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BIODIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN SWEET CHERRY (*PRUNUS AVIUM* L.) OF KULLU DISTRICT OF HIMACHAL PRADESH

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ABSTRACT
The present investigation was carried out to study the biodiversity of endomycorrhizal status in sweet cherry. Ten efficient indigenous AM fungi were isolated and characterized from various geographical locations of Kullu district of Himachal Pradesh, India. AMF spore population varied from 120.00 to 275.00 spores/50 g of soil and the percentage of root colonization were recorded between 37.96 to 52.00 per cent. The *Glomus* spp. showed 80 per cent frequency of occurrence, *Acaulospora* spp. showed 10 per cent frequency of occurrence, while the minimum occurrence of 5 per cent was shown by *Gigarpora* and *Scutellospora* spp. in soil. These AM spores were identified up to species level as *Glomus macrocarpum, G. mosseae, G. constrictum, G. fasciculatum, G. clarum, G. epigenum, Acaulospora scorbiculata, A. bireculata, Gigaspora albida, G. heterogama* and *Scutellospora pellucida*. Therefore, the superior indigenous strains of AM fungi were selected for future inoculum studies that can enhance productivity and protection of cherry crop under high hills conditions of Himachal Pradesh.

INTRODUCTION

Cherries are extensively grown in all the temperate countries of the world and the leading cherry producing countries are USA, Germany, Italy and France. In India, it occupies an area of about 3,264 hectares with a production of 12,690 metric tonnes and is extensively grown on commercial scale in Jammu and Kashmir, Himachal Pradesh and to limited extent in Kumaon hills of Uttranchal. In Himachal Pradesh it is grown in Shimla and Kullu districts and occupies an area of 450 hectares with fruit production of 202 metric tonnes fruit (Anonymous, 2014). Cherries are rich source of protein, sugars, carotene, and folic acid and also have more calories than apple. It is mainly consumed as dessert and also in confectionary, ice-cream, bakery, juice making and various other purposes. Cherry fruits have antioxidants like pectin, polyphenol and anthocyanin compounds that reduce the risk of degenerative diseases caused by oxidative stress such as cancer, cardiovascular diseases and stroke (Kang *et al*., 2003). Arbuscular mycorrhizal fungi (AMF) are soil microbes forming symbiotic association with plant root system of utmost plant species including sweet cherry. They are formed by the group of fungi that are usually present in all soils from the phylum Glomeromycota, including nine genera; *Glomus, Paraglomus, Sclerocystis, Acaulosopora, Entrophospora, Gigaspora, Scutellospora, Diversispora* and *Archaeospora* (Schuessler *et al*., 2001). Distribution and diversity of vesicular arbuscular mycorrhizal fungi have been documented by many workers (Kullu and Behra, 2012; Kavitha and Nelson, 2013; Kirti *et al*., 2016). AM fungi are known to enhance plant biomass through better uptake of P and other mineral nutrients by increasing the absorbing surface area of the root system and fungal hyphae serve as the extension of the root system. AM fungi are also known to produce plant growth hormones, protection of plant roots from pathogens (Seguel *et al*., 2013), alleviate heavy metal toxicity in the host plants (Meier *et al*., 2015) and provides tolerance against drought and salinity (Auge *et al*., 2015). Many studies on various agricultural crops have utilized AM fungi to increase the growth, reduce mortality during transplantation and also helped in their subsequent acclimatization in that soil (Azcon *et al*., 2005; Guissou, 2009; Syafruddin *et al*., 2016). Now days our commercial horticultural systems mainly rely on heavy chemical inputs that has not only degraded the soil and environment but also decreased the productivity levels. So, in the current scenario, the most acceptable and environmentally conscious approach to solve this problem is to manipulate the plant rhizosphere population. Keeping this in view, the present investigation was aimed to select and characterize the efficient AM fungi for future inoculum for biomass production and growth promotion of sweet cherry.

MATERIALS AND METHODS

The experiment was conducted at laboratory of Department

of Basic Sciences, Dr. Y. S Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India.

Collection of soil samples

The rhizospheric soil along with the feeder root samples of sweet cherry plants were collected at 0-30 cm depth from the tree basin about 45 cm away from the tree trunk with the help of soil auger from four locations of Kullu district *viz*., Patlikulh, Katrain, Naggar and Mashalaghat. The samples were then carried to the laboratory in polythene bags and were stored in refrigerator at 4ºC till further analysis.

Estimation of AMF spore population

The spore population of AM fungi was determined by wet sieving and decanting method of Gerdemann and Nicolson (1963). 50 g rhizosphere soil was suspended in 200 ml water and the suspension was thereafter decanted through the series of sieves (500-45μm size). The residue left in each sieve was collected in the beaker and the final volume was made to 20 ml. The known quantity of suspension *i.e.* 2 ml from the above suspension was transferred to the counting plate and examined under stereoscopic binocular microscope and the number of spores per 50 g air dry soil basis was thus calculated.

Estimation of AMF root colonization

The root samples were washed carefully to remove the adhering soil particles. The tertiary roots were cut into small pieces (1 cm in length) and were subjected to differential staining as described by Phillips and Hayman (1970). Then root samples were observed under stereoscopic microscope for the presence of vesicles, mycelium, arbuscules, spores or sporocarps. The per cent colonization was assessed in accordance with gridline intersect method as described by Giovannetti and Mosse (1980) and was calculated as:

$$\% \text{ root colonization} = \frac{\text{No. of mycorrhizal infected root segments observed}}{\text{Total no. of root segments examined}} \times 100$$

Identification and characterization of AM spores

AM spores were picked up with auto pipette and mounted in lactophenol (Omar *et al*., 1979). Measurement of mounted spores was done at 10-40x magnification under biological microscope model LEICA, DMLB with image analysis software system.The isolated spores were identified on the basis of spore size, colour, shape, wall layer characteristics and attachment of subtending hyphae with the help of synoptic key of Trappe (1982), Hall (1984), Schenck and Perez (1990) and Benny *et al*. (2001).

Statistical analysis

The data recorded for various parameters under laboratory conditions were subjected to statistical analysis as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Estimation of AMF spore population and root colonization

The AMF spore population and cherry plant root colonization varied significantly with the location (Table 1.). The AMF spore population varied from 120.00 to 275.00 spores per 50 g of soil. The maximum spores were observed from cherry orchards at site-I (275.00 spores/50g of soil) of Katrain location. However, minimum spores per 50 g of soil were observed at site-III of Patlikulh location (120.00). There was registered no significant effect of aspect and their interaction with sites on AM spore counts. The AMF root colonization of cherry roots under natural conditions varied from 37.96 to 52.00 per cent. The root colonization was maximum (52.00 %) at site-II of Katrain location and the minimum (37.96 %) percentage was observed at site-II of Naggar location. Presence of more root surface area and root exudates may play an important role to have higher association of mycorrhizal fungi with the roots of mature trees (Wilcox, 1991). The higher root colonization at Katrain location may be described due to the presence of more spore counts at the site. Similar trend have also been observed by Kirti *et al*. (2016) and Kumar *et al*. (2016) who depicted a significant variation in root colonization ranged from 19.22 to 29.33 and 3.3 to 90.0 per cent for the cherry and litchi orchards, respectively. Further, Khakpour and Khara (2012) have also revealed a significant positive correlation between number of spores and root colonization. While observing the root colonization, mycelium of AM fungi was found to grow

Table 1: AMF spore counts and root colonization of sweet cherry (*Prunus avium* L.) from various locations of Kullu district (H.P.)

Location		AMF spore counts (spores/50g soil)			Root colonization (%)		
		Northern Aspect	Southern aspect	Mean	Northern aspect	Southern aspect	Mean
Patlikulh	Site-I	150	180	165	47.29	48.44	47.87
	Site-II	210	200	205	49.65	46.14	47.89
	Site-III	140	100	120	47.29	46.14	46.71
Katrain	Site-I	270	280	275	47.29	48.49	47.89
	Site-II	250	260	255	49.65	54.36	52
	Site-III	200	180	190	50.8	53.16	51.98
Mashalaghat	Site-I	140	130	135	42.68	42.68	42.68
	Site-II	180	190	185	41.52	42.68	42.1
	Site-III	150	160	155	41.52	42.68	42.1
	Site-I	120	170	145	41.52	44.98	43.25
Naggar	Site-II	140	160	150	36.81	39.12	37.96
	Site-III	220	200	210	36.81	39.17	37.99
Mean		180.83	184.17		44.4	45.67	
$CD_{0.05}$		44.2			3.45		
Location							
Aspect		NS			NS		
Location × Aspect		NS			NS		

Table 2 :Morphological characteristics of AM fungal spores associated with cherry (*Prunus avium* L.) at different locations of district kullu in (H.P.)

AM SPECIES		Morphological characters Spore colour	Spore shape	Spore diameter (μm)	Spore wall thickness (μm)	Spore wall Number	Spore wall cavity	Subtending hyphae	Frequency occurrence (%)
Glomus spp.	*G. macrocarpum*	Brown	Round	284.57	11.09	Fused	Absent	Straight or curved	
	G. mosseae	Brown	Round	174.76	8.21	Fused	Absent	Funnel shaped	
	G. constrictum	Brown	Globose	213.45	16.22	Fused	Absent	Straight or curved, markedly curved at the spore base	
	G. fasciculatum	Brown	Globose	92.33	7.65	Fused	Absent	Straight	
	G. clarum	Golden brown	Oval	43.24	6.72	Fused	Absent	Simple	
	G. epigenum	Brown	Round	98.81	6.32	Fused	Absent	Simple	80
Acaulospora spp.	*A. scorbiculata*	Brown	Round	224.89	12.43	Fused	Absent	Absent	10
	A. bireculata	Brown	Round	189.72	4.28	Fused	Absent	Absent	
Gigaspora spp.	*G. albida*	Brown	Round	432.12	11.62	Two	Bulbous type	Suspensor cell at the tip	
	G. heterogama	Light brown	Elliptical	132.42	8.25	Two	Present	Suspensor cell at the tip	5
Scutellospora spp.	*S. pellucida*	Dark Brown	Round	189.46	15.7	Two	Present	Suspensor cell at the tip	5

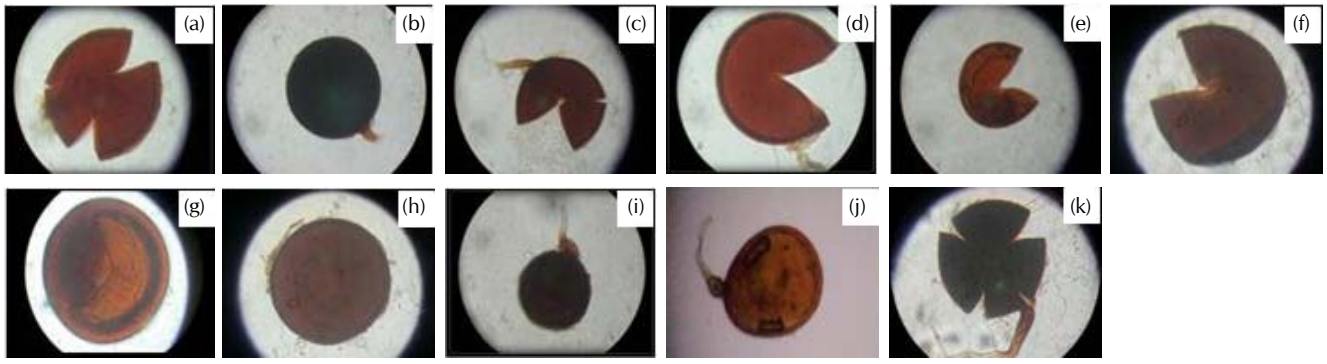

Figure 1: Spores of different AM fungi isolated from sweet cherry orchards in Kullu district of Himachal Pradesh (a) *Glomus macrocarpum* (b) *G. mosseae* (c) *G. constrictum* (d) *G. fasciculatum* (e) *G. clarum* (f) *G. epigenum* (g) *Acaulospora scorbiculata* (h) *A. bireculata* (i) *Gigaspora aibida* (j) *G. heterograma* and (k) *Scutellospora pellucida*

intercellularly in the root tissues. These AM fungal mycelium produced vesicles and arbuscules in roots. The presence of these structures in roots is considered as an indicator of AM fungal association with plant roots. Similar heavy arbuscular and vesicular infections were also observed by Yang *et al*. (2008) and Bharti and Parvesh (2014).

Characterization of AM fungi

In the present studies ten species of AM fungi related to four genera *viz., Glomus, Acaulospora, Gigaspora* and *Scutellospora* were found associated with the cherry plantation in Kullu district of Himachal Pradesh. The *Glomus* spp. showed 80 per cent frequency of occurrence in soil collected from various locations. *Acaulorpora* spp. showed 10 per cent frequency of occurrence, while the minimum occurrence of 5 per cent was observed in case of *Gigarpora* spp. and *Scutellospora* spp. On the basis of their identifying characters as depicted in Table 2, these AM fungi were identified as *Glomus macrocarpum, G. mosseae, G. constrictum, G. fasciculatum, G. clarum, G. epigenum, Acaulospora scorbiculata, A. bireculata, Gigaspora albida, G. heterogama* and *Scutellospora pellucida* (Fig. 1.). The variation in the occurrence of different genus and species of AM fungi associated with the cherry roots might be due to biological characteristics of rhizosphere under host species, mycorrhizal dependency and host plant mediated alteration of the soil microenvironment (Wu *et al*., 2009). These results are in confirmation with the findings of Bharat and Bhardwaj (2001) and Dohroo *et al*. (2013). Under present investigations the most frequently occurring AM fungal species in cherry rhizosphere was found to be *Glomus* as compared to other genera. These results are in corroboration with the findings Moreira *et al*. (2015) and Kirti *et al*. (2016) who reported that *Glomus* is most widely distributed AMF species among different ecosystems.

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