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Population dynamics of VAM-fungi in temperate soils of Himachal Pradesh

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Abstract : Representative rhizosphere (0-15 cm) soil samples were collected from different locations/land uses(s) in Himachal Paradesh and efficient isolates of VAM strains (*Glomus* spp.) were subjected to VAM spore count and root infectivity studies in maize and soybean crops in a pot experiment. Maximum spore count (200 per 250 g soil) and root infectivity (38% in maize, 40% in soybean) were observed in soils of vegetable fields of Sihunta area of Chamba district as against the lowest VAM spore count and root infectivity in soils of cereal based-cropping systems.

Additional key words: *VAM, maize, soybean, biofertilizers, acid soils.*

Introduction

About 82 per cent of the arable area in Himachal Pradesh is rainfed. Maize is one of the most important *Kharif* crops in the acid soils of the region and it is commonly grown under rainfed conditions. Soybean is now gaining popularity as a rainfed crop. The soils of hilly region of Himachal Pradesh are phosphorus deficient due to their acidic nature. These soils have high phosphorus fixing capacity due to the presence of Fe and Al ions. The Vesicular-Arbuscular Mycorrhizae (VAM) are potential fungi in mobilizing phosphorus to the crops. The preparation of an efficient mycorrhizal biofertilizer for phosphorus deficient acid soils of Himachal Pradesh would be a boon for sustainable mountain agriculture. An extensive VAM resource survey of acidic soils of wet temperate zone of Himachal Pradesh was, therefore, carried out to isolate the efficient VAM strains on the basis of spore count and infectivity studies.

Materials and Methods

VAM resource survey of wet temperate soils of Himachal Pradesh was undertaken during 2001-2003. Six hundred fifty rhizosphere soil samples (0-15 cm) were collected from different locations/soils/land uses having varied crop management practices.

i) VAM spore isolation and spore count

The VAM fungal spores (chlamydospores) were isolated from collected soil samples following standard wet sieving and decanting procedure (Pacioni, 1992). It involved weighing out 250 g of the given soil into a glass beaker, contents of which were stirred constantly. The majority of the chlamydospores floated on the surface of water soon.

The soil suspension thus prepared was passed through a series of sieves having 1000 to 100 meshes. The sieves of 1000 to 500 mesh sizes retained the coarse refuse/ particles whereas those from 500 to 100

mesh size sieves retained the chlamydospores. The VAM spores retained by subsequent sieves as mentioned above were collected separately in various beakers making required washings with distilled water. From above, the most probable number of spores was worked out employing standard procedure. The identity of VAM (*Glomus* spp.) fungi was established by observing temporary mounts under dissecting and compound microscope. The identified spores of *Glomus* spp. were multiplied on their respective host as pure culture for further studies.

ii) Root infectivity studies

The spore propagules of the VAM fungi (*Glomus* spp.) isolated from different rhizosphere soils were inoculated to maize and soybean crops. For the above tests, field soils were collected in bulk amounts followed by removal of coarse fractions through dry sieving. After sieving, the soil was prepared sandy loam soil by mixing soil, sand and farm yard manure (3:1:1) and packed into 2 to 5 kg capacity cotton bags for utoclaving twice at 15 lbs/psi for one hour, each time. The autoclaved sterilized sandy loam soil was then filled in small surface sterilized pots (4" height x 4" internal diameter) with $HgCl_2$ (0.02%) and also surface sterilized seeds of maize and soybean with $HgCl_2$ (0.02%) were sown. The VAM spore propagules separated from 250 g soil were taken from maintained pure culture of VAM on respective host and suspended in a beaker containing water (served as the VAM inoculum). The pots were then placed under natural environmental conditions in a cage house and watered/maintained as required.

Table 1. VAM resource survey of wet temperate zone of Himachal Pradesh (Kangra district)

Villages	Crops/Land use	No. of spores per 250 g of soil	Root infectivity in maize (%)	Root infectivity in soybean (%)
Kangra district				
Lower Banoori	Vegetable area	160	32	30
Draman	Maize harvested, manuring practice	160	18	16
Jhilki Bhet	Grass-wheat cropping system	200	14	20
Utarala	Maize-wheat cropping	150	16	16
Ehju	Soybean harvested fields	160	25	24
Billing	Wheat grown earlier. But at the time of sampling fallow land.	100	22	20
Bir	Maize harvested fields	120	20	22
Gopalpur	Maize-wheat cropping. FYM & 12:32:16 fertilizer @ 5 kg/manal are added	120	20	22
Bhagsu Nag	Maize-barley cropping. FYM alone is added	150	22	24
Barla	Maize-vegetable fields. FYM and 12:32:16 mixed.	160	28	30
Darang	Maize-wheat cropping. Apply FYM only.	150	30	25
Malan	Vegetable fields. Apply FYM only.	240	24	24
Pathiar	Maize-potato fields. Apply FYM only.	240	16	16
Thrind	Maize-Toria/potato/vegetable fields. Apply mainly FYM and a little amount of urea and 12:32:16 mixed fertilizer.	170	18	20
Upper Banoori	Maize-vegetable fields. Apply FYM only.	200	22	20
Basaal	Maize-wheat cropping. Apply FYM, urea and 12:32:16 mixed fertilizer.	200	28	29
Thanpuri	Maize-vegetable fields. Apply FYM only.	150	25	22
Dnamnala	Maize-wheat cropping system.	120	10	8
Jongta	Maize-wheat cropping system.	110	18	18

Chamba district				
Barahi	Maize-wheat cropping system, FYM and urea @ 30 kg N ha^{-1}	120	26	24
Panchpula	Maize-wheat cropping system	90	28	30
Banikhet	Pasture land	80	20	30
Mangla	Garlic sown field	90	10	12
Mangla	Maize-wheat cropping system, FYM applied	95	20	24
Gajnoi	Maize-wheat cropping system	100	18	20
Khajiar	Vegetable fields	160	24	20
Khajiar	Maize harvested barley sown	130	20	20
Salooni	Maize harvested un-sown field	75	10	8
Surangani	Maize-wheat cropping sequence in apricot orchard	110	12	8
Sundla	Wheat-sarson intercropping	125	13	10
Sihunta	Maize-wheat cropping system. Apply FYM only	160	19	18
Sihunta	Vegetable area (Kitchen garden)	200	38	40
Mandi district				
Urla	Maize-wheat cropping system. FYM added	95	10	8
Sambal	Maize-wheat cropping	95	8	12
Rassog	Wheat sown	115	16	16
Kanda	Pea-potato cropping system	190	20	22
Baggi	Wheat planted field	170	22	24
Randhera	Wheat sown field	160	28	26
Ghatasani	Maize-wheat cropping system	150	18	16
Tikkam	Maize harvested unsown fields (A bit gravelly)	80	18	20
Diyot	Vegetable fields (Chilli)	150	22	18
Multhan	Maize/potato-barley cropping system. Apply 12:32:16 mixed fertilizer and urea	160	20	22
Hamirpur district				
Kuthera	Maize-wheat cropping system	140	14	20
Bilaspur district				
Berthin	Pea fields	190	20	24
Shimla district				
Sunarbhati	Cabbage field, FYM used	180	28	32
Kumarsain	Wheat field	80	12	10
Kullu district				
Banjar	Maize-garlic cropping system in apple orchard	170	22	28
Chalog	Pea fields, FYM used	180	24	28
Bandrolla	Vegetable fields	195	20	22

* *Figures in per cent are transformed angular values*

In order to find out the potential of VAM fungi in inducing root infection, the plants grown in pots was detached after 8-10 weeks. The plant roots were cleaned, chopped into small pieces and then subjected to fixation, cleaning, rinsing and bleaching in KOH solution following standard techniques for microscopic observations (Rajapakse and Miller 1992). In case of infected roots, the presence of VAM fungi fortifications was observed. On the basis of root cuts (1 cm size), infected and uninfected, the degree of root infectivity was worked out in terms of percentage. The infection causing rhizosphere soil samples and respective VAM spore count and root infectivity data were recorded. On the basis of spore count and root infectivity status, the most efficient VAM strains were finally screened out for developing an efficient local mycorrhizal biofertilizer. The percentage root colonization by the VAM was determined using Nicholson's formula (1960), given below :

$$\text{Colonization per cent (\%)} = \frac{\text{Number of positive colonized segment with VAM}}{\text{Total number of positive colonized segment with VAM}} \times 100$$

Results and Discussion

i) VAM isolation and spore count

From the resource survey conducted in the field and the actual laboratory studies, *Glomus* spp. were found to be the pre-dominant rhizosphere soil samples of wet temperate zone of Himachal Pradesh (Table 1). The maximum spore count was observed in vegetable field of Malan (240) followed by green grassy fodder-wheat cropping system at Jhikli Bhet (200), maize-vegetable system fields at Upper Banoori (200), maize-wheat cropping at Basaal (200) and vegetable soils at Sihunta (200) supplied with FYM as organic manure input. Minimum spore count was observed under mycorrhizal soil samples from maize harvested rabi unsown fields at Salooni (75), Tikkam (80) and Kumarsain (80). The spore count for rest of the soil samples varied from 100-170/250 g soil. It is noticed that organic matter rich vegetable soils and FYM amended soils resulted in high VAM fungus spore population owing to improvement in soil physical and biological properties. The results are in close conformity to those reported by Galvez *et al.* (2001).

ii) Root infectivity studies

The relevant information about rhizosphere mycorrhizal soil samples is presented in table 1 and 2. The root infectivity studies were carried out as per the procedure outlined by Rajapakse and Miller (1992). Maximum root infectivity (38% in maize, 40% in soybean) was recorded in case of mycorrhizal soil from vegetable fields of Sihunta area of Chamba district followed by vegetable fields of Lower Banuri of Kangra district. It is concluded that organic matter rich soils (vegetable fields) having high VAM fungi spore population resulted in higher VAM infectivity in roots of both maize and soybean crops owing to improvement in soil physical and biological properties due to addition of organic matter (Galvez *et al.* 2001).

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