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Growth and iron nutrition of broccoli (*Brassica oleracea* L. var. *italica* Plenck), grown in a Typic Ustochrept, as influenced by vesicular-arbuscular mycorrhizal fungi in the presence of pyrite and farmyard manure

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Abstract Greenhouse experiments were conducted using potted soil (Fe-deficient Typic Ustochrept) to study the influence of the vesicular-arbuscular mycorrhizal fungi (VAM), *Glomus macrocarpum* and *G. fasciculatum*, on the mobilisation of Fe in broccoli (*Brassica oleracea* L. var. *italica* Plenck) in the presence of pyrite and farmyard manure (FYM). Individual applications of either VAM or pyrite with NPK fertiliser significantly enhanced both the Fe^{2+} content in leaf tissue and total uptake of Fe and resulted in increased curd and straw yields of broccoli compared to those observed with NPK alone. Though the application of FYM decreased the Fe^{2+} content in leaf tissue relative to plants supplied NPK alone, this result was not statistically significant. The available Fe content in soil, after harvest of broccoli, was found to be lower in the presence of VAM than in the control.

Key words Vesicular-arbuscular mycorrhizal fungi · *Glomus macrocarpum* · *Glomus fasciculatum* · *Brassica oleracea* L. var. *italica* Plenck · Iron nutrition

Introduction

Improved efficacy of nutrient absorption and, in turn, enhanced growth of plants can occur due to root colonisation by vesicular-arbuscular mycorrhizal fungi (VAM) (Li et al. 1991; Tarafdar 1995; Ghandour et al. 1996; Singh 1996). There have been few studies to ascertain directly the role of VAM in plant uptake of nutrients other than that of PO_4^{3-} and N. Many agricultural crops worldwide, especially in semiarid climates, suffer from Fe deficiencies (Chen and Barak 1982; Morris et al. 1990). There is a paucity of information available on the involvement of VAM in the uptake of Zn and Cu, trace elements with low mobility in soil (Harley and Smith 1983). It has also been reported that certain mycorrhizal fungi are capable of producing hydroxamate-type siderophores which may solubilise Fe and make it available to plants (Schuler and Haselwandter 1988). However, sound information on the role of VAM in alleviating Fe deficiency is lacking. Therefore, an attempt has been made to investigate the role of VAM on the mobilisation of Fe in broccoli (*Brassica oleracea* L. var. *italica* Plenck) in the presence of pyrite and other sources of Fe, such as farmyard manure (FYM).

Materials and methods

Surface soil (0–15 cm) collected from the Indian Agricultural Research Institute (IARI) farm, New Delhi, was used for the present study. The sandy clay loam soil is an illitic, mixed hyperthermic Typic Ustochrept. The physico-chemical properties of the soil are given in Table 1. Four kilograms of sieved soil (5-mm sieve) mixed with a basal dose of diammonium phosphate (at the rate of 58.9 mg P pot^{-1}) and KCl (at the rate of 111.2 mg K pot^{-1}) was placed in each pot. Diammonium phosphate supplied two-fifths of the total N (at the rate of 214.3 mg N pot^{-1}) applied as a basal dose. Furthermore, wherever necessary, the remaining N was supplied as urea (two-fifths and three-fifths, respectively, in the treatments receiving FYM and minus FYM) or FYM (one-fifth). FYM that was collected from the IARI dairy was prepared by com posting the mixture of cow dung and left-over wheat straw in an open trench for 1 year. FYM contained 5000 mg N kg^{-1}, 6000 mg P kg^{-1}, 8000 mg K kg^{-1}, 1300 mg S kg^{-1}, 24.7 mg Fe kg^{-1} and 375 mg available N kg^{-1} on an oven-dry-weight basis. Pyrite (total S 22% and Fe 20%) was applied at the rate of 446.4 mg pot^{-1} as a basal dose. The

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Table 1 Physico-chemical properties of the soil (air-dried)

Properties	Value	Method	Reference
pH	8.1	1:2; w:v Soil:water suspension	Jackson (1967)
Organic C (g kg^{-1})	3.0	Chromic acid oxidation	Walkley and Black (1934)
Total N (g kg^{-1})	0.4	Acid digestion	Bremner and Mulvaney (1982)
Available P (mg kg^{-1})	5.0	0.5 M $NaHCO_3$, pH 7.5	Olsen and Sommers (19829)
Available K (mg kg^{-1})	76.1	1 N CH_3COONH_4, pH 7.0	Knudsen et al. (1982)
Total S (mg kg^{-1})	219.0	Acid digestion	Tabatabai (1982)
Available S (mg kg^{-1})	14.0	0.15% $CaCl_2$	Williams and Steinbergs (1959)
Available Fe (mg kg^{-1})	3.9	0.005 M DTPA	Lindsay and Norvell (1978)

Table 2 Yield, Fe^{2+} content of leaf tissue (determined 35 days after transplanting) and uptake of Fe by broccoli (*B. oleracea* L. var. *italica* Plenck) as influenced by a mixed inoculum of vesicular-arbuscular mycorrhizal fungi (*VAM*), *Glomus macrocarpum* and *G. fasciculatum*, in the presence of (*NPK fertiliser*) pyrite (*PYT*) and farmyard manure (*FYM*). *CD* Critical difference

Treatment	Yield (g pot^{-1})		Leaf tissue Fe^{2+} content	Uptake of iron (mg pot^{-1})		
	Curd*	Straw**	(μg g^{-1})	Curd	Straw	Total
VAM uninoculated						
NPK	3.12	15.98	39.4	0.49	1.79	2.28
PYT+NPK	3.56	17.66	50.1	0.80	2.00	2.88
FYM+NPK	3.00	13.56	37.0	0.40	1.19	1.59
FYM+PYT+NPK	3.05	13.89	38.6	0.41	1.66	2.07
VAM inoculated						
NPK	3.43	17.43	45.8	0.65	2.06	2.71
PYT+NPK	3.96	18.98	56.4	1.19	2.08	3.27
FYM+NPK	3.02	14.30	41.2	0.57	1.57	2.14
FYM+PYT+NPK	3.37	14.69	43.7	0.85	2.00	2.85
CD ($P=0.05$)	0.30	1.30	5.4	0.14	0.25	0.35

* Curd = Influorescence
** Straw = Step plus leaf

experiment was laid out as a complete factorial design and replicated three times.

A soil- and sand-based mixed inoculum of the VAM *Glomus macrocarpum* and *G. fasciculatum* (15 chlamydospores g^{-1}), obtained from the Division of Microbiology, IARI was used to inoculate broccoli seedlings. Processed, air-dried surface soil (5 kg) was placed in a polythene bag and 20 g of mixed inoculum was uniformly spread over it. Then 100 broccoli seeds were uniformly applied over the inoculum and were subsequently covered with a thin layer of soil. The moisture content of the soil was maintained at field capacity (–10 kPa). The same procedure was followed for the control treatment which did not receive any inoculum. Four, 1-month-old, nursery-raised broccoli seedlings, either inoculated with the mixed inoculum or left uninoculated, were transplanted into the pots containing soil, and were subsequently thinned to give two plants pot^{-1}. Before transplanting, the roots of the seedlings were checked for root colonisation by VAM (Phillips and Hayman 1970).

The plants grew in the greenhouse at a temperature of 28±2 °C for 90 days. Plants were irrigated with deionised water in order to minimise the incorporation of any further micronutrients.

Leaves (2nd and 3rd from the top) were collected 35 days after transplanting and the Fe^{2+} content was determined colorimetrically following extraction with orthophenanthrolin (Katyal and Sharma 1980). Yields (dry weight) of curd and straw were measured when the plants were mature. For estimation of total Fe uptake by broccoli at maturity, the curd and straw samples were digested in a tri-acid mixture (H_2SO_4:HNO_3:$HClO_4$; 9:4:1) and the Fe content was determined by atomic absorption spectrophotometry, model Pye-Unicam-SP9 (Bhargava and Raghupati 1983). The available Fe content of the soil after harvest of broccoli was also determined by the diethylene triamine penta acetic acid method (Lindsay and Norvell 1978).

Results and discussion

It is apparent from the results (Table 2) that pyrite amendment increased curd and straw yields of broccoli grown on FYM due to the acquisition of Fe from pyrite, solubilised in the soil through oxidation. Here, the probability of a plant response to S was reduced because the soil used in this treatment was not deficient in S. However, in the VAM treatments excluding FYM the curd, yield was higher as a consequence of a relatively high level of root colonisation (60%) by *G. macrocarpum* and *G. fasciculatum* compared to native VAM which only colonised 4% of the root. Similar results have been found for varius crops by several workers (Cress et al. 1986; Rai 1988; Srinivasa et al. 1993). It is of note that maximum yields were recorded when plants were supplied with NPK and pyrite, both under inoculated and uninoculated conditions.

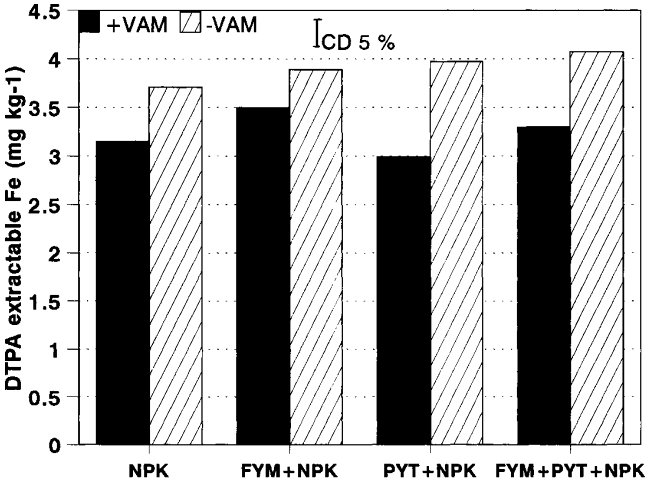

Fig. 1 Influence of vesicular-arbuscular mycorrhizal fungi (*VAM*) on diethylene triamine penta acetic acid (*DTPA*)-extractable Fe content of soil after harvest of broccoli (*B. oleracea* L. var. *italica* Plenck). *FYM* Farmyard manure, *PYT* pyrite, *CD* critical difference

In general, the Fe^{2+} content of leaf tissue 35 days after transplanting, and total as well as individual uptake of Fe by curd and straw of broccoli at maturity were increased due to the application of pyrite (Table 2). However, levels of Fe^{2+} in the leaf tissue and Fe uptake by the curd and straw were higher when soil had been inoculated with VAM, even in the treatments, not provided with pyrite. This could have been due to the production of hyphal networks in the rhizosphere, which extracted Fe more efficiently and made it available to the plants. Moreover, Fe^{2+} in the leaves and Fe uptake by the curd and straw were highest when broccoli was grown on inoculated soil amended with NPK plus pyrite; this may have been due to the acquisition of more Fe solubilised by siderophores produced by VAM. Similar observations have been reported for certain VAM (Cress et al. 1986; Clark and Zeto 1996) and ericoid mycorrhizal fungi (Schuler and Haselwandter 1988). Though the application of FYM along with NPK did not show any effect on leaf tissue Fe^{2+} content, it did lead to a reduction in the total uptake of Fe by broccoli; this may have been due to the low availability of N (7.5%) in FYM. While inoculated broccoli grown in the presence of pyrite and FYM showed increased levels of total uptake of Fe, as well as Fe uptake by curd and straw, the yield did not increase. It is worth noting that the total Fe content of the leaf tissue of broccoli ranges between 89 and 113 mg kg^{-1} at the stage when 5% of the heads have been formed (Gupta 1990, 1992). In our experiment, instead of estimating the content of total Fe (Fe^{2+}+Fe^{3+}) in leaf tissue 35 days after transplanting, metabolically active Fe, which included only Fe^{2+} extracted with ortho-phenanthrolin, was estimated, and this resulted in a lower value than that given by Gupta (1990, 1992).

The levels of available Fe in the soil when broccoli was harvested were lower in the inoculated treatments as compared to the uninoculated control (Fig. 1); this may have been due to the extraction of Fe from the soil by VAM. Similar results for P have been reported by Li et al. (1991). Another possible reason could be the storage of Fe in VAM endophyte vesicles. In this experiment, the available Fe levels of the soil were higher in the treatments applied with pyrite and FYM as compared to NPK alone, because the two former amendments act as a source of Fe.

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