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New Report on AMF Colonization in Root Parasite Striga Gesnerioides and Its Host *Lepidagathis hamiltoniana* From High Altitude Region of Maharashtra

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Abstract

Present paper deals with in-depth analysis of AMF colonization reported in root parasite *Striga gesnerioides* (Willd) Vatke. Oester, Var *gesnerioides* and its host *Lepidagathis hamiltoniana* Wall. ex Nees collected from Alang, and Madan Forts situated at high altitude region of Maharashtra. Very few studies on the association of Host – mycorrhizal fungi – parasitic plan are known. Although AM fungal colonization in *S. gesnerioides* and its host *L. cuspidata* has been recently reported, this is the new report with reference to host- parasite system of *S. gesnerioides* - *L. hamiltoniana* from high altitude region of Maharashtra. The tripartite interaction between *L. hamiltoniana*, *Striga gesnerioides* and arbuscular mycorrhizal fungi is discussed in present work. We have presented evidence of significant colonization of AMF in roots of host parasite as well as host - parasitic plant root interaction zone on the basis of root screening at an intimate anatomical level. The AMF species encounterd in soil samples were *viz., Gigaspora* spp, *Glomus epigaeum*, *G. macrocarpum*, *G. occulatum*. Amongst the four AMF species *G. macrocarpum* showed highest (639) spore count 100 g^{-1} soil sample.

Keywords: Arbuscular mycorrhizal fungi, AMF colonization, *Striga gesnerioides*, *Lepidagathis hamiltoniana*

INTRODUCTION

Striga gesnerioides (Willd) Vatke. Oester, Var *gesnerioides* (Scrophulariaceae) is common root parasite on *Lepidagathis cuspidata* Nees (Acanthaceae) roots at high altitudes in open areas throughout Maharashtra and grows in very poor gravelly soil of hill-slope [1]. Although *Striga* has reported as root parasitic plant, the ethnobotanical data on *S. gesnerioides* revels that fruits [2-4] and whole plant is considered as anti diabetic [5]. *S. gesnerioides* Var *gesnerioides* is distributed at Amba, Ajara, Dajipur, Gaganbavda of Kolhapur district [1]. However, Kamble and Agre [6] first time collected *S. gesnerioides* from Panhala hills of Maharashtra and reported AM colonization. Arbuscular mycorrhizal fungi (AMF) normally colonizes almost all tropical plants, however the incidence of mycotrophy in medicinal plants is less documented compared to studies on forestry and crop species [7]. Previously Govinda Rao et al. [8] showed occurrence of AMF in the roots of several medicinal plants. However, there is a little work on medicinal plants in relation to AM colonization [9]. In past few years some Indian workers have reported AMF distribution and root colonization in medicinal plants from Tamil Nadu [10], Karnataka [11] and Kerala [9]. Recently from Central Himalaya [12], Madhya Pradesh [13] and Maharashtra [14-17]) investigations were made on AMF root colonization in medicinal plants. "The AM symbiosis is found in roughly 70% of all plant species and is the major type of mycorrhizal symbiosis found in most tropical forests, in which there is also the greatest diversity of myco-heterotrophs. Most of the myco-heterotrophs in the families Gentianaceae, Triuridaceae, Burmanniaceae and Corsiaceae are found in tropical forests and other environments in which the majority of plants have arbuscular mycorrhizas" [18]. However, host and parasitic plant association has remained unfocused for AM colonization with very few exceptions such as Woltz et al. [19]; Feild & Brodribb, [20] and de Vega et al. [21].

AM fungi have played a key role in plant evolution on earth as well as on the development and maintenance of the structure and diversity of terrestrial ecosystems [22]). Most of the plants depend on mycorrhizas to thrive particularly in fragile and stressed environment. In continuation with earlier investigations [6] present research work was planned with aim to explore AMF colonization and spore density in *S. gesnerioides* from other high altitude regions of Maharashtra such as Alang, and Madan Forts region of Nasik District, situated in Kalasubai ranges, Located North west of Nasik at Altitude: 4852' (1479 m) Latitude: 19°34'50"N Longitude: 73°39'38"E.

MATERIALS AND METHODS

The roots and rhizospheric soil samples of host - parasite association were collected from study area during Dec-Jan 2010 and 2011. At least ten associations of plants were screened for mycorrhizal colonization.

AM colonization

The method used by de Vega et. al. [21] to investigate tripartite association in host (Cistaceae species) – mycorrhizal fungi – holoparasitic plant *Cytinus hypocistis* (a rootless, stemless and leafless parasite grows exclusively inside its host root) could not be applicable in present material as *S. gesnerioides* is a hemiparasite having distinct root system and attachment with host (*L.*

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hamiltoniana) roots. Hence during present study the roots of host, parasite and host – parasite root junction regions were separated carefully thereafter screened for AM colonization by using recently used technique by Kamble and Agre [6]. Roots and host-parasite root junction from all ten associations were cleaned with tap water washed separated and collected individually. These roots were processed and stained for microscopic observation and occurrence intensity [23-24]). The root segments of 100 of 1 cm length were randomly selected for and quantification of mycorrhizal percentage colonization [25].

AMF spores

The wet sieving and decanting method was applied to recover mycorrhizal spores [26]) from soil. Synaptic keys of Trappe [27] and Gerdmann & Trappe [28] were used for spore identification. Association with species was considered mycorrhizal if the root samples showed presence of hyphae, vesicles or arbuscules [29-30].

Table 1- AMF Colonization in *S. gesnerioieds* and *L. hamiltoniana* from high altitude region of Maharashtra

Sr. No.	Plant species	*Occurrence intensity of AMF			AMF coloni -zation (%)	AMF species (spores 100 g^{-1} soil in parenthesis)
		V	A	H		
1.	*Lepidagathis hamiltoniana* Wall. ex Nees (Host)	+++	-	+	80	*Gigaspora* spp [142], *Glomus epigaeum* Daniels & Trappe [340], *G. macrocarpum* Tul. & C. Tul. [639], *G. occulatum* C. Walker [417]
2.	*Striga gesnerioides* (Willd) Vatke. Oester, Var *gesnerioides* (Parasite)	+++	-	++++	100	
3.	*S. gesnerioides* – *L. hamiltoniana* (Host-parasite Root interaction zone)	+++	-	+++	95	
						Total AMF Spores 100 g^{-1} = 1538

RESULTS AND DISCUSSION

The occurrence of mycorrhizal components *viz.,* vesicles, arbucules and hyphae was categorized into four grades based on the intensity - i) Excellent : Mycelia, vesicles or arbuscules present on more than 75 % of root bits ii) Good : Mycelia, vesicles or arbuscules present on more than 50 - 75 % surface of root bits iii) Moderate : Mycelia, vesicles or arbuscules present on more than 25 - 50 % surface of root bits iv) Poor : Mycelia, vesicles or arbuscules present on more than 1 - 25 % surface of root bits. The results obtained showed *S. gesnerioides*, and *L. hamiltoniana* have established AMF association with their roots (Table 1). Colonization of AM fungi was indicated by the presence of darkly stained vesicles and hyphae in the roots. Percentage colonization in both the host and parasite was 80% and 100% respectively. Whereas, 95% colonization was observed in host-parasite root interaction zone. Arbuscular colonization was absent in host, parasite and host-parasite interaction zone of root. In *S. gesnerioides* roots, vesicles were found comparatively smaller in size than host *L. hamiltoniana* and *L. hamiltoniana - S. gesnerioides* root junction region (Fig 1). "Although most parasitic plants being traditionally described as nonmycorrhizal, many have been observed parasitizing mycorrhizal host species [31-33]. Field observations also showed that some parasitic plants are found more often on plants associated with mycorrhizae [34]. In previous studies [33] [35-36] it is reported that AM association of the host plant is indirectly useful for parasite by increasing biomass and flower production. Whereas, in some cases it was contradictorily proved that AMF of the host plant can reduce the germination, attachment, and emergence of the parasite, reducing the damage caused to the host [37-39]. Besides the effect of mycorrhizal fungi on the parasite, it has been traditionally stated that parasitic plants, which establish a close connection with their host's roots do not interact directly with the mycorrhizal fungi of their host species [40-41]. *Parasitaxus ustus* (Podocarpaceae) is the only parasitic gymnosperm known to be closely associated with the mycorrhizal fungi of its host plant under natural conditions [19-20]" (with courtesy of de Vega et al.) [21]. Li & Guan [42] proved *Pedicularis* (Orobanchaceae), a genus of photosynthetic, root hemiparasitic plants associations with AM fungi. Whereas, de Vega [21] recently investigated possibility of direct tripartite association under natural conditions in Cistaceae: Host (Cistaceae: rockrose family, Order Malvales)) – mycorrhizal fungi – *Cytinus*: holoparasitic plant (Cytinaceae; Order Malvales).

In present study mycorrhizal association in *L. hamiltoniana:* Host – root junction region of host & parasite – *S. gesnerioides:* parasitic plant has been proved based on root screening (Table 1, Figure 1) which makes a new addition to existing data on host – mycorrhizal fungi – parasitic plant. The results obtained suggested that, there is variation in size and shapes of vesicles as well as percentage colonization (Table 1, Figure 1). Vesicles were moderate (host), smaller (parasite) and larger (Host – parasite root junction region) in size. (Figure 1). which agree with earlier report [6]. "In the arbuscular mycorrhizal symbiosis, plants obtain water and mineral nutrients from their fungal partners, enabling them to survive under various stressful conditions [43-45]" (with courtesy of Bouwmeester et al.) [46]. According to Feldmann [47] the partnership between AM fungi and host roots can lead to an enhanced tolerance of the plants to abiotic and biotic stresses. In light of abiotic stresses "it is conceivable that mycorrhizal fungal proliferation *i.e.* colonization is encouraged as a survival mechanism under carbohydrate stress" [48]). Likely, the stress buffer effect of the arbuscular mycorrhyzal fungi and their colonization behavior was studied by Biró et al. [49] in heavy-metal spicked soil on a long-term level, but among controlled conditions. Since, biological stress or biotic stress problems in plants are caused by 'living, organisms such as fungi, bacteria, viruses, nematodes, insects, mites and animals' [50]); In this scenario, one could speculate that host - parasitic plant association is also under influence of biological stress. Hence, probable explanation put forward for variation found in vesicular appearance and percentage colonization during present study might be that, the present biological association of host and parasitic plant is a kind of 'natural biological stress condition' in which AM fungus has tried to establish colonization. However, more studies are needed to evaluate possibility of many other factors potentially responsible for these variations. Thus, to understand possible factors inducing colonization variations in currently investigated tripartite association of *L. hamiltoniana* (host) – mycorrhizal fungi – *S. gesnerioides* (parasite), we strongly admire that it needs *in vitro* study and further investigations on molecular mechanisms involved under such a interesting biotic stress condition

Recently we [6] have showed AMF colonization 30.76% (*L. cuspidata*), 53.33% (*S. gesnerioides*), and 83.87% (*L. cuspidate* - *S. gesnerioides* root interaction zone) from Panhala hill. Whereas, during present investigation we observed more percentage colonization (Table 1) with change in host species and study area. In India Ragupathy et al. [51] investigated seven Scrophulariaceae members *viz., Lindernia antipoda* (L.) Alston, *L. hysopiodes* Haines, *Verbascum Chinese* (L.) Santapau (Non mycorrhizal at river bank habitat); *Lindernia anagallis* (Burm. f.) Pennell, *Striga angustifolia* (D. Don) C.J. Saldanha (Mycorrhizal in Coastal dry evergreen forest habitat); *Limnophila indica* (L.) Druce & *Peplidium martimum* (L.) Aschers. (Mycorrhizal & nonmycorrhizal respectively in aquatic and semi aquatic habitat) from Thanjavur District, in Tamil Nadu. But these results were neither supported with percentage colonization, nor with AMF spore species associated with plants. Hence it serves as only a preliminary data. Hence our observations on AMF species and mycorrhizal association in Scrophulariaceae parasite: *S. gesnerioides* and *L. hamiltoniana* host makes worthy contribution in existing knowledge. This study agree with the report [51] that, Scrophulariaceae members of diverse habitat shows variation in AMF colonization.

The results obtained suggest that, AMF spores of two genera *viz., Gigaspora* and *Glomus* were found in high density at the rate of 1538 spores per 100 g soil. The AMF spores belonging to four species *viz., Gigaspora* spp, *Glomus epigaeum, G. macrocarpum* and *G. occulatum* were recorded during spore count study. Among these *G. macrocarpum* was found in abundance followed by *G. occulatum* and *G. epigaeum* (Table 1). Although, AMF are an important part of host-parasite association in plants but their role is still not fully understood [6]). Hence, it is necessary to plan out further studies to explore morphological and physiological effects caused by AMF association in host and parasitic plants. Efficacy of AMF species *viz., Glomus intraradices, G. albidum, G. mosseae, G. fasciculatum*, and *G. etunicatum* to control parasitism of *Striga hermonthica* in sorghum [*Sorghum bicolor* (L.) Moench] is now well understood [38]). Studies on the tripartite interaction between cereals (sorghum or maize), AMF and *S. hermonthica* have demonstrated that levels of *S. hermonthica* seed germination can be significantly reduced with AMF root colonization [52-53]). Recent studies [54]) have also proved negative impact of AMF and plant growth promoting bacteria on *S. hermonthica* germination and hence recommended for *Striga* control in sorghum crop through integrated management with bacteria. Although AMF root colonization reduces stimulation of seed germination of the plant parasite *S. hermonthica* and *S. gesnerioides,* which can affect not only host plants by resulting in a lower parasite incidence [52-53], [55]) but also false hosts or trap crops such as *Vigna unguiculata* (L.) Walp. [56]) which induce suicidal *Striga* seed germination, thereby diminishing their effectiveness. Thus AMF inoculums can be significantly used in cash crops parasitic weed management. However, in present study we have observed significant colonization of AMF in roots of host as well as parasite plant.

The discovery of the parallelism between AMF and parasitic weeds in their signaling with roots constitutes a milestone in AMF research [57]. It is well established that, the same host-derived compounds strigolactones play a critical role as rhizospheric signaling molecules for seed germination of parasitic weeds [57]) and induce hyphal branching of AM fungi to establish mycorrhizal symbiosis [58] has opened up new lines in AMF research. However, simultaneous AMF colonization in host plant and its interactions between both is not understood perfectly. The newly recorded host plant in present paper *L. hamiltoniana* colonized with AMF which is economically neither cash crop nor a plant of positive economic value but is considered as weed. Thus, the implication for cultivation of *S. gesneroides* on *Lepidagathis* in relation to recover large biomass for antidiabetic components need further experimental testing under laboratory and field conditions by using native AMF inoculums .

[*Occurrence intensity of AM F : (+) poor , (+++) good, (++++) excellent, (-)absent; V: Vesicles, A: Arbuscules, H: Hyphae]

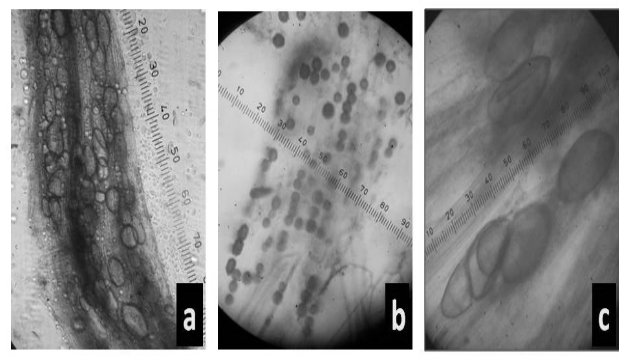

Fig 1. AMF Colonization showing Vesicles in: a) Host - *L. hamiltoniana* b) Parasite - *S. gesnerioides* c) Host- Parasite root junction - *L. hamiltoniana* - *S. gesnerioides.*

CONCLUSION

Although it is known that, AMF root colonization reduces stimulation of *Striga* seed germination, however we present evidence of significant colonization of AMF in roots of host parasite as well as host - parasitic plant root interaction zone on the basis of root screening at an intimate anatomical level. Thus it is worthy to note that newly recorded host (*L. hamiltoniana*) is a weed which can be served as model root surface for growth of antidiabetic parasite (*Striga*) in near future. However, to tap the mycorrhizal potential and to introduce cultivation practice there is urgent need to produce pure inoculums of native AMF species and various trials from laboratory to field.

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