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# MECHANISM OF ENHANCED NODULATION IN VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) PIGEONPEA *CAJANUS CAJAN* (L.) Millsp.

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It is well documented that dual inoculation with *Rhizobium* and vesicular arbuscular mycorrhizal (VAM) fungi enhances the nodulation and nitrogen fixation in legumes (Crush, 1974; Sivaprasad *et al.*, 1983). This increase in nodulation is generally attributed to improved soil nutrient uptake, particularly phosphorus, conferred by mycorrhizal association (Carling *et al.*, 1978; Asimi *et al.*, 1980). However, no information is available about the exact mechanism of enhanced nodulation in tripartite symbiosis of legume, VA-mycorrhiza and *Rhizobium.* Normally rhizobia trigger nodule initiation through the production of auxins and cytokinins. Root cortical cells which receive adequate amount of auxins and cytokinins starts multiplying. Thus the nodule initiation takes place. Whether or not the cell divide in direct response to the rhizobial trigger depends on their position within a pre-existing transverse gradient system of stimulatory host factors; probably nutrient and/or cytokinin. The failure of an infection thread to induce cell divisions may be due to quantitative variation of the gradient along the root and/or inadequate concentrations of auxins and cytokinins (Libbenga *et al.*, 1973; Libbenga and Harkes, 1973). Influence of VA-mycorrhizal association on these factors is reported here.

## Materials and Methods

Pots of 30 cm diameter were filled with 10 kg of P deficient unsterile Alfisol soil with 50 percent sand (pH 5.6, 0.3 *M* $NH_4F$+HCl extractable P 2.4 ppm) for raising the crop. Surface sterilized spores of *Glomus fasciculatum* extracted from guinea grass (*Panicum maximum)* rhizosphere served as mycorrhizal inoculum. Fifty ml of water suspension containing 12 spores/ml was poured 5 cm below the soil surface and covered with a layer of soil before sowing the seeds. Selfed seeds of pigeonpea (variety Pusa Aganthi) were sown over the mycorrhizal inoculum and 0.5 ml of the broth culture of *Rhizobium* strain IHP 100 was added over it. Four treatments viz., (i) no inoculation (c), (ii) *Rhizobium* alone (R), (iii) VAM fungusalone (M) and (iv) dual inoculation (RM) were included in the study.

The number of lateral roots and nodules initiated in a specific area, at the collar region i.e., 8 cm of the tap root, and 1 cm of all the lateral roots formed from 8 cm tap root, before the mycorrhizal colonization (11th day) and affer

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the formation of effective mycorrhizal association (25th day) was recorded, using stereomicroscope. Mycorrhizal observations were made following the procedure of Phillips and Hayman (1970).

Cytokinin activity of root and stem exudate of dually inoculated and *Rhizobium* alone inoculated plants was estimated on 20th and 40th day by the cucumber cotyledon bio-assay following Udayakumar and Krishnasastry (1973). Cytokinin activity was computed from standard curve obtained for benzyladenine.

## Results and Discussion

Root initiation study indicated no significant difference among the treatments in number of lateral roots initiation in the specific area subjected to the observation (Table 1). The number of nodules initiated upto 11th day in this region was significantly high in dual and *Rhizobium* alone inoculation treatments. On 25th day, dually inoculated plant recorded 27 nodules as against 13.2 of *Rhizobium* alone inoculation. Although there was a notable increase in the number of nodules initiated between the days 11 and 25 in plants inoculated with mycorrhiza and *Rhizobium,* no difference was noticed in root initiation. This could be probably because of the different physiological basis existing for nodule and root initiation (Libbonga and Harkes, 1973).

Mycorrhizal association was noticed from 12th day onwards in inoculated plants, while it took 23 days in uninoculated plants. Mycorrhizal infection per cent was 42 on 25th day in dual inoculation. VAM fungal structures like vesicles, arbuscules, mycelium etc. were present predominantly in cortical region of the root (Fig 1). Further, the nodule initiation and VAM fungal colonization were taking place simultaneously, as mycorrhiza was noticed as early as 12th day. In the present study the rhizobial infection and infection thread formation should have been uniform in dually and *Rhizobium* alone inoculated plants, since the number of roots initiated and rhizobial inoculum density were similar. When the rhizobial infection and infection thread forming process were going on, VAM fungus was also entering the root and this might have led to enhanced nodule formation as noticed on 25th day. Thus mycorrhizal association increased the success of nodule initiation.

Phosphorus (Gates, 1974), phytohormones like auxins and cytokinins (Libbenga *et al.*, 1973; Libbenga and Harkes, 1973) are essential for nodule initiation Improved soil nutrient uptake, particularly phosphorus, through enhanced soil exploration due to VA mycorrhizal association has been well documented (Sanders and Tinker, 1971; Hayman and Mosse, 1972).

Cytokinin content of the root of plants inoculated with VAM fungus and *Rhizobium* (1.3 $\mu$g/10 g) was higher than that of *Rhizobium* alone inoculation (0 84 $\mu$g/10g) on 20th day of plant growth. Similarly the total cytokinin activity was higher in the stem exudates of mycorrhizal plants (Table 1). Enhanced cytokinin activity due to mycorrhizal colonization has been reported in *Boutelous gracillis* (Allen *et al.*, 1980).

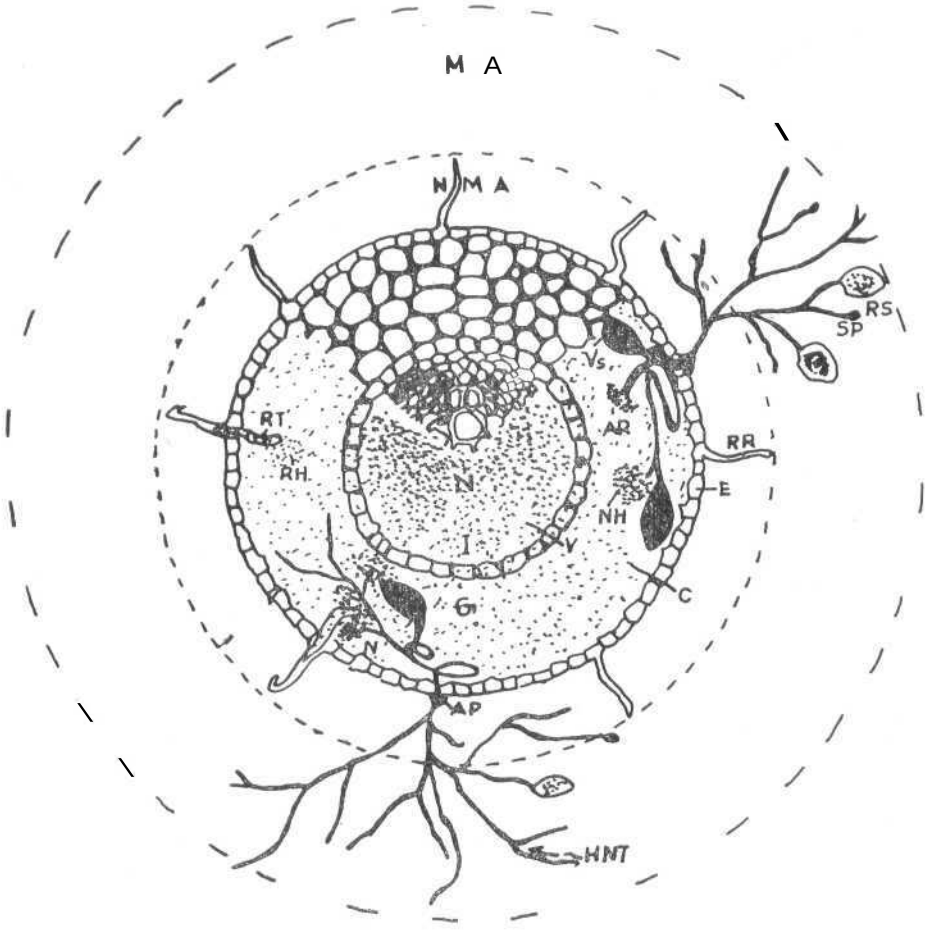


Fig. 1 Diagramatic representation of VAM fungus and *Rhizobium* interaction in nodule formation

| | | | |
|---|---|---|---|
| MA | Mycorrhizal absorption zone | RR | Root hair |
| NMA | Non-mycorrhizal absorption zone | E | Epidermis |
| RS | Resting spore | C | Cortical region |
| SP | Secondary spore | NH | Nutrient and (hormone) release by VAM in NIG |
| VS | Vesicle | V | Vascular region |
| AR | Arbuscule | | |
| NIG | Nodule initiation factor gradient | HNT | Hyphal nutrient translocation |
| AP | Appressorium | N | RH+NH |
| RH | Rhizobial hormone | RT | Rhizobia enfolded in the infection thread |

Table 1

Effect of dual inoculation of *Rhizobium* and mycorrhizal fungus on root and nodule initiation and cytokinin content of *Cajanus cajan*

| Treatments | Observation on 11th day at collar region (8x1 cm) | | Observation on 25th day at collar region (8x1 cm) | | Cytokinin content 20th day | | Cytokinin content 40th day | |
|---|---|---|---|---|---|---|---|---|
| | No. of roots | No. ot nodules | No. of roots | No. of nodules | Root (μg/ 10 g) | Stem Exudate (μg/) 2 pl/3h | Root (μg/ 10 g) | Stem Exudate (μg/) 2 pl/3h |
| RM | 35.2 | 7.0 | 50.00 | 27.0 | 1.3 | 0.11 | 0.17 | 0.12 |
| M | 35.8 | 3.2 | 52.00 | 7.4 | — | — | — | — |
| R | 36.2 | 6 2 | 49.00 | 13.2 | 0.84 | 0.06 | 0.13 | 0.09 |
| C | 35.8 | 3.6 | 53.00 | 6.2 | — | — | — | — |
| CD (0.05) | NS | 1.79 | NS | 3.63 | NA | NA | NA | NA |

NS = Not significant
NA = Not analysed

Rhizobia trigger cell division (nodule initiation) by increasing the concentration of auxins and cytokinins in the cortical cells to an optimum level (Libbenga *et al*, 1973). When mycorrhizal association exists, extra cytokinin and other unknown factors may be produced. This may add to the pool of hormones (rhizobial) and other factors involved in the nodule initiation. Thus concentration of these factors may increase sufficiently so as to trigger the cell division and hence nodule initiation (Fig 1). However, it needs to be confirmed with better technique whether VAM fungus releases phytohormones in root cortical region.

Host control over nodule initiation is through a pre-existing transverse gradient system of unknown host cell division factor (s); probably nutrient and cytokinin (Libbenga *et al*, 1973). In addition to increase in cytokinin production VAM fungi observed in the root cortical region transfer nutrient and other unknown factors in the root cortical region (Fig 1). This gives an evidence that association of VAM fungi tilts the physiological control of host on nodule initiation. Hence, it could be hypothesized that in tripartite symbiosis VAM fungi enhance the success of nodule initiation through enhanced hormone production and altered host physiological control on nodule initiation. This is the first hypothesis proposed on the mechanism of enhanced nodulation in mycorrhizal legumes.

## Summary

Nodule and root initiation in *Cajanus cajan* plants inoculated with vesicular—arbuscular mycorrhizal fungus and *Rhizobium* was studied Mycorrhizal association enhanced the success of nodule formation with no significant difference in number of root initiated in a specific area. Further, there was increased cytokinin

activity in stem exudate and root of mycorrhizal plants. Based on the observations a hypothesisis proposed for the mechanism of enhanced nodulation in mycorrhizal legumes.

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