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Mycorrhizal association of Solanaceous vegetables from vast Deccan Plateau Zone of India

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Arbuscular mycorrhizal fungal (AMF) root and root length colonization percentage of in Tomato (*Lycopersicum esculentum* Mill.), Chilli (*Capsicum annuum* L.) and Brinjal (*Solanum melongena* L.) roots were studied during 2010-2012 from Vast Deccan Plateau (Marathwada, Maharashtra) zone of India. Study revealed that percentage of root colonization in every species studied varies significantly in the region; it was highest in Chilli (82.83%) followed by Tomato (73.77%) and Brinjal (72.87%). Average percentage of root colonization at every site studied varies significantly it was highest in Latur (87.9 %) followed by Osmanabad (85 %), Beed (82.2 %), Parbhani (74.5%), Jalna (74.3%) and Aurangabad (55%).The results of root colonization (rc) and root length colonization (rlc) significantly varied. Percentage of root colonization (rc) was more than root length colonization (rlc). All the three plants showed arbuscular, vesicular and hyphal colonization and intraradical and extraradical AMF spore as well in variable amount. Arbuscular colonization was found frequently than vesicular colonization.

Key words: Solanaceous Vegetables, Arbuscular Mycorrhizal Fungi, root colonization, Vast Deccan Plateau

INTRODUCTION

Marathwada is the region comprising of eight districts i.e. Aurangabad, Jalna, Parbhani, Beed, Osmanabad, Latur, Nanded and Hingoli of Maharashtra State, India. The region is rocky and dry with low and uncertain rainfall. The rivers and rivulets remain dry for major part of the year. Soils of Marathwada region are vertisols. Vertisols is classified as course shallow soils, medium black soils and deep black soils. There are nearly 12.4 million hectare course shallow brownish red soils, 32.5 million hectare of medium black soils and 12.1 million hectare deep black soils. The fertility index with respect to Nitrogen and Potash varies in all the districts of Marathwada. Major crops in this region are Sorghum, Cotton, Pigeonpea, Sunflower, Groundnut, Beans and Sugarcane. Region also contributes to fruit crops like Banana, Orange, Grape, Mango, Papaya, Guava, Ber, Lime and vegetable crops like Tomato, Brinjal, Chilli, Cucurbits, Cauliflower, Cabbage, Onion, Garlic, Leafy Vegetables like Spinach, Fenugreek etc.

The above studed vegetables are important for human diet therefore for their high yield and nutritive value therefore these are selected to study with respect to colonization of arbuscular mycorrhizal fungi. Arbuscular mycorrhizal fungi by asset

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of their symbiotic associations with roots are the most significant microbes in agriculture ecosystem which improve the growth of entire plant and often control certain plant pathogens (Muthukumar and Udaiyan, 2000). Other potential benefits of AMF colonization include increased tolerance of roots to soil-borne pathogens (Gianinazzi-Pearson and Gianinazzi,1983; Heald *et al*., 1989), improved drought stress (Davies *et al*, 1992) and increased protection from salt stress (Rosendahl and Rosendahl, 1991).These fungi are beneficial to plants as extraradical hyphae explore more amount of soil than plant roots. In India, the positive effect of AM fungi in crop plants has been reported from Uttar Pradesh (Hasan, 2002), Karnataka (Shivaputra *et al*, 2004), West Bengal and Assam (Roy *et al,* 2002), Maharashtra (Borde et al, 2009).Tamilnadu (Muthukumar and Udaiyan, 2006), Goa (Khade and Rodrigues, 2009) and Delhi (Kapoor *et al,* 2004). Therefore present research were to determine the availability of AMF colonization in drought prone region of Marathwada, Maharashtra.

MATERIALS AND METHODS

Sampling location

A survey was conducted from 2010 to 2012 in Osmanabad, Latur, Parbhani, Beed, Jalna and Aurangabad districts in the farmers' ,field of Marathwada region (17^0 35' N to 20^0 40 N Latitude and 70^0 40' E to 78^0 15' E Longitude) of Maharashtra state. Frequent visits were made to study mycorrhizal association at different growth stages of plants.

Root clearing

At the time of root colonization assessment, preserved roots of Brinjal was washed in water to remove traces of FAA. Nearly 20-30 root segments of 2-3 cm length were placed in 50 ml beaker half filled with 10 % KOH to facilitate stain penetration in cortex tissue. Beaker was placed in oven for two hours at 70^0 C. Roots were heated till depigmentation. In some cases microwave oven (30 seconds) was used for KOH treatment.

Rinsing and acidification of root tissues

The root segments were rinsed 2-3 times in water to dilute KOH residue and then immersed in 15 mL of Hydrochloric acid (5 %) for 2 minutes at room temperature to improve the root staining efficiency.

Root staining

Acidified roots were washed 2 to 3 times in water and immersed in trypan blue (0.05 %) for overnight period. De-staining was done to remove excess stain from root tissues using water.

Slide mounting

Stained root segments were mounted on microscopic slides in polyvinyl lactic acid glycerol (PVLG) [(Polyvinyl alcohol 8.33 g, distilled water 50mL, lactic acid 5ml, glycerine 5ml) (A dry powder polyvinyl alcohol was added to the water in beaker and kept in oven at 60^0 C to dissolve it. Lactic acid and glycerine was then added. Solution was used after 24 hours)] medium (Omar *et al,* 1979; Koske and Tessier, 1983). Roots were observed under the compound microscope (LOBAMED Vision 2000) and photographed with a Sony digital camera (DSC-W310/BC E37).

Root Colonization

A root was considered mycorrhizal when hyphae, vesicles and arbuscules alone or in any combination of these were present. Root colonization was measured according to the Giovannetti and Mosse (1980) using the following formula AMF colonization of roots collected from different study sites were studied according to Phillips and Hayman (1970) and Dalpé and Séguin (2013). Mycorrhizal root length colonization was quantitatively calculated according to McGonigle and Miller (1999) as well as Bierman and Linderman (1981).

$$\text{Root colonization (\%)} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

The data collected from the experiment were analyzed statistically following the procedure given by Mungikar (1997).

RESULTS AND DISCUSSION

Root colonization

The results of root colonization percentage observed in Tomato (*Lycopersicum esculentum* Mill.),

Table 1 : Percentage of AMF root colonization

Study sites	Percentage of root colonization (%)			
	Tomato (n* = 500)	Chilli (n* = 500)	Brinjal) (n* = 500)	Average for site
Osmanabad	79.6	88.00	87.4	85.0
Latur	87.4	94.4	81.8	87.9
Beed	68.8	94.8	83.0	82.2
Parbhani	81.2	73.0	69.4	74.5
Jalna	88.0	62.6	72.4	74.3
Aurangabad	37.6	84.2	43.2	55.0
Average for species	73.77	82.83	72.87	-
S. E. ±	7.77	5.20	6.55	-
C.D. 5%	19.97	13.37	26.38	-

(Values represent average of five replicates, n = Number of root segments assessed for root colonization)

Table 2 : Percentage of AMF root length colonization

Study sites	Percentage of root length colonization (%)			
	Tomato (n* = 500)	Chilli (n* = 500)	Brinjal) (n* = 500)	Average for site
Osmanabad	51.90	66.77	61.04	59.90
Latur	46.72	63.55	57.55	55.94
Beed	81.94	73.36	34.56	63.29
Parbhani	54.25	38.54	47.03	46.61
Jalna	43.35	47.43	81.93	57.57
Aurangabad	43.03	51.26	30.12	41.47
Average for species	53.53	56.82	52.04	-
S. E. ±	5.97	5.39	7.780	-
C. D. 5%	15.35	13.85	19.96	-

(Values represent average of five replicates, n = Number of root segments assessed for root colonization)

Chilli (*Capsicum annuum* L.) and Brinjal (*Solanum melongena* L.) were described in Table1and Fig.1

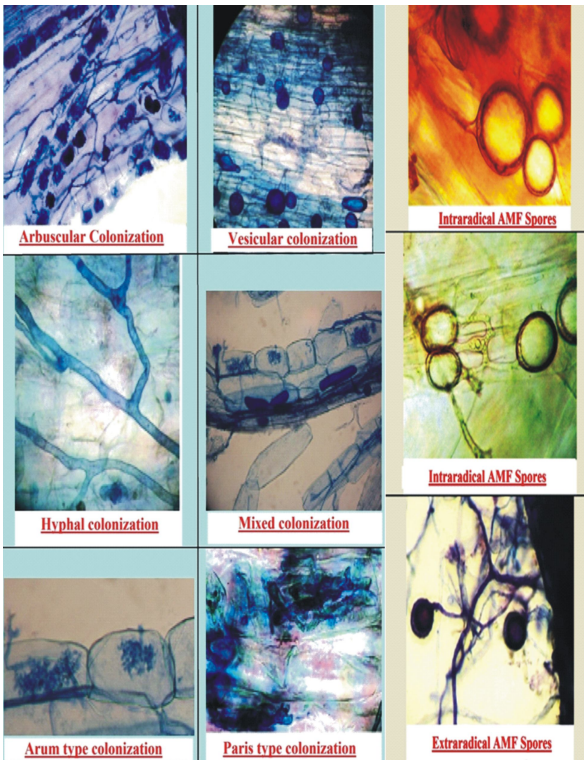

Fig. 1 : Root colonization types

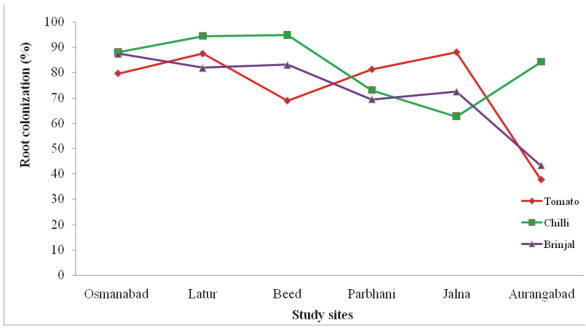

Fig. 2 : Percentage of AMF root colonization in Tomato, Chilli and Brinjal collected from different study sites

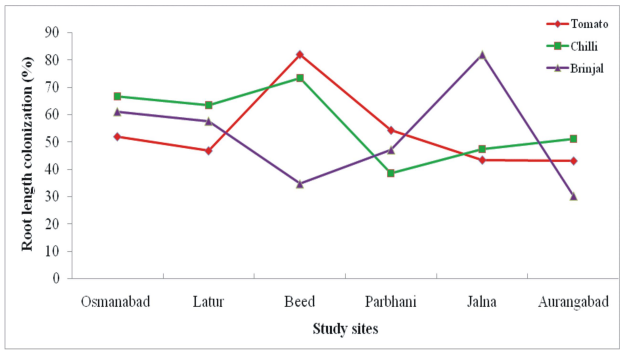

Fig. 3 : Percentage of AMF root length colonization in Tomato, Chilli and Brinjal collected from different study sites

Table 3: Status of AMF root colonization types

Study Sites	Type of root colonization (%)											
	Tomato				Chilli				Brinjal			
	NM	M			NM	M			NM	M		
	-	H	A	V	-	H	A	V	-	H	A	V
Osmanabad	20.4	27.4	43.8	8.4	12.0	44.2	43.8	0.0	12.6	19.2	63.8	4.4
Latur	12.6	25.0	46.6	15.8	5.6	25.4	57.8	11.2	18.2	9.0	49.0	23.8
Beed	31.2	59.4	9.4	0.0	5.2	60.6	4.2	30.0	17.0	32.2	50.8	0.0
Parbhani	18.8	33.6	47.6	0.0	27.0	56.0	17.0	0.0	30.6	37.4	22.4	9.6
Jalna	12.0	17.4	70.6	0.0	37.4	60.4	2.2	0.0	27.6	28.6	43.8	0.0
Aurangabad	62.4	21.4	16.2	0.0	15.8	12.8	69.4	2.0	56.8	33.4	9.8	0.0
S. E.±	7.77	6.16	9.22	2.72	5.20	8.17	11.67	4.89	6.55	4.33	8.17	3.83
C.D. 5%	19.96	15.83	23.69	7.00	13.37	21.01	30.00	12.58	16.83	11.13	20.99	9.84

(NM= Non-mycorrhizal, M = Mycorrhizal, H = Hyphal colonization, A = Arbuscular colonization, V = Vesicular colonization)

and 2. One hundred root segments from individual plant (100 x 5 individual plants) were subjected for study from every species. In tomato, root colonization percentage was significantly increased (p<0.05) in the area of Jalna (88 %) which was followed by Latur (87.4 %), Parbhani (81.2 %), Osmanabad (79.6 %), Beed (68.8 %) and Aurangabad (37.6 %). In chilli, the maximum root colonization was in the area of Beed (94.8 %) and Latur (94.4 %) significantly increased (p<0.05) than Osmanabad (88 %), Aurangabad (84.2 %), Parbhani (73.0 %) and Jalna (62.6 %). In brinjal, root colonization percentage was considerably highest (p<0.05) in the area of Osmanabad (87.4 %) than Beed (83 %), Latur (81.8 %), Jalna (72.4 %) and Parbhani (69.4 %). It was lowest in Aurangabad (43.2 %).

Average percentage of root colonization in every species studied varies significantly (p<0.05) in the region; it was 73.77 % in Tomato, 82.83 % in Chilli while in Brinjal it was 72.87 %. Average percentage of root colonization at every site studied varies significantly; it was 85 % in Osmanabad, 87.9 % in Latur, 82.2 % in Beed, 74.5 % in Parbhani, 74.3 % in Jalna and 55 % in Aurangabad.

Root Length colonization

Root length colonization percentage in Tomato, Chilli and Brinjal were observed. One hundred root segments from individual plant (100 x 5 individual plants) were subjected for study from every species.

Percentage of root length colonization of plants collected from different sites of Marathwada region is shown in Table 2 and Fig 3.

In tomato, root length colonization percentage was maximum in the area of Beed (81.94 %) which was followed by Parbhani (54.25 %), Osmanabad (51.90 %), Latur (46.72 %) and Jalna (43.35 %). It was minimum in Aurangabad (43.03 %). In chilli, plant the maximum root colonization was in the area of Beed (73.36 %) which was followed by Osmanabad (66.77 %), Latur (63.55 %), Aurangabad (51.26 %), Jalna (47.43 %) and Parbhani (38.54 %). In brinjal, root length colonization percentage was maximum in the area of Jalna (81.93 %) which was followed by Osmanabad (61.04 %), Latur (57.55 %), Parbhani (47.03 %) and Beed (34.56 %), it was minimum in Aurangabad (30.12 %).

Average percentage of root length colonization in every species studied varies significantly in the region; it was 53.53 % in Tomato, 56.82 % in Chilli while in Brinjal it was 52.04 %. Average percentage of root length colonization at every site studied varies significantly; it was 59.90 % in Osmanabad, 55.94 % in Latur, 63.29 % in Beed, 46.61 % in Parbhani, 57.57 % in Jalna and 41.47 % in Aurangabad.

Type of root colonization

While studying colonization in all species some non-mycorrhizal segments were found. Mycorrhizal segments showed hyphae, arbuscles and vesicles. When arbuscles were observed colonization was taken as arbuscular, when vesicles were observed it was taken as vesicular, when both arbuscles and vesicles were found it was also taken as vesicular, when arbuscles or vesicles were not observed colonization was taken as hyphal. AMF and formed typical AM structures including intra- and intercellular hyphae, arbuscules, and vesicles (Figure 1). Occasionally, intraradical and extraradical spores were observed alone or together in the root tissues. The results of type of colonization are described in Table 3.

In mycorrhizal segments of tomato, arbuscular colonization was highest in Jalna (70.6%) and lowest in Beed (9.6%), Vesicles were found in Latur (15.8 %) and Osmanabad (8.4 %) only, at all sites hyphal colonization were found in variable percentage. In Tomato the percentage of non-mycorrhizal segments was highest in Aurangabad (62.4%), whereas it was least in Jalna (12%).

In mycorrhizal segments of chilli, arbuscular colonization was highest in Aurangabad (69.4 %) and lowest in Jalna (2.2 %) and Beed (4.2 %), Vesicles were found in Latur (11.2 %) , Beed (30 %) and Aurangabad (2 %) only, hyphal colonization were found in variable percentage at all sites. In Chilli the percentage of non-mycorrhizal segments was highest in Jalna (37.4%), while it was lowest in Beed (5.2 %).

In mycorrhizal segments of brinjal, arbuscular colonization was highest in Osmanabad (63.8%) and least in Aurangabad (9.8%). Vesicles were found in Latur (23.8 %), Parbhani (9.6 %) and Osmanabad (4.4 %), hyphal colonization was observed at all sites in variable amount. In Brinjal the percentage of non-mycorrhizal segments was highest in Aurangabad (56.8%).

Edathil *et al.* (1994) observed VAM colonization in Tomato (87.8 %), Brinjal (96.1 %) and Chilli (87.4 %) in pot culture. Gallou et al. (2010) examined 1.9 % hyphal colonization after 2 days, 3.9 % arbuscles and 0.3 % vesicles after 6 days of contact with extraradical mycelium network of *Glomus intraradices* in Potato (*Solanum tuberosum*) plantlets. Dark septate endophytes observed during investigation in Tomato, Chilli and Brinjal play a positive role in improving the nutritional status of the host plant (Li and Guan, 2007; Newsham, 1999). Colonization and dark septate endophytes were supported by results of Muthukumar and Tamilselvi (2010) in *Capsicum annuum, Solanum melongena, Solanum nigrum, Solanum torvum and Nicotiana tobacum* of family Solanaceae. Ten plants belonging to different families growing in various parts of Osmanabad and Beed districts of Marathwada region in Maharashtra, India were studied for their Arbuscular Mycorrhizal (AM) association and found maximum percentage of root colonization in *Oxalis corniculata* (40%) and minimum in *Lantana camara* (4%) (Bhale *et al,* 2011). The maximum percent of root colonization was found in *Eucalyptus globulous* (96%) and minimum in *Glossocardia bosvallea* (8%) was reported by Bhale (2013).

The root colonization percentage clearly indicates that plants are mycorrhizal and root colonization depends on plant species as well as on geographical coordinates of plant. Soil microorganisms that form mutually beneficial relationships with plant roots have become a target of increasing interest in agricultural research and development because they offer a biological alternative to promote plant growth and reduce inputs in sustainable cropping systems. AM fungi at the interface between soil and plant roots makes them a key functional group of soil biota which by their nutritional and non-nutritional activities profoundly influence ecosystem processes that contribute to the ecosystem services in agro-ecology.

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