Phosphorus uptake of *Sesbania cannabina* and *Crotalaria juncea* associated with root nodule bacteria

Hiroyuki Daimon, Kazue Nobuta, Masamichi Ohe, Jiro Harada and Toshinobu Morikawa

Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1-1, Gakuncho, Sakai, Osaka 5998531,
Japan www.osakafu-u.ac.jp Email daimon@plant.osakafu-u.ac.jp

Abstract

To evaluate plant growth and phosphorus (P) uptake of two green-manure legumes, *Sesbania cannabina* and *Crotalaria juncea*, a pot experiment under different P conditions was conducted. Dry weight and P content of *S. cannabina* were not affected by the rate of applied P. On the other hand, applying P at the rate of 8.0 g/pot significantly increased the P content of *C. juncea* at 50 days after sowing. The difference in P content between the two species was larger at lower P supplies (3.2 and 4.0 g/pot). The rhizobial strain of *S. cannabina*, U9709-SC, showed high solubilization of tricalcium phosphate in the medium accompanied by a lowering of the medium pH, but the strain of *C. juncea*, USDA-3024, did not show this ability. A contribution of P to a succeeding crop by incorporation of these legumes would be expected as a result of P recycling.

Media summary

Sesbania cannabina associated with the phosphate-solubilizing rhizobial strain, U9709-SC, has a high potential to access immobilized forms of soil phosphorus.

Key Words

Cropping system, green manure, P-solubilization, legume, nitrogen fixation

Introduction

Recycling of plant nutrients such as nitrogen and P is one of the most important concepts of sustainable cropping systems. We have tried to improve the nitrogen nutrition of crops by the incorporation of some tropical green-manure legumes into a range of cropping systems (Yano et al. 1994; Ohdan et al. 1995; Daimon and Kotoura 2000; Shiba and Daimon 2003). We are also attempting to recover P accumulated in the soil under intensive cultivation systems as a result of the previous application of chemical P fertilizer. The tropical green-manure legumes, *Sesbania* and *Crotalaria* have been recently introduced into temperate regions as green manure and/or nematocidal plants. The high potential biomass production and vigorous root growth of these plants appear to be desirable traits to increase the uptake of P accumulated in soil, and their incorporation as green manure has the potential advantage of being able to sustain soil productivity. In this experiment, we investigated the growth and P-uptake of the two leguminous species, *Sesbania cannabina* and *Crotalaria juncea* grown under different P conditions, and also determined the solubilization of sparingly available P by their associated rhizobia, *Rhizobium* and *Bradyrhizobium*.

Materials and methods

Growth and P uptake under different P application rates

In July 2002, seeds of *Sesbania cannabina* (Retz.) Pers. and *Crotalaria juncea* L. were sown in 1/5000a Wagner pots containing a mixture of "Akadamatuchi" (subsoil of Andosol with a pH 5.2, EC 0.06 ds/m, total nitrogen 0.11%, Truog-P 0.06 mg/100g, Ca-P 1.07 mg/100g, Al-P 15.0 mg/100g, Fe-P 5.24 mg/100g) and vermiculite at the rate of 3:1. The seeds were inoculated with the *Rhizobium* sp. strain U9709-SC for *S. cannabina* and *Bradyrhizobium* sp. strain USDA-3024 for *C. juncea*. There were three P

fertilization levels consisting of mixtures of insoluble phosphate (IP) and soluble phosphate (SP), i.e. 3.2 g P (IP2.4 + SP0.8), 4.0 g P (IP2.4 + SP1.6), and 8.0 g P (IP2.4 + SP5.6) per pot. Mixtures with $Ca_3(PO_4)_2$, $AlPO_4$ and $FePO_4 4H_2O$ at the rate of 1:1:1 as P were used as the IP sources, and superphosphate was applied as the source of SP. The plants were grown for 50 days under natural conditions.

Tricalcium phosphate solubilizing activity of rhizobia

Two rhizobia strains, U9709-SC and USDA-3024, were routinely cultured in YM broth at 27^{o}C in the dark. The rhizobia were then cultured for 6 days in a modified AYG broth according to Halder and Chakrabartty (1993) supplemented with 0.2% $Ca_3(PO_4)_2$. The cultures were centrifuged at 1000xg for 15 min, and then the supernatant was used for determination of pH and soluble P concentration released into the medium. The rhizobial cultures were also streaked on agar plates containing $Ca_3(PO_4)_2$ for detection of clear zones showing solubilization of the tricalcium phosphate (Illmer and Shinner 1992).

Results

Growth and P uptake under different P application rates

Seedlings of *S. cannabina* and *C. juncea* emerged at 2 and 4 days after sowing (DAS), respectively. In both species, there were no differences in the length and number of nodes of the main stem among the different P treatments. Dry weights and P contents of shoot and root in *S. cannabina* were not affected by the applied P level (data not shown). On the other hand, applying P at the rate of 8.0 g/pot significantly increased the P content of *C. juncea*. The dry weights and P contents of *S. cannabina* were greater than those of *C. incana* at 50 DAS. The difference in P content between the two species was greater at the levels of 3.2 and 4.0 g P/pot. Root nodule formation of *S. cannabina* was not influenced by applied P, but that of *C. juncea* was greatly increased at the highest P rate.

Tricalcium phosphate solubilizing activity of rhizobia

The rhizobial strain U9709-SC showed high solubilization of tricalcium phosphate in the medium which was accompanied by a lowering of the medium pH. The rhizobial strain used for *C. juncea*, USDA-3024, did not show an ability to solubilize tricalcium phosphate. The ability of U9709-SC to solubilize tricalcalcium phosphate was also indicated by the clear zones around the colonies, which were developed on the agar medium supplemented with tricalcium phosphate (Figure 1).

Figure 1. Tricalcium phosphate solubilizing activity of *Rhizobium* sp. strain U9709-SC. The strain was grown on Illmer and Shinner agar medium supplemented with $Ca_3(PO_4)_2$ at 27 ^{O}C in the dark for 3 days (right) and 12 days (left). Clear zones around colonies reveal the areas where the tricalcium phosphate has been solubilized.

Conclusions

As shown by our results, both species differed in their response to P application. The dry weight and P content of *C. juncea* increased considerably at the highest P level, but this increase in dry matter and P uptake did not occur in *S. cannabina*. We conclude that *S. cannabina* has a high ability to access poorly available forms of inorganic phosphates and that this depends on its association with P-solubilizing rhizobia. However, this trait needs to be further evaluated in terms of the mechanisms involved, as does P cycling to the succeeding crop after incorporation of *S. cannabina* as a green manure plant.

Acknowledgements

We thank to Dr. Velazquez and Institute of Tokachi Nokyoren for supplying the rhizobia strains PECA21 and J802b, respectively. This work was supported by Iwatani Naoji Foundation and MEXT, KAKENHI (14656009), Japan.

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