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STUDIES ON HOST PREFERENCE OF *GLOMUS* SPP AND THEIR SYNERGISTIC EFFECT ON SAPOTA [*MANILKARA ACHRAS* (MILL) FORSBERG] SEEDLINGS GROWTH

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Abstract

Standardizing nursery practices for vigorous and healthy seedling production is one of the important requirements in sapota germplasm maintenance and hybridization programme. An experiment was initiated with main aim to understand the native Arbuscular Mycorrhizal (AM) fungi host preference and their effect on growth promotion of sapota seedling under nursery condition. A survey was conducted in three sapota growing districts in Karnataka and isolated the commonly occurring AM fungi in sapota cropping system. Twenty one different AM fungal species comprising *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* were commonly recorded, however *Glomus* spp was found to be dominant in sapota cropping system. All the AM fungal isolates were tested for their colonization potential in sapota (variety: Cricket Ball) seedlings and the following three *Glomus* species *viz.*, *G. mosseae, G. fasciculatum* and *G. intraradices,* were selected based on their host preference and colonization efficiency. The selected three *Glomus* species were evaluated individually and in combination for their growth promoting effects in Sapota (variety: Cricket Ball) under glass house conditions. The results indicated that the combined application of *Glomus* spp significantly increased plant height, total plant fresh &dry biomass and total leaf area compared to individual *Glomus* isolate. The plant dry biomass and leaf area increased 14.6 and 12.96 per cent higher respectively in combined *Glomus* spp inoculated treatmnet compared to application of individual treatment. Similarly, the combined inoculation of *Glomus* spp increased in the range of 11-21 % AM fungal root colonization and spore population in rhizosphere of sapota seedlings compared to individaul isolate. This finding evident that *Glomus* spp. is the dominant AM fungal genera in sapota cropping system, but the colonization and host preference varying within *Glomus* spp. The mixed *Glomus* spp. application showed better performance of sapota seedling growth and AM fungal colonization compared to individual inoculum.

Key words : Sapota, *Glomus*, mixed inoculum, seedling growth.

Introduction

Sapota belongs to sapotaceae family, which is slow growing and long-living tree mainly got attraction for its sweet and delicious fruits. India is one of the largest producers of sapota in the world, this crop has the ability to adapt in different agro-climatic conditions and hence the area and production is increasing at a large scale. In India, it is cultivated in an area of 0.82 lakh ha with a productivity of 14.19 t ha^{-1} (Shirol *et al*., 2009). Sapota is propagated both by seeds and vegetative methods; the vegetative propagation is generally used for better growth and development. However, the seed propagation is the only method for maintaining germplasm and hybridization programme; but poor growth of the seedling is one of the major problems in this method. Hence, there is a need to improve the seedling growth at nursery stage.

In general, production of high quality, vigorous healthy seedlings in nursery is a pre-requisite for better establishment of plants under field condition. Many reports indicated that application of microbial inoculants is essential to improve the seedlings growth at nursery stage (Panneerselvam *et al*., 2012 and 2013). But the farmers are not generally practicing any bio-inoculants at nursery

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stage as well as during the time of maintenance of germplasm and hybridization programme particularly in fruit crops seedlings production. In nursery, the soil mixtures used for raising seedling production may have lower microbial population particularly AM fungi, it might be due to use of subsoil from barren surroundings or use of long time storage soils (Muthukumar *et al*., 2001). The AM fungal inoculum is required in large quantities for field application, this is a burden for the farmers and therefore, it is imperative to apply AM fungi at nursery stage.

AM fungi form obligate associations with a vast majority of agronomic plants except some of the families *viz*., *Brassicaceae*, *Chenopodiaceae*, *Ericaceae* and *Proteaceae*. AM fungi play an important role in the uptake of major and micronutrients by plants. It is mainly reported that AM fungi enhance the availability of soil P and also reported to mobilize other nutrients *viz*., K, Ca, Mg, S, Zn, Cu and Fe from soil. In addition, AM fungi are inovolved in soil aggregation, biotic and abiotic plant stress management. To manage the transplantation shock, the plant seedlings need to be associated with AM fungi (Urgiles *et al*., 2009) besides gaining other beneficial effects. Many research findings proved the importance of AM fungi in nursery seedling production of perennial fruit crops (Sukhada Mohandas, 2012a and 2012b; Panneerselvam *et al*., 2012 and 2013). Though, the AM fungal associations have been reported in many crops, the information on sapota is inadequate.

AM fungi are one of the important components in soil microbial biomass as they influence some of the essential process in soil and plant interface (Bagyaraj, 1984). But, the formation and function of mycorrhizas may vary from species to species (Smith *et al.*, 2000) apart from environmental conditions. Further, it was reported that the plant growth performance and nutrient uptake will vary from species to species, hence there is a need for selection of efficient AM fungi for better plant growth and development (Bagyaraj *et al*., 1989). The beneficial effects of AM fungi in plants may also depend on their host preference for particular soil or host (Dhillon, 1992), rate of infection (Rajan *et al*., 2000) and competitive ability (Michelsen, 1993). In view of above, the present study was initiated with the aim to understand the native AM fungi host preference, colonization potential and their effect on plant growth improvent in sapota seedlings under nursery conditions.

Materials and Methods

Survey was conducted at major sapota growing regions, *i.e*., Bangalore rural, Chikkaballabur and Kolar districts of Karnataka and root and soil samples were collected from the rhizosphere in different age group plants at two different depths *i.e*. 15-30 cm and 30-45 cm to understand the occurrence of AM fungi. AM fungal spores in rhizosphere soil were isolated by adopting wet sieving and decantation technique (Gerdemann and Nicolson, 1963). The isolated spores were identified based on spore morphology (Schenck and Perez, 1990) by using Stereozoom microscope (Olympus SZX9-Japan). The soil chemical properties *viz*., pH, OC, available N, P and exchangeble K (Jackson, 1973) were measured by adopting standard methods. The identified AM fungal spores were maintained in sterilized sand and soil (1:1) by using Rhodes grass as host plant. Further the *Glomus* spp identification was confirmed through molecular technique. In this method, genomic DNA was isolated from the purified *Glomus* spore by using the standard C-TAB method (Rogers and Bendich, 1994) and amplified with ITS 1(TCCGTAGGTGAACCTGCGG) and ITS 4(TCCTCCGCTTATTGATATGC) primers (White *et al.*, 1990). The amplified product was further cloned into vector (PTZ 57R/T-Fermentas), sequenced and Blast analyzed for the molecular identity.

After identification, all the AM fungal spores were multiplied in sterile sand: soil mixture (1:1) by using Rhodes grass (*Chloris Gayana Kunth*) as a host plant. After 4 months, the top vegetative portion of Rhodes grass was removed and the roots were thoroughly mixed with the substrate. The inoculum mixture containing 78-80 *Glomus* spores per gram of soil was used for the study. In this experiment, the genus *Glomus* was selected for further colonization and host preference studies as it was dominant genera under sapota cropping system. All the isolated *Glomus* spp were tested their colonization potential and host preference in sapota (variety: Cricket Ball) seedlings under glass house condition for four months. After 120 days, the seedling roots were removed carefully and analysed for AM fungal infection by using Phillips and Hayman (1970) method. The rhizosphere samples were collected from each *Glomus* sp inoculated sapota seedlings and analysed the proliferation of AM fungal spores by following Wet sieving and Decandation technique (Gerdemann and Nicolson, 1963). Based on colonization efficiency of *Glomus* spp, the following three species *viz.*, *G. mosseae*, *G. fasciculatum* and *G. intraradices* were selected for further evaluation studies.

The selected *Glomus* spp. were evaluated individually and in combination for their performance on enhancement of sapota seedling growth under glasshouse condition. The sapota seeds (variety: cricket ball) were sown in pots containing the sterilized cocopeat allowed for germination upto 45 days and then seedlings were gently

transplanted to pots containing (1.5 kg capacity) sterilized soil: sand: FYM (1:1:1) mixture. At the time of transplanting, inoculum of *Glomus* species (78 -80 spores g^{-1} soil) was applied at the rate of 20 gm per seedling nearer to roots. Control treatment received no inoculum. The following were the five treatments in this experiment *i.e. G. mosseae* (G1), *G. fasciculatum* (G2), *G. intraradices* (G3), combination of *Glomus* spp. (G1+ G2+ G3) and un-inoculated control. After 180 days, five seedlings from each treatment were randomnly selected and recorded all the growth parameters *viz.*, plant height, root length, stem girth, plant fresh and dry biomass and total leaf area. The plant dry biomass was measured by following oven drying method. The AM fungal colonization and spore load was estimated as described by above methods.

Statistical analysis

The data were analyzed using Web Agri Stat Package version WASP1.0 software. The visual indication of data dispersion on bar graphs was arrived by means of standard error of the mean. In the nursery evaluation of Sapota, the experiment comprised of five treatments replicated five times and each treatment consisted of 25 seedlings, one seedling per poly bag. Data were subjected to one way analysis of variance (ANOVA). Per cent AM colonization and spore numbers were transformed into arc-sine and square root value respectively to ensure homogeneity of variance before analysis (Gomez and Gomez, 1984). Treatment difference were evaluated using least significant difference (LSD) at $p<0.05$.

Results and Discussion

Chemical properties and occurrence of AM fungi in sapota cropping system

The chemical properties of soils in different sapota growing regions are presented in table 1. There was lot of variation in organic carbon (OC) and nutrient content between different sapota growing regions. The pH, OC, N, P and K recorded were in the range of 5.11-7.40, 0.31-0.97%, 50.2-157.1 ppm, 2.69-7.49 ppm and 50.0-306.2 ppm respectively in surface soil (15-20 cm) of different sapota cropping system. In general, surface soil (15-20 cm deep) recorded higher OC and other major nutrients compared to 30-45 cm depth. This variation might be due to management practices being adopted by the farmers. The AM fungal spore in different sapota growing regions indicated that the surface soil (15-20 cm deep) had higher AM fungal spore (1.2-8.1 spores g^{-1} soil) compared to 30-45 cm depth soil in most of the sapota orchards. In most of the orchards, nearly fifty percent reduction of AM fungal spore load was recorded at 30-45 cm depth in the sapota rhizosphere. This might be due to the presence of more secondary and feeder roots on the surface soil. A total of 21 AM fungal species belonging to five genera namely *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* were recorded in sapota rhizosphere, irrespective of the age group of sapota plant. However, the *Glomus* spp. was found dominant in all the orchards (table 1). This finding also revealed that occurrence of AM fungi was higher in multiple cropping systems in sapota as compared to monocrop, which indicates that the multiple cropping systems in sapota orchard may improve the diversity of AM fungi.

Mycorrhizal colonization and their host preference in sapota seedlings

Twenty one AM fungal isolates isolated were tested for their root colonization and host preference in sapota seedlings and the results of the selected isolates are given in table 2. Among the different *Glomus* spp., *G. mosseae* had significantly higher root colonization (78.8%) followed by *G. fasciculatum* (75.2%) and *G. intraradices* (66.4%). Similar trend was noticed in AM fungal spore population (13.6 -15.1 g^{-1} dry soil). All the AM fungal isolates were able to colonize the sapota seedlings, but the colonization and spore proliferation varies from species to species. The present findings indicated that the host preference varied from species to species within the same genus of *Glomus*, hence selection of specific species for specific crop is very important. Based on root colonization rate and spore proliferation, three best *Glomus* species *i.e. G. mosseae*, *G. fasciculatum* and *G. intraradices* were selected for further studies. This observation was corroborated with earlier findings of Banerjee *et al.* (2013), who reported that *G. intraradices* and *G. mosseae* were found to be the best AM fungal species for neem plants in terms of the growth promotion.

***Glomus* spp and their effect on sapota seedling growth (180 DAP)**

In this experiment, the individual and combined *Glomus* spp were evaluated for improving sapota seedling growth and the results are presented in table 3. The seedlings treated with *Glomus* spp. either individually or in combination significantly increased all the growth parameters compared to uninoculated control. Similar kind of observation was reported by many researchers (Plenchette *et al.*, 1981; Rupnawar and Navale, 2000; Druzic *et al.*, 2006) in different crop plants.

Among the three *Glomus* spp, there was no significant variation in plant growth. But, the combined

Table 1 : Occurrence of AM fungi in sapota cropping system.

Age of the plants (Years)	Different Districts	Soil samples collected from two different depths (cm)	Chemical properties of Rhizosphere soil					AM spore load (No. per g soil)	Cropping system	Dominant genera
			pH	OC (%)	N (ppm)	P (ppm)	K (ppm)			
10	Doddabellapur	15-20	7.40	0.97	157.1	4.60	112.5	7.0	Guava-Mango-Sapota	*Glomus* sp
		30-45	7.13	1.09	176.5	2.40	75.0	4.0	„ „	*Glomus* sp
12	Doddabellapur	15-20	5.90	0.31	50.2	6.00	50.0	4.0	Guava-Sapota	*Glomus* sp
		30-45	5.92	0.43	69.6	2.54	68.75	2.8	„ „	*Glomus* sp
15	Doddabellapur	15-20	6.49	0.92	149.04	4.19	163.2	1.2	Sapota	*Glomus* sp
		30-45	6.27	0.74	119.8	2.54	150.0	0.7	„ „	*Glomus* sp
20	Kolar	15-20	6.31	0.34	55.08	2.69	31.25	8.1	Sapota-Mango	*Glomus sp*
		30-45	6.83	0.29	49.98	1.35	137.5	2.0	„ „	*Glomus* sp
35	Kolar	15-20	5.11	0.72	116.6	7.49	306.2	1.92	Sapota	*Glomus* sp
		30-45	4.77	0.40	64.8	1.05	256.2	1.1	„ „	*Glomus* sp
40	Kolar	15-20	5.96	0.77	124.7	2.69	162.5	2.8	Sapota	*Glomus* sp
		30-45	5.76	0.57	92.3	3.74	18.7	5.6	„ „	*Glomus* sp
15	Bangalore	15-20	6.32	0.76	121.6	3.12	110.6	4.3	Sapota	*Glomus* sp
		30-45	6.21	0.68	95.1	2.89	95.2	3.2	„ „	*Glomus* sp
18	Bangalore	15-20	6.71	0.72	124.2	6.56	283.2	7.5	Sapota	*Glomus* sp
		30-45	6.68	0.65	100.3	3.29	145.2	5.2	„ „	*Glomus* sp
35	Bangalore	15-20	6.31	0.81	138.5	4.31	138.2	6.8	Sapota	*Glomus* sp
		30-45	6.20	0.73	114.2	4.12	124.2	4.3	„ „	*Glomus* sp

Table 2 : Evaluation of AM fungal colonization potential and sporulation in Sapota seedlings (120 DAP).

Different *Glomus* spp	AM colonization (%)	AM Spore load (number g^{-1} dry soil)
G. mosseae	78.8 (62.58)*	14.3 (3.85)**
G. fasciculatum	75.2 (60.13)	15.1(3.95)
G. macrocarpum	62.0 (51.94)	11.0(3.39)
G. etunicatum	58.2 (49.72)	10.1(3.26)
G. intraradices	66.4 (54.57)	13.6(3.75)
SEM	0.61	0.07
CD (p=0.05)	1.29	0.15

Values are mean of five replications.
SEM – Standard error means, CD (p=0.05) - Critical difference at 5 % level
* Values in the parenthesis are arcsine transformed values.
**Values in the parenthesis are square root transformed values.

inoculation of three *Glomus* spp. significantly increased plant height (14.8cm), shoot and root fresh weight (3.9 g /plant and 0.89 g /plant), shoot and root dry weight (1.38g /plant and 0.39g/plant respectively), total biomass (1.77 g/plant) and total leaf area (141.2 cm^2) compared to the individual *Glomus* spp. The plant total dry biomass was 14.6 % higher in combined *Glomus* spp. (G1+G2+G3) treated sapota seedlings as compared to individual application of *Glomus* spp. Similar to plant growth promotion, the AM fungal colonization was significantly higher (70.3–86.8%) in either individual or combined application of *Glomus* spp. treated seedlings as compared to uninoculated control (28.6%) (table 4). However, the combined *Glomus* spp. (G1+G2+G3) application recorded significantly higher AM fungal root colonization (86.8%) and spore population (15.7 g^{-1} dry soil) in sapota seedlings compared to individual *Glomus* spp. Among the *Glomus* spp., *G. fasciculatum* and *G. mosseae* inoculated sapota seedlings had significantly higher root colonization and spore population in rhizosphere as compared to *G. intraradices.* This experiment finding revealed that the combined inoculation of *Glomus* spp. (G1+G2+G3) increased 11.0–20.0% higher AM fungal root colonization and spore population compared to individual *Glomus* sp application (table 4).

In AM fungal application, selection of efficient host preferring species is very important than merely application of any AM fungal species. In most of the findings, the consortia of AM fungal inoculums enhanced plant growth performance than individual species

Table 3 : Effect of *Glomus* spp on enhancement of sapota seedling growth (180DAP).

Treatments	Shoot height (cm)	Stem girth (cm)	Shoot fresh weight (g/plant)	Root fresh weight (g/plant)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total biomass (g/plant)	Total leaf area (sq.cm)
G. mosseae (G1)	13.2	0.6	3.4	0.80	1.21	0.32	1.53	124.4
G. fasciculatum(G2)	12.1	0.6	3.2	0.74	1.16	0.34	1.50	118.2
G. intraradices (G3)	12.5	0.6	3.0	0.71	1.20	0.31	1.51	126.2
G1+G2+G3	14.8	0.6	3.9	0.89	1.38	0.39	1.77	141.2
Control	10.2	0.5	2.7	0.65	0.92	0.27	1.19	96.2
SEM	0.61	NS	0.16	0.04	0.06	0.02	0.07	5.94
CD (p=0.05)	1.29		0.33	0.08	0.12	0.03	0.16	12.59

Values are mean of five replications. SEM – Standard error means, CD (p=0.05) - Critical difference at 5% level.

Table 4 : Effect of mixed inoculum of *Glomus* spp on root colonization and sporulation in spota seedling production (180 DAP).

Treatments	AM colonization (%)	AM spore load (number g^{-1} dry soil)
G. mosseae (G1)	75.8(60.5)*	13.9 (3.79)**
G. fasciculatum (G2)	77.1(61.3)	14.1(3.82)
G. intraradices (G3)	70.3(56.9)	12.49(3.59)
G1+G2+G3	86.8(68.6)	15.7(4.02)
Control	28.6(32.3)	6.1(2.57)
SEM	1.31	0.08
CD (p=0.05)	2.77	0.17

Values are mean of five replications.
SEM – Standard error means, CD (p=0.05) - Critical difference at 5% level
* Values in the parenthesis are arcsine transformed values.
** Values in the parenthesis are square root transformed values.

(Banerjee *et al.*, 2013; Daft and Hogarth, 1983). In mycorrhizal association, the host plant root colonization plays an important role in deciding plant growth parameters. The higher root colonization allows more host fungus contact and exchange of nutrients and helps in better growth (Mallesha and Bagyaraj, 1990). In present investigation, the higher root colonization in combined *Glomus* spp. treatment (G1+G2+G3) might have exchanged more nutrients from soil, which inturn might have increased all the growth parametes in sapota seedlings as compared to individual species application. Some earlier findings proved that mixed inocula containing AM fungi provide more consistent benefits to the host plant (Daft, 1983) than individual species. AM fungal spores and hyphae provide sites for some beneficial bacteria to thrive on their surfaces. These associated bacteria are commonly referred to as mycorrhizal helper bacteria and play a very vital role in promoting mycorrhizal colonization of host plants by the secretion of enzymes and phytohormones that help in the proliferation of the fungal hyphae and promote the colonization of host plant roots (Panneerselvam *et al.*, 2012 and 2013). In the present studies, the enhancement of sapota seedling growth in combined application of *Glomus* spp. may be due to various beneficial bacteria on the surface of spores. The better plant growth promotion in combined *Glomus* spp treated seedlings is not fully explicable, but it needs further research.

Conclusion

The present finding indicated that the different AM fungal genera have been recorded in sapota cropping system, however *Glomus* spp. was found dominant in all the sapota orchards irrespective of the age group of plants. The mycorrhizal root colonization and host preference varied from species to species in sapota seedlings. The following *Glomus* spp. *viz.*, *G. mosseae*, *G. fasciculatum* and *G. intraradices* efficiently colonized sapota seedlings compared to other AM fungal species. The combined application of *Glomus* spp @ 20g per seedlings (*G. mosseae*, *G. fasciculatum* and *G. intraradices*) significantly improved the sapota seedling growth, AM fungal root colonization and spore proliferation compared to individual species application, which can be used as efficient bio-inoculants for sapota seedlings production as well as field establishment.

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