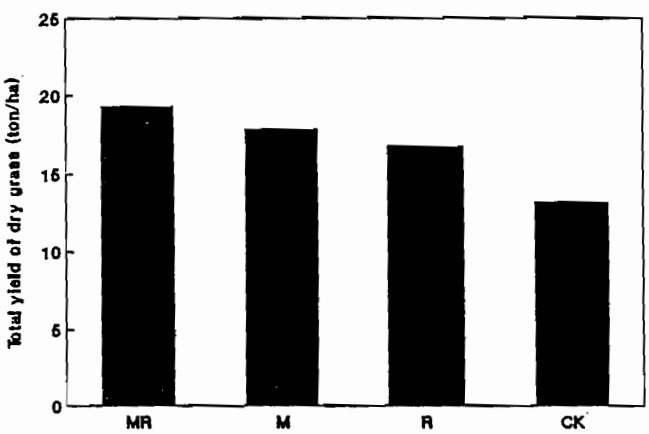

Fig. 3: Effect of inoculation on total yield of white clover (field experiment)

TABLE 4

Effect of inoculation with VAM on total uptake of other element of white clover (microplot)

Treatment	P_2O_5	N	Mo	Co	Cu	B	Zn	Mn	Fe	Ca	Mg	K
	g/plant					μ g/plant				g/plant		
CK	0.06	0.37	0.63	0.12	2.97	3.50	5.79	5.98	116	0.11	0.08	0.15
R	0.09	0.51	0.96	0.25	9.34	6.13	2.66	6.83	115	0.19	0.10	0.20
M	0.21	1.59	3.01	0.33	15.17	14.02	9.98	19.68	321	0.50	0.31	0.69
MR	0.91	1.35	3.75	0.39	18.67	27.90	10.53	21.42	539	0.57	0.33	0.60

TABLE 5

Effect of inoculation on plant growth (microplot)

Treatment	80 days		240 days	
	Shoot dry wt. g/20 plants	Root dry wt. g/20 plants	Shoot dry wt. g/20 plants	Root dry wt. g/20 plants
CK	0.48A	0.47A	0.10A	0.03A
R	1.21B	0.91B	0.14A	0.04A
M	4.68C	4.60D	0.48B	0.09B
MR	5.60D	3.65C	0.49B	0.12C

The effect of mycorrhizal inoculation and M+R dual inoculation on the recovery of phosphorus by plants is shown in Table 6. The maximum phosphorus recovery appeared at the level of 60 kg P_2O_5 ha^{-1}, 35.6% and 43.6% of P recovered by M and M+R inoculated plants. The mean recovery by non-mycorrhizal plants was only 7.9%. These results demonstrated that inoculation with either M or M+R improved phosphorus uptake of white clover, and increased the recovery of phosphorus. As a result, the requirement of phosphate fertilizer was greatly reduced for maximum plant growth.

TABLE 6

Effect of inoculation on recovery of phosphorus

Phosphorus level (P_2O_5 kg/ha)	Recovery of phosphorus (%)			
	CK	R	M	MR
60	7.9	18.6	35.6	43.6
180	4.9	23.8	27.9	25.8

Conclusions

Synergistic action of the tripartite symbiotic associations among legumes, VAM fungi and Rhizobium play a very important role in the improvement of plant growth. VA mycorrhiza greatly improved plant growth through the increase of the uptake of phosphorus and other mineral nutrients as well as the encouragement of nodulation and nitrogen fixation in soils of low fertility. The optimum amount of P fertilization to mycorrhizal infection in fluvo-aquic soil was 60 kg P_2O_5 ha^{-1}, by the application of which, VAM infection was greatly promoted and the efficiency of P recovery was increased simultaneously. From the results obtained, it is indicated VAM inoculation and the dual inoculation with VA mycorrhiza and Rhizobium are very important for the establishment of white clover in this region, and their symbiotic associations make great contribution to the increase of yield and decrease of the requirement of fertilizer for maximum plant growth.

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