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HISTOCHEMICAL CHANGES IN NODULES OF *CAJANUS CAJAN* (L.) Mill sp. INOCULATED WITH VESICULAR—ARBUSCULAR MYCORRHIZAL FUNGUS AND *RHIZOBIUM*

P. Sivaprasad[1], S. S. Joshi and P. V. Rai

Department of Agricultural Microbiology, U. A. S., G. K. V. K., Bangalore 560 065, India

Improved nodulation and nitrogen fixation in legumes due to vesicular-arbuscular mycorrhizai (VAM) association have been reported (Daft and El-Giahmi, 1976; Azcon ef *al.,* 1979; Sivaprasad ef *al.,* 1983). Mycorrhizai effect on nodulation and nitrogen fixation has generally been attributed to enhanced soil nutrient uptake, particularly phosphorus, conferred by mycorrhizal association (Asimi ef *al.,* 1980). Carbon availability and number and spread of bacteroids in the nodule tissue have a direct relationship with the amount of nitrogen fixed. However, no information. is available about the changes in root nodule due to VA mycorrhizai association, We report here for the first time the histochemical changes in root noduie of pigeon pea (*Cajanus cajan)* inoculated with VA mycorrhiza and *Rhizobium.*

Materials and Methods

Selfed seeds of *Cajanus cajan* (L) Mill sp. variety Pusa Agathi, vesicular-arbuscular mycorrhizai fungs *Glomus faciculatum* and *Rhizobium* strain IHPIOO wore used for the experiment conducted in pots of 30 cm diameter filled with 10 kg of P deficient unsterile Alfisol (pH 5.6 and available phosphorus 2.4 kg soil) for raising the crop. Surface sterilized mycorrhizai spores extracted from guinea grass *(Panicum maximum)* rhizosphere served as mycorrhizai inoculum. Fifty ml of water suspension containing about 600 spores was poured 5 cm below the soil surface and covered with a layer of soil. *Rhizobium* treated seeds were sown over the mycorrhizai inoculum. Four treatments viz (i) no inoculation (MoRo), (ii) *Rhizobium* clone (MoR), (iii) VAM fungus alone (MRo) and (iv) dual inoculation (MR) were included in the study. Plants were harvested after 40 days. Nodules from all treatments were fixed in Carnoy's B (6:3:1 ethno!: chloroform: acetic acid) for 1 h, dehydrated using n-butanol series and embedded in paraffin at 56"C. Serial sections of 5-6 μm thickness were made and were subjected to histochemical studies. Observations were made on volume of bacteroidal zone, transformed bacteroidal cell and nucleus with micrometer, using the formula $4/3r^3$. The intensity of polysaccharide accumulation in the nodule tissue was also observed

1 Present address: College of Agriculture, Vellayani 695 522, Trivandrum, India

Staining for insoluble polysaccharide

Per-iodic and Schiff's test was followed as outlined by Jensen (1962). Sections were deparaffinized and hydrated using butanol, alcohol series and finally with water. Sections were then treated with 1 per cent per-iodic acid for 15 minutes, differentiated in water, leached in 2 per cent potassium metabisulphate, dehydrated using alcohol, butanol, xylol series, cleaned and mounted. Insoluble polysaccharide appeared majenta.

Results and Discussion

Mycorrhizal association enhanced the percentage of bacteroidal zone in the nodule tissue (Table 1). Tripartite system transformed 70 per cent of the nodule tissue into bacteroidal zone as against 54 per cent recorded in the case of inoculation with *Rhizobium* alone. Combined inoculation with microsymbionts significantly increased tho size of transformed bacteroidal cell at the central and peripheral region of the bacteroidal zone (Table 1 and Fig. 1 to 4). Lone inoculation of VAM fungus also increased the transformed cell size of native rhizobia. Irrespective of the treatment, transformed cell present at the peripheral region was bigger than the one present at the central region of the nodule tissue. Size of the nuclei of bacteroidal cell also increased due to mycorrhizal association.

VAM fungus alone inoculated plans showed maximum polysaccharide accumulation in the bacteroidal zone (Fig. 3), followed by control (Fig. 1). Plants received dual or *Rhizobium* alone inoculation showed very little polysaccharide accumulation particularly in the bacteroida! cells (Fig. 2 and 4).

Mycorrhizal influence on enhancing the transformed bacteroidal zone can be attributed to factors like increase in initial multiplication of bacteria and nodule cells due to better availability of nutrients and/or hormonal effect. Mycorrhizal plants are known to have more hormone activity (Allen *et al.* 1980; [illegible]sad, 1983). Mycorrhiza induced transformed cell size is probably due to increased metabolic activity of the cell conferred by mycorrhizal association. Further, the increased nuclear size in the transformed cell indicated the effective replication of DNA which is perhaps necessary for enhancing tho metabolic activities of the transformed cell Thus, the increased size of tho transformed cells in the transformed area helped in accommodating the increased number of bacteroids, Since nitrogen fixation is very much dependent on number and spread of bacteroid in the nodule tissua (Bergersen, 1974; Rao, 1976) the nitrogen fixation will also increase. Consistent increase in transformed cell size observed at the peripheral region of the nodule might be due to close proximity of vascular tissue, particularly phloem, and hence better nutrient availability.

Polysaccharide accumulation was maximum in VAM fungus alone inoculated plant nodules. This indicates that in mycorrhiza alone inoculated plant eventhough there was an improved carbohydrate flow into the nodule, the native rhizobia could not utilize the accumulated carbohydrate effectively. In contrast to this, the carbohydrate flown into the nodule is very efficiently utilized by the nodule microsymbiont in dual inoculation treatments. Hence, synergestic interaction of VA mycorrhiza and *Rhizobium* resulted into, in addition to increased bacteroid content, efficient utilization of carbon available in the nodule tissue. Higher bacteroid content together with improved carbon utilization might have enhanced the nitrogen fixing efficiency of mycorrhizal *Cajanus cajan*.

Table 1

Histological changes in redgram root nodule due to dual inoculation with VAM fungus and *Rhizobium*

Treatments	Transformed bacteroidal zone (%)	Size of transformed bacteroidal cell (x 10 μm³)		Size of nucleus in the transformed bacteroid cell (x 10 μm³)	
		Peripheral	Central	Peripheral	Central
MoRo	33.67	20.18	8.03	0.14	0.12
MoR	58.42	59.42	31.78	0.27	0.31
MRo	54.57	66.59	31.62	0.79	0.74
MR	70.06	131.83	65.67	0.74	0.69
CD (0.05)		12.29	5.38	0.08	0.09
CD (0.01)		17.69	7.42	0.11	0.12

Volume of the cell calculated using the formula 4/3 r^3

Summary

Symbiosis of *Glomus fasciculatum* (Thaxter sensu Gerd) and Trappe, *Rhizobium* and *Cajanus cajan* (L.) Millsp. was studied in relation with bacteroidal zone and polysaccharide accumulation in nodule tissue. Tripartite symbiosis enhanced the area of bacteroidal zone in nodule tissue and size of bacteroidal cell and nucleus. Association of vesicular-arbuscular mycorrhizal fungus alone had maximum polysaccharide accumulation in the nodule tissue. Dual inoculation with microsymbionts showed very little polysaccharide accumulation.

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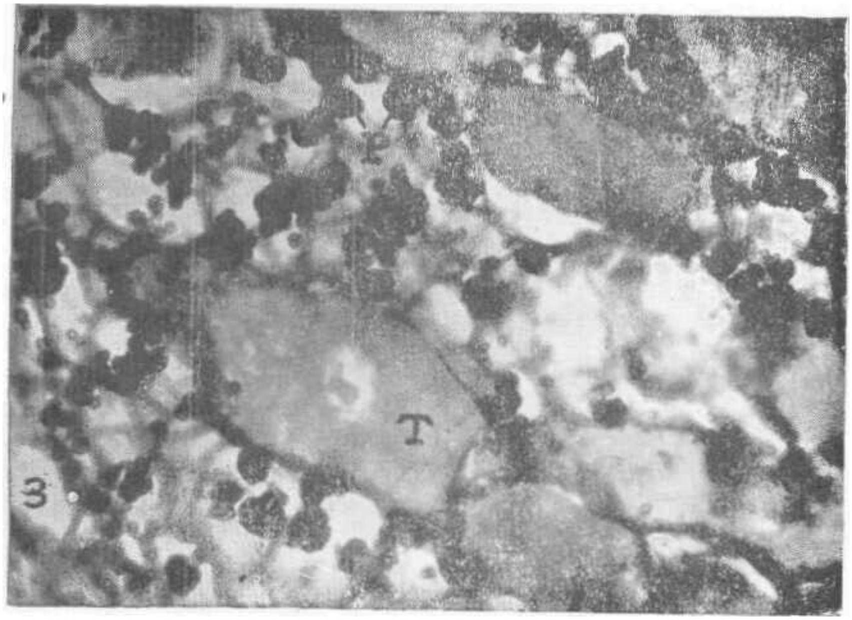
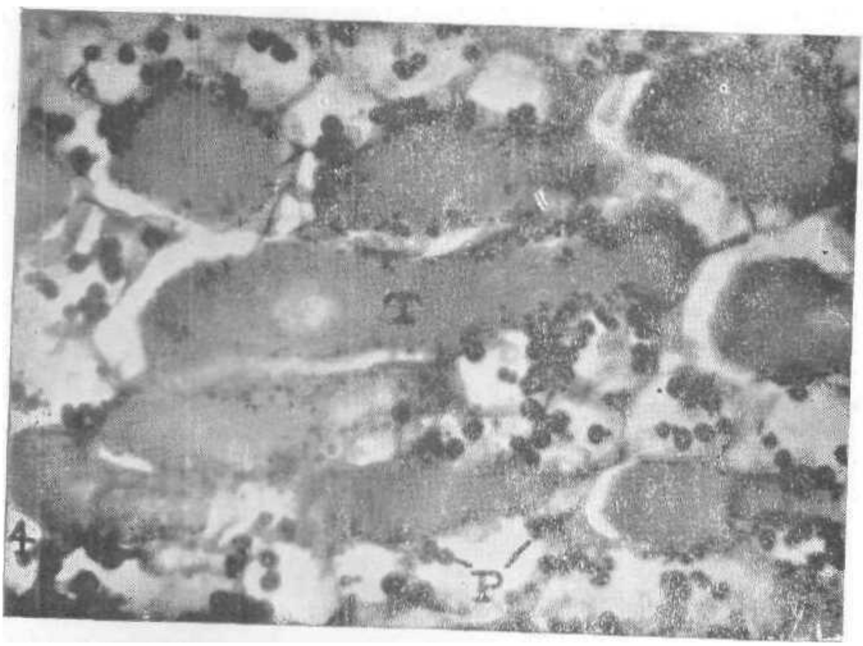
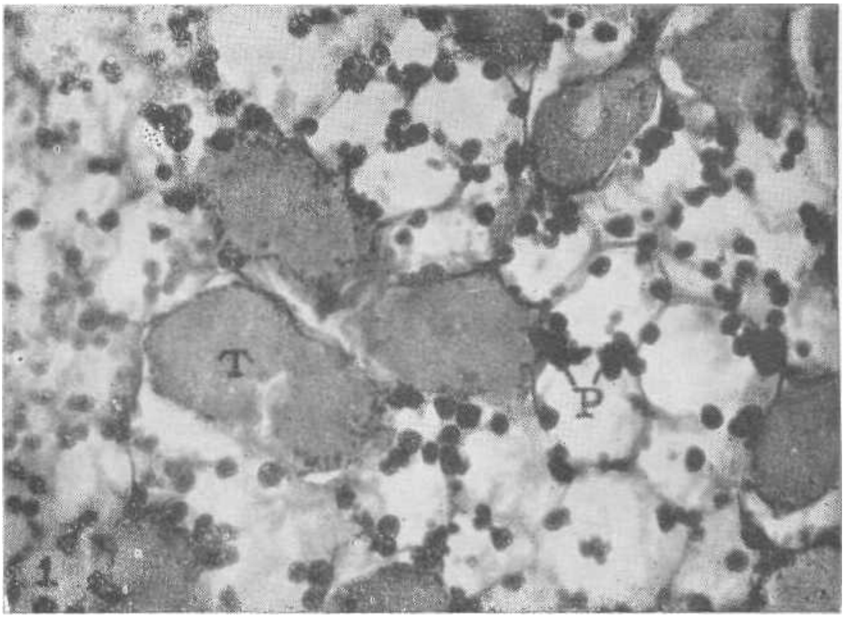




Rate 3 and 4. ' '*Janus cajan* root nodule sections stained for poiysi . V. A. mycorrhiza alone. 4. Dual inoculation = Polv 'ide, T = transformed nodule cells containing bacteroids x 500)



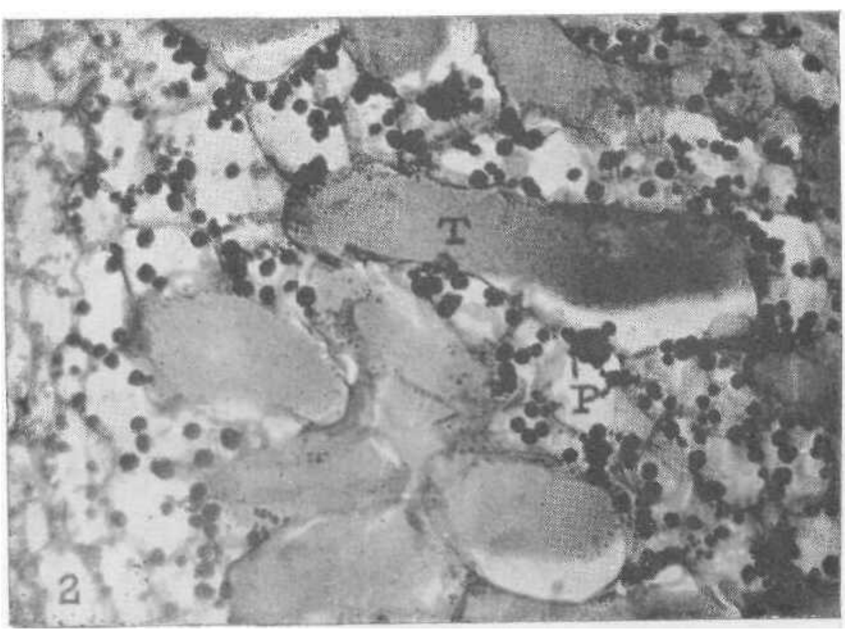


Plate 1 and 2. *Cajanus cajan* root nodule sections stained for polysaccharides. 1. No *Rhizobium* and V. A. mycorrhiza inoculation. 2. *Rhizobium* alone (P=polysaccharide, T=transformed nodule cells containing bacteroids x 500)

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