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Association and diversity of Arbuscular Mycorrhizal Fungi in potato of Gwalior Chambal region of India

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ABSTRACT: The present study was undertaken to evaluate the AMF association in three potato varieties Kufri Sindhuri (KS), Kufri Chipsona-3 (KC-3) and Kufri Lauvkar (KL) grown at the Central Potato Research Station, Maharajpura, Gwalior, (M.P.), India. Sampling of soil and roots was done fortnightly from sowing to harvesting. Spores were found both in the rhizospheric and bulk soils of potato varieties. The spore density was maximum in rhizosphere soil than the bulk soil. Root colonisation and spore density were observed higher in Kufri Lauvkar variety followed by Kufri Sindhuri and Kufri Chipsona-3 respectively. Following 105 days after planting, the AMF spore density in soil peaked. Spores predominantly belong to five genera of *Glomus, Acaulospora, Gigaspora, Scutellospora,* and *Entrophospora* when both the rhizo and bulk soil were mixed. *Glomus* sp. showed dominance in both rhizo and bulk soils, around the three potato varieties.

Key words : Potato, AMF, Rhizosphere, soil, *Glomus*.

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) are now established plant root symbionts and have widespread symbiotic association with the roots of plants belonging to angiospermophyta, pteridophyta, bryophyta and coniferophyta. Except for plant species mainly belonging to the families cruciferae, chenopodiaceae, cyperaceae, caryophyllaceae and juncaceae, which do not show AMF association, almost all other plant species are known to be associated with AMF (Harley and Smith, 1983; Smith and Gianinazzi-Pearson, 1988; Azcon-Aguilar and Bago, 1994). AMF belong to class Zygomycetes with order Glomales, and families Glomaceae (G*lomus* and *Sclerocystis*), Acaulosporaceae (*Acaulospora* and *Entrophosphora*) and Gigasporaceae (*Gigaspora* and *Scutellospora*) (Morton and Benny, 1990).
Symbiosis between AMF and the plant involves a mutual sharing of assimilated carbon from the plant to AMF in exchange for soil-derived nutrients from the AMF. Extensive networks of mycelia external to roots in the soil enables the AMF to take up and subsequently translocate nutrients to inside roots through arbuscules and hyphal mass into the plant. The hyphal networks help increase the fungal surface area thereby increasing availability of soil nutrients to the host plant (Smith and Read 2008). Improved water relations by AMF association can be another functional benefit to the host plant (Auge, 2001).

Inventory of AMF biodiversity within agricultural soils is still wanted (Singh and adholeya, 2013). AMF being obligate symbionts, improve plant growth by absorbing and also increasing available phosphorous to the plant. They also improve element absorption of N, K, Ca, S, Cu, and Zn from the soil to the plant (Jiang *et al.,* 2013). AMF also produce glomalin (Guo *et al.,* 2012); which is now known involving enhance salt (Evelin *et al.,* 2009); heavy metals, and drought stress tolerance (Li *et al.,* 2011) and in the

regulation of synthesis and distribution of plant hormones (Barker and Tagu, 2000). AMF are therefore, important for sustainable farming due to their efficient nutrient availability to the plants. This they achieve by freeing nutrients bound to soil particles and/or organic matter. Agricultural plants which are already known to be benefiting from AMF association include maize, potato, sunflower, wheat etc. especially when these crops are under conditions where availability of nutrient concentration could be limiting for the plant growth (Halder *et al.,* 2015).

Potato (*Solanum tuberosum* L.) is a tetraploid herbaceous perennial plant belonging to the family solanaceae. Main propagule is a tuber which is an underground modified stem. The tuber in itself constitutes the major edible crop of the world. Wu *et al.,* (2013) reviewed that the potato plants have a low root colonisation and spore density and that phosphate fertilisation suppressed AMF colonisation in potato. Different phosphorus concentration may affect the root colonisation in maize plant. The level of phosphorus and root colonisation are inversely related (Naghashzadeh *et al.,* 2013).

Present study was planned to focus on the status of occurrence, root colonisation and AMF spore diversity in the rhizospheric and bulk soil around roots of three potato varieties grown in Gwalior Chambal region of central India.

MATERIALS AND METHODS

Soil samples were collected from at the Central Potato Research Station, Maharajpura, Gwalior, (M.P.), India from different soils where potato cultivars Kufri Sindhuri, Kufri Chipsona-3 Kufri Lauvkar were grown. Samples were taken from a depth down to approximately 12 cm, and then stored at 4^0C in a refrigerator till subjected to analysis, specifically for spore isolation. Soil closely clinging to the roots constituted rhizospheric soil and the soil 5-10 cm around roots the non-rhizospheric or bulk soil in this study.

Roots from each potato variety were used for observing colonisation in them. The method as described by Phillips and Hayman (1970) and later modified by Kormanik *et al*., (1980) was employed for root clearing and staining. Tender roots at the deepest to middle level were selected randomly from the plants of each variety and then cut into 3cm segments. After washing thoroughly with water, roots were cleared in 10% KOH over a water bath for 15-20 minutes at 100 ^{0}C and time was determined according to the tenderness of roots.

Cleared roots were rinsed 4-5 times with distilled water and transferred to 1% HCl for 5 minutes. The solution was decanted. The cleared roots were then placed overnight in 0.05% trypan blue staining solution and subsequently destained 1-2 times in a glass petri plate containing destaining solution of 50% lacto glycerol. The cleared roots were cut into one cm segments and placed on clean glass slides and followed by mounting in 1% (v/v) glycerol. Once mounted, the excess mountant was drained out and subsequently covered with an another clean slide. 10 pieces of cleared roots were mounted on each glass slide. Ten slides were prepared per sample and observed under a compound light microscope. The extent of root colonisation was quantified by using frequency distribution of Biermann and Lindermann, (1981). It was assessed as proportion of root length colonised by mycorrhizal fungi. Slides were observed under compound microscope for locating any of the AMF associated structures such as hyphae, vesicles or arbuscules.

Per cent root colonisation was calculated using following relation:

$$\text{Percent root colonisation} = \frac{\text{Total number of colonised roots}}{\text{Total number of roots examined}} \times 100$$

Separation of AMF spores from the soil was achieved by wet sieving and decanting method of Gerdemann and Nicolson (1963). 100 gm rhizosphere soil was suspended in 1000 ml of tap water. The mixture was stirred for half an hour and the heavier soil and sand particles were allowed to settle down to the bottom. The soil water mixture was decanted through sieves of descending mesh in the order of 240µm, 120µm, 60µm and 30µm. Top sieve captured roots and debris. Debris from the remaining sieves was collected separately in beakers. Spores were collected in a petridish of 10 cm diameter and were examined under leica stereo-microscope.

For the quantification of AMF spore density the protocol as proposed by The Energy and Resource Institute (TERI), New Delhi, was employed (Gaur and Adholeya, 1994). The girded petri plate along with the sievate from each sieve was examined under stereo microscope. Spore density was calculated as the total number of AMF spores recorded in all petri plates. Quantification was carried out in 10 cm dia petri dishes with a girdline of 1 cm square under a stereo microscope at 50x (Lugo and Cabello, 2002).

Ten divisions were counted and related to the total number of spores by using the modified method of McKenney and Lindsey (1987). The surface sterilisation of spores was done by transferring these to the sterile water in a petri plate, using micropipette for picking, and then transferring these for 2-minutes these to an another petri plate containing 0.05% cetrimide solution. After a thorough washing 5 to 6 times with sterile distilled water, different types of spores were collected with the help of a micropipette and mounted in a drop of polyvinyl lactoglycerol (PVLG) on a glass slide (Omar *et al.,* 1979; Koske and Tessier, 1983) with or without Melzers reagent (Morton, 1988). Cover slip of one of the slides was gently pressed to break open the spores and observed under a compound microscope.

The spore identification was done using manual of Schenck and Perez, (1990) after comparisons with original spore descriptions and reference isolates from the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi. AMF spores were identified according to their morphology and wall characters (Walker and Treppe 1993; Wu, 1993; Schenck and Perez, 1990; Morton and Benny, 1990).

RESULTS

In the early stages of growth till 30 days after planting (DAP) of the tuber propaugle only hyphae were found in root segments of all the three varieties of potatoes. In Kufri Sindhuri (KS) arbuscule were found after 45 DAP. In varieties Kufri Chipsona-3 (KC-3) and Kufri Lauvkar (KL) arbuscule were seen after 60 and 105 DAP stage respectively. Vesicle like structures were seen both in KS and KC-3 at 75 DAP (Figs 1 and 2). In KL however, vesicle like structure were seldom seen. Root colonisation in all three potato varieties initiated between 15 and 30 DAP. Percentage colonisation was 23, 20 and 26 which peaked between 90 and 105 DAP to 66, 34 and 76 in KS, KC-3 and KL respectively (Table 1).

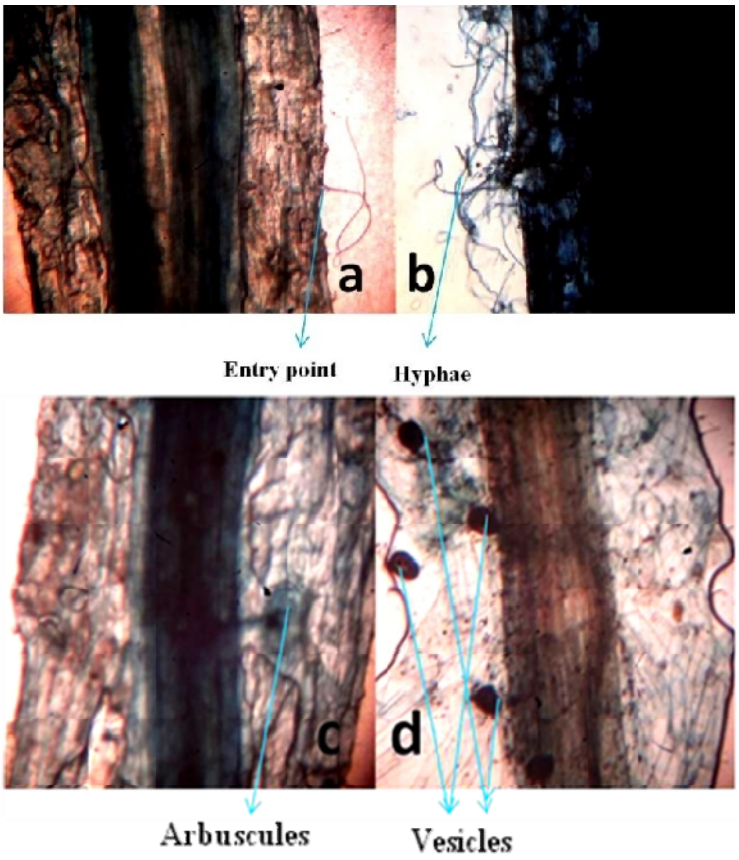


Fig. 1. Potato roots showing presence of Arbuscular Mycorrhizal Fungi and various structures associated (a=Entry point, b=Hyphae, c= Arbuscules, d=Vesicles).

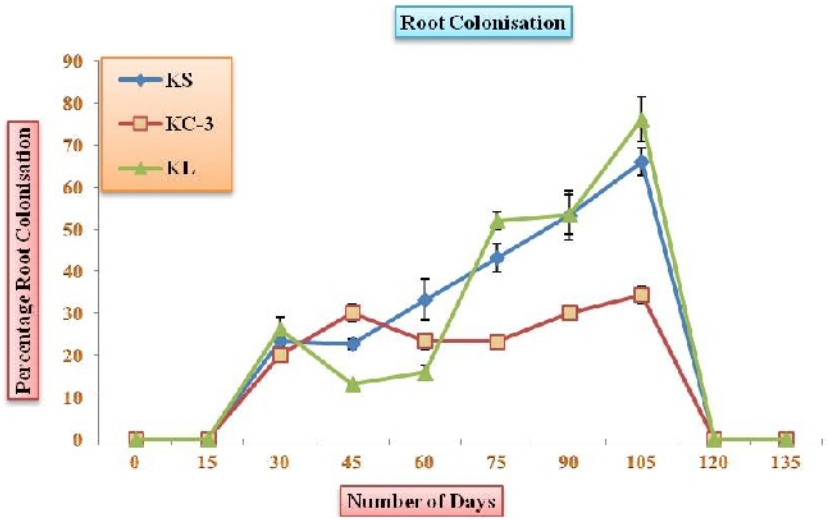


Fig. 2. Percentage of root colonisation in the rhizospheric soil of potato by AMF with increasing days of the plating.

Table 1: Percentage root colonisation and spore density in the rhizo and bulk soil in the three potato varieties.

Days after planting (DAP)	Potato varieties								
	% root colonisation of rhizo soil			Spore density (100^{-1} gm soil)					
				Rhizo soil			Bulk soil		
	KS	KC-3	KL	KS	KC-3	KL	KS	KC-3	KL
0	0±0	0±0	0±0	1.43±0.07	2.03±0.07	2.46±0..02	1.03±0.01	1.93±0.04	1.46±0.33
15	0±0	0±0	0±0	14.16±1.82[a]	20.27±1.17[a]	30±1.55[a]	2±0.57	1.96±0.33	2.16±0.33
30	23.34±1.46[a] (H)	20±1[a] (H)	26.22±2.71[a] (H)	41.34±1.86[a]	46.53±3.8[a]	50.23±2.89[a]	5.34±0.88[a]	5.19±1.2[a]	6.56±0.88[a]
45	22.67±1.21[a] (H,A)	30±2.02[a] (H)	13.13±0.34[a](H)	30.67±1.73[a]	33.16±2.18[a]	40.56±1.21[a]	4.14±1[a]	5.32±0.88[a]	5.44±1.2[a]
60	33.13±4.85[a] (H)	23.34±1.82[a] (H,A)	15.81±1.86[a] (H)	27.34±1.21[a]	54.14±2.03[a]	33.17±1.3[a]	2.02±0.57	2.15±0.57	2.11±0.57
75	43.15±3.34[a] (H,V)	23.19±0.7[a] (H,V)	52±2.03[a] (H)	18±1.74[a]	20.22±1.92[a]	22.88±1.92[a]	1.11±0	1.31±0.33	2.87±0.57
90	53.28±5.9[a] (H)	30.06±1.67[a] (H)	53.44±4.7[a] (H)	22.76±1.49[a]	23.55±1.1a	26.73±2.34[a]	3±0.57[a]	2.99±0.33	2.67±0.88
105	66.04±3.34[a] (H,A)	34.37±1.94[a] (H)	76.11±5.46[a] (H,A)	48±2.47[a]	45.12±2.25[a]	68.14±3.49[a]	6.54±0.88[a]	5.73±1.2[a]	8.88±1.2[a]
120	0±0	0±0	0±0	30±2.78[a]	42±1.59[a]	35.81±2.49[a]	2.16±0.41	3.1±0.57[a]	3.07±0.88[a]
135 (post harvesting)	0±0	0±0	0±0	28.09±1.34[a]	44.05±0.93[a]	30.67±2.34[a]	2.30±0.21	3.0±0	2.36±0.33

Data Represent Mean ± SEM, whereas 'a' represents significance ($p<0.05$). KS= Kufri Sindhuri, KC-3= Kufri Chipsona3, KL= Kufri Luvakar, H=Hyphae, A=Arbuscules, V= Vesicles, DAP= Days after planting.

AMF spores associated in the soil and in vicinity of potato roots showed diversity of colours ranging from just off white to yellow, orange, brown and black. Based on INVAM compares on the morphological basis, the spore species belonged to five genera *viz Glomus, Acaulospora, Gigaspora, Scutellospora,* and *Entrophospora* (Table 2). Yellow and orange colored spores always dominated amongst the entire array. In all three varieties of potato the spore density varied with soil pH which ranged between from 6.3 and 7.6. On observation from sowing to harvest, AMF spore density around the rhizosphere of each variety was 68, 48 and 45 in KL, KS and KC-3 respectively at 105 DAP (Table 1 and Fig. 3). The potato rhizosphere of all three varieties showed that the spore density trends with two peaks, one between 30 and 60 DAP and the other just before harvesting at 105 DAP. At final stages, before crop harvest, coinciding with the second peak, rhizosphere of KL variety had 41% more spore number per 100 g soil than the rhizosphere soil of KS and KC-3 varieties. The KC-3 variety rhizosphere however, showed nearly 50 percent higher number of spores per 100 g soil at the first density peak after 60 DAP (Table 1 and Fig. 3). Species of genus *Glomus* dominated and were being followed by the species of genus *Acaulospora* whereas, the species of *Gigaspora, Scutellospora and Entrophospora* were meagerly present in the potato rhizospheric soil (Table 2).

Table 2: Number and Distribution of Arbuscular Mycorrhizal Fungi (AMF) species.

S/N	AMF spices	Number of species		Distribution of AMF spices	
		Rhizo soil	Non-rhizo (bulk) soil	Rhizo soil	Non rhizo (bulk) soil
1	*Glomus sp*	6	3	a, b, c, d, e, f	a, f, g
2	*Aculospora sp*	2	1	i, j	k
3	*Gigaspora sp*	1	0	g	-
4	*Entrophosphora sp*	1	1	k	i
5	*Scutellospora sp*	1	0	h	0
	Total	**11**	**5**		

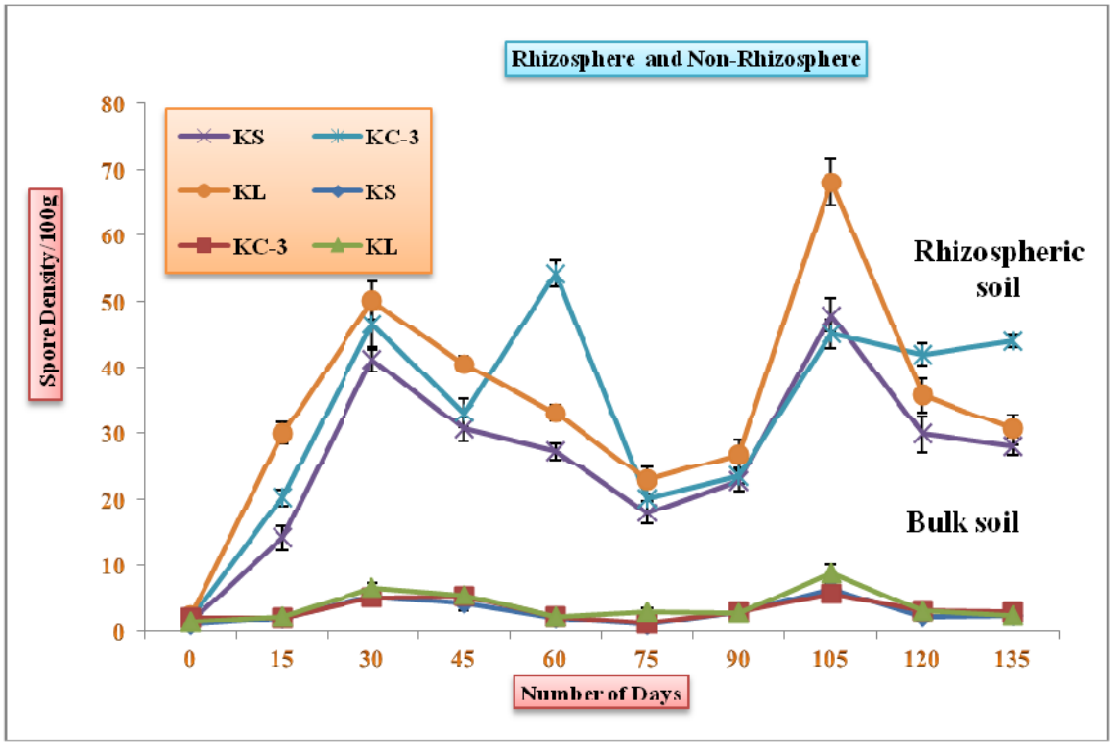
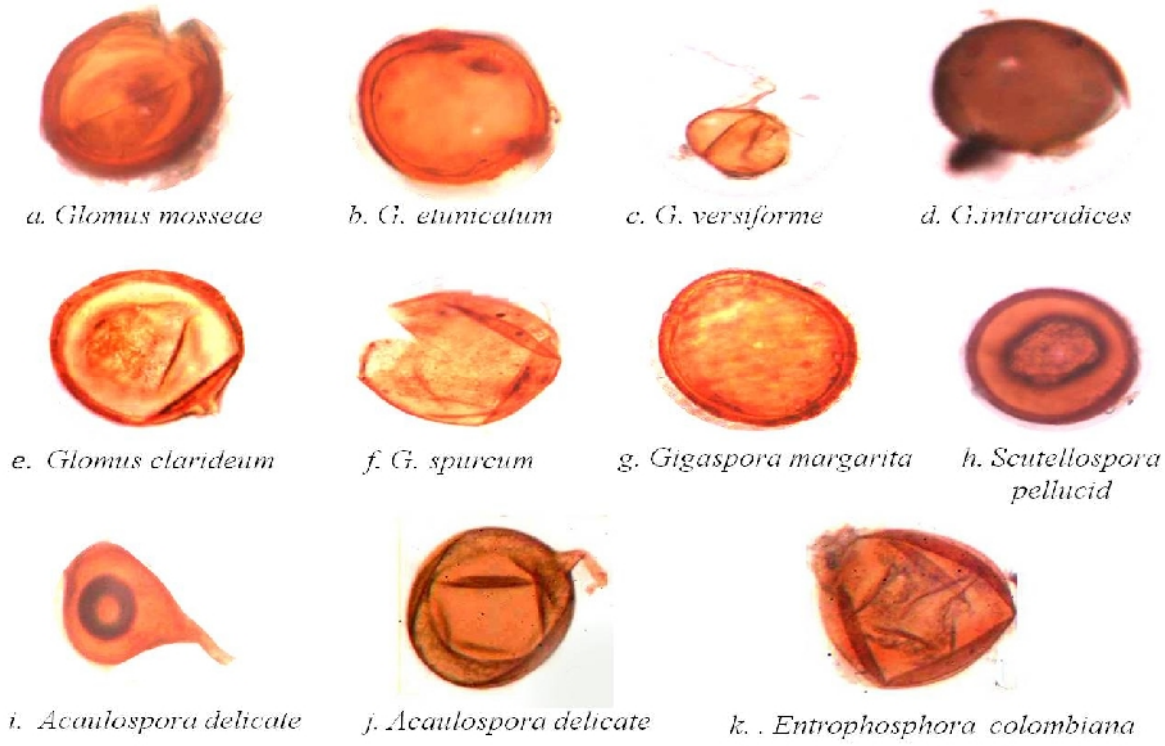


Fig. 3. Spore density of rhizosphere and non-rhizosphere (bulk) soils of three varieties of potato.

In non-rhizospheric (bulk) soil similar coloured spores, as in rhizospheric soil were observed. These seem belonging to the three genera *Glomus, Acaulospora,* and *Entrophospora*. Compared to rhizosphere, the bulk soil had extremely low number of AMF spores ranging from 1.03 to 6.54 in KS, 1.93 to 5.73 in KC-3 and 1.46 to 8.88 in KL per 100 g of soil. In all three varieties, the maximum spore density was observed at 105 DAP averaging to 6.54 in KS, 5.73 in KC-3 and 8.88 in KL. At 135 DAP, that is harvest, the spore number in bulk soil receeds to almost absence (Table 1 and Fig. 3). As far as trends are concerned, the non-rhizospheric spore number between the potato varieties have no similarities. However, the two peaks of higher numbers are similar to the rhizospheric stages.

At all stages the non rhizo bulk soils are poorly represented by AMF than their rhizospheric soils. This is true for all the three potato varieties and differences range between 10 to 25 fold between the two. Species of *Glomus* was dominant species in bulk soil too.

Based on morphological characterisation 11 species of AMF, were identified in rhizo and 5 in non rhizo (bulk) soil of potato plant. Species of genus *Glomus* were maximally present in rhizo soil and representing genera *Gigasopra*, *Scutellospora, Entrophosphora* were also identifiable, besides species of genus *Glomus*, and the species of genus *Acaulospora*, and *Entrophosphora* were also present in the non rhizo soil (Table 2; Fig. 4).



a. Glomus mosseae, b. G. etunicatum, c. G.versiforme, d. G.intraradices, e. G.clarideum, f. G.spurcum, g. Gigaspora margarita, h. Scutellospora pellucid, i.. Acaulospora delicate j. Acaulospora sporocarpia, k. Entrophosphora colombiana

Fig. 4. Some Arbuscular Mycorrhizal Fungi (AMF) spores isolated from the rhizo bulk soils of potato plants roots at various growth stages.

DISCUSSION

The study implies that the Arbuscular Mycorrhizal Fungi have a strong association with potato grown in the Gwalior-Chambal region of India. The association it seems is assured in the potato roots despite varied edaphic conditions. The presences of AMF, barring plants belonging to few angiosperm families is ubiquitous in the plant roots (Koul *et al.,* 2012). These are known to synergise the various growth and developmental parameters (Lone *et al.,* 2015). The observations here, are therefore, in conformity with other earlier studies where the presence of AMF in the potato plant rhizosphere are already reported (Baradar *et al.,* 2015; Wu *et al.,* 2013; Lakshmipathy *et al.,* 2012; Das and Kayang, 2010). These reports also pertain to various geographical regions of the world; hence being reports from diverse environmental conditions.

There are studies where AMF inoculations of the soil have led to the improved growth and development of the plants. In certain cereal crops the use of AMF inoculations are now becoming a common practice for better crop yield. Singh and Adholeya, (2013) reported the AMF diversity of wheat crop soils in India. Priyadharsini *et al.,* (2012) have inventorised the AMF associated with the *Allium cepa* crop. The dominance of *Glomus intraradices* amongst the AMF associated with potato was established by Cesaro *et al.,* (2008) and as also reported by others (Bhat *et al.,* 2014; Sheikh *et al.,* 2013; Das and Kayang 2010; Singh *et al.,* 2007). In this study too the genus *Glomus* and it species dominate both rhizospheric and the bulk soil which therefore, seems one of the adaptive convenience of this genus with potato. *G. intraradices* here shows highest spore numbers intra *Glomus* species. This condition is related to various other crops and also potato where *G. intraradices* is the dominant species compared to all other species of *Glomus* and plus those of other genera. Some workers explain this as a co-evolution of genus *Glomus* with plants over varied niches and therefore, applicable to association with potato too (Baradar *et al.,* 2015; Lone *et al.*, 2015; Cesaro *et al.,* 2008; Bharadwaj *et al.,* 2007).

Results show that in potato propagules the AMF hypha penetration is steady which becomes subsequently vigorous once the potato plant is established and shoots emanate from the propagules. However, the differentiation of other AMF structures in the roots *viz* arbuscules or vesicles seems to be cultivar oriented, since their presence in the roots varies with time after propagule plantation. Since AMF symbiotic association with plant roots is a mutuality of carbon skeleton sharing as photosynthates from the plant to AMF in exchange with the soil water carried nutrient enhancement. It therefore, is obvious that immediately after the potato plants are established, irrespective of the cultivars, the AMF hyphal establishment in roots is demonstrated. This function of symbiosis being primary, the secondary structures wait and vary in formation either due to both internal physiological responses of the cultivars or due to external factor like pH, soil conductivity or even mineral and other microbial organisations in the potato root vicinity. Such inferences for other crop plants are already available, for example that of Wu *et al*., (2013) saying that the level of mineral availability specifically phosphorus are inversely related to the AMF colonisation and more recently by Halder *et al.,* (2015).

Despite varietal differences in the extent of root colonisation or soil sporal density, there seems a similarity of trends. All the varieties show two peaks of spore densities commensurate with the beginning of the sharp shoot and root growth after plantation and the other at the time of underground tuber formation. Obvious inference could that these are two important stages in the life cycle of the potato plant when roots have to organise nutrients and translocate these maximally first for growth and development of the plant followed by the formation of underground tuber acting as the nutrient and photosynthate sinks. Once, the tuber formation has matured and the shoot root disfunction is organised before harvest, the living roots as remnants remain interestingly bereft of any AMF infestation. This seems an acquired character since all the cultivars follow this trend despite differences in the extent of AMF root colonisation at various stages of growth. Interestingly the presence of AMF hyphae has been reported in the potato peels also which is it seems a phenomenon of a post tuber differentiation (Lone *et al*., 2014).

Rhizo soil AMF, seem play dominant role in the root penetration as the spore density around potato roots almost all through, is 5 to 10 folds higher than the bulk non-rhizo soils.

Bulk soil spore density is almost negligible at the time of planting subsequently remains constant till the time of harvest.

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