Original Research Article

Diversity of Arbuscular Mycorrhizal Fungus Inhabiting the Rhizosphere of *Panax pseudoginseng* and *Solanum khasianum* Plants Growing Under Natural Conditions in Ziro, Arunachal Pradesh, India

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Abstract: Worldwide, the demand for herbal products is increasing which may result in over exploitation of many traditionally used and pharmaceutically important plant species and destruction of their habitat. On the other hand, there is mounting evidence that arbuscular mycorrhizal fungi (AMF) enhance the growth and productivity of medicinal plants. This calls for the need for identification and culture of AMF present in roots of naturally occurring plants. With this background, the present study was aimed to investigate the diversity of AMF inhabiting the rhizosphere of *Panax pseudoginseng* and *Solanum khasianum* plants growing under natural conditions in Ziro, Arunachal Pradesh, India. Rhizosphere soil of the *Panax pseudoginseng* and *Solanum khasianum* growing areas were collected from five sites in Ziro and were subjected to wet sieving and decanting method to isolate AMF spores. The identity of spores was identified morphologically by comparing the descriptions in INVAM. Spore density and species richness were also calculated. The rhizosphere of both the plants was populated with AMF spores belonging to *Glomus, Acaulospora, Gigaspora, Entrophspora* were found. *S. khasianum* plants harboured more AMF spores than the *P. pseudoginseng. Glomus and Acaulospora* were the most commonly found AMF species for both plants. These findings indicate that *P. pseudoginseng* and *Solanum khasianum* plants are rich in AMF diversity, therefore selecting and inoculating the plants with suitable microbial strains could be of immense importance in enhancing their growth and productivity.

Key words: Arbuscular mycorrhizal fungi (AMF), Diversity, *Panax pseudoginseng, Solanum khasianum*

Introduction

More than 35,000 medicinal plant species are reported to be used in medicine for treating a variety of diseases around the world (Zubek *et al.,* 2011). Medicinal plants occur naturally and these herbal wealth are receiving a great deal of attention all over the world because of their natural healing power. They are also gaining increased recognition as being non-toxic with no side effects and easy availability at affordable prices (Radhika and Rodrigues, 2010). They are regarded as nature's chemical industry since they produce essential phytochemicals or medicinally active compounds (Singh, 2005). Thus, it is very necessary to improve growth and quality of medicinal plants as they are used for the treatment of many diseases and it is advised that the medicinal plants should be raised through organic cultivation practices to prevent the accumulation of residual toxicity of fertilizers in the raw drugs (Thenmozhi *et al.,* 2011).

Panax pseudoginseng Wall. (Nepal ginseng, Himalayan ginseng) (Syn. *Panax wangianus*) is a perennial herb belonging to the family Araliaceae. It is barely found in temperate regions of the Himalayas and North East India, mainly Sikkim, Arunachal Pradesh, Manipur and Meghalaya between altitudinal ranges of 2900 – 4000 m. Globally, they have been reported from China, Tibet, Nepal, Bhutan and Myanmar. The plant has been declared critically endangered and is on the verge of extinction in North-East India (Venugopal and Ahuja, 2010). The plant has a horizontal knotted rootstock, producing a single knot per year (Venugopal and Ahuja, 2011). Its rhizome has been used as medicine for thousands of years in Asian countries, especially in Korea, China, and Japan (Kim, 2007). The medicinal properties of ginseng owe to the presence of ginsenosides found mainly in their roots (Kim, 2007). The biochemical properties of the ginsenosides and their abundance are influenced by many factors, such as, the genetic composition of ginseng, the environmental conditions like chemical composition of the soil, climate and interaction with other organisms including soil microorganisms (Lee 1987).

Solanum khasianum, belonging to the family Solanaceae is also known as Dutch egg plant, is an important medicinal plant. It is generally distributed throughout the North-east parts of India. The plant is stout, branched, woody shrub reaching a height of 0.75 to 1.5 m. The stem has spines, leaves are ovate to lobed with spines on both the surfaces, flowers are white, hermaphrodite and borne on axillary clusters, the berries are greenish in young stage and turn yellowish when ripe; seeds are small, brown in colour, abundant and entrenched in a sticky mucilage (Sunitha and Swapna, 2014). Traditionally, berries of the plant are used for treating toothache, small pox and fever. It has been reported for antifertility, anabolic and anti-inflammatory properties (Jarald *et al.,* 2008) due to the presence of glyco-alkaloids like solasodine (a nitrogen analogue of diosgenine), solakhasanin, solamargine, and khasinin (Putalun *et. al*., 2000). The extracts obtained from berries are also known to have high anti-helmintic activity which may be attributed to the presence of tannins (Jarald *et al.,* 2008 and Sunitha and Swapna, 2014). Besides, antioxidant activities, hepatoprotective activities of berry extracts have also been reported (Jerald *et al.,* 2013).

Arubuscular mycorrhizal fungi (AMF), belonging to phylum Glomeromycota establishes a symbiotic association with the roots of most terrestrial plants (Smith and Read, 2008). AMF are known to enhance the efficacy of nutrient uptake by the host plant; improve tolerance to abiotic stresses, such as drought, heavy metal and salt stress as well as biotic stresses, such as, resistance to leaf herbivory by insects and diseases caused by soil pathogen resistance (Auge *et al.*, 1994; Gange *et al.,* 1994; Kurle and Pfleger 1994; Leyval *et al.*, 1997). Hence, AMF play significant roles in plant physiology, plant health and nutrient cycling of the ecosystem (Gosling *et al.,* 2006).

Worldwide, the demand for herbal products is increasing which may result in over exploitation of many traditionally used and pharmaceutically important plant species and destruction of their habitat (Fuchs and Haselwandter 2005). Keeping in view the beneficial effects of AMF on medicinal plants, it seems significant to study soil-mychorrhizal development during the process of their growth (Songachan and Kayang, 2012). In addition to this, the mounting evidence of the AMF enhancing the growth and productivity of medicinal plants inoculated with them accentuate the need for identification and cultivation of AMF present in roots of naturally occurring plants (Ryszke *et al.*, 2010). Although, it is estimated that 80% of terrestrial plants establish symbiosis with AMF, only a small fraction of plant species have been actually examined for AMF status. It is also reported that each AMF species affects plant growth and nutrient uptake differently. There are only a few reports of AMF association in the rhizosphere of *Panax* and *Solanum* species (Cho *et al.*, 2004; Eom *et al.,* 2004; Kim *et al.*, 2004; Songachan and Kayeng, 2012). Thus, the study was aimed to analyze the diversity of two medicinal plants *P. psuedogensing* and *S. khasianum* growing under natural condition in Ziro, Arunachal Pradesh.

Materials and methods

Sampling site

The rhizosphere soil of *Panax pseudoginseng* and *Solanum khasianum* (Fig. 1) were sampled from Ziro valley, Arunachal Pradesh. The Ziro valley, also known as Apatani valley lies between 27° 38' 0" North and 93° 50' 0" East, surrounded by the Panyor and Kamla (Kuru) rivers at an altitude of 1524 to

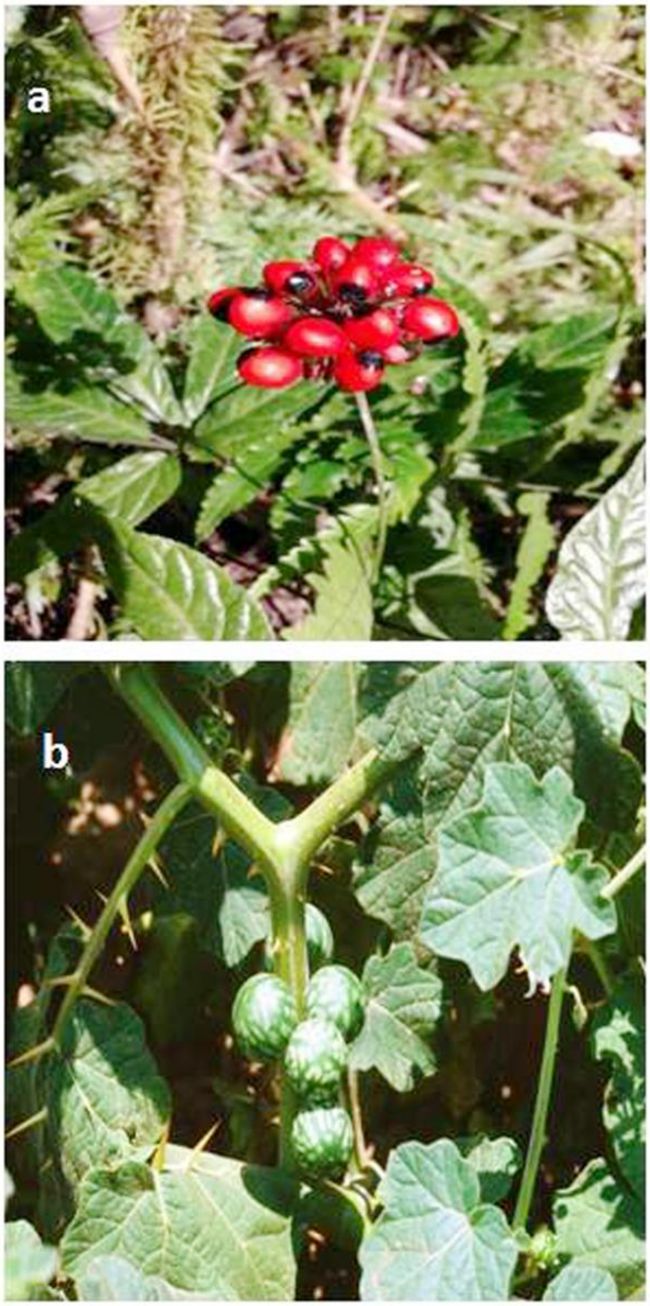

Fig. 1. *Panax pseudoginseng* (a) and *Solanum khasianum* (b) plants.

2738 m asl. The Ziro valley has a total geographical area of 3460 sq km of which 277.94 sq km area is covered by forests. The valley exhibits a humid sub-tropical to temperate type of climate with an annual rainfall of 108.1 cm and a temperature ranging from a maximum of 30.6°C to minimum of 1.1°C. The climatic, altitudinal and geo-morphological variations have shaped the two major vegetation types in and around the Ziro area – sub-tropical forest and temperate forest.

Collection of rhizosphere soils

The rhizosphere soil sample of *Panax pseudoginseng* and *Solanum khasianum* were collected randomly from five different sites during October 2014. Rhizosphere soil was dug at the depth of 0-15 cm after removing topsoil layer. Then the collected soil samples were taken in sealed plastic bags, labelled and was transported to the laboratory. The soils were air dried for isolation of AMF spores.

Isolation, Enumeration and identification of AMF spores

Spores were isolated by using the 'wet sieving and decanting method (Gerdemann and Nicolson, 1963). The isolated spores were mounted on clean slides using PVLG as mountant. The identification of AMF spores was based on morphological descriptions provided by the international collection of vesicular and arbuscular mycorrhizal fungi (INVAM) and originally published species descriptions. AM fungi species were identified after observing and imaging using photomicroscope (LEICA DM5000 LED). Spore density was calculated from direct counts of AMF spores under a binocular stereomicroscope. Species richness was calculated by counting the number of species present in the site. For each site, isolation and counting of spores was done five times each.

Results

The study is a first attempt to describe the diversity of AM fungi in the rhizhosphere soil of *Panax psuedoginseng* and *Solanum khasianum* of Ziro Arunachal Pradesh. AMF association in the rhizospheres of both the plants were observed at all sites. AMF spore density in 10 g soil sample was higher in *S. khasianum* as compared to *Panax psuedogensing*. The average number of AMF spores in the rhizosphere of *S. khasianum* was 72 as compared to 42 of *Panax pseudoginseng*. Similarly, species richness was higher in *S. khasianum*, averaging 37 species at each site compared to *P. pseudoginseng,*

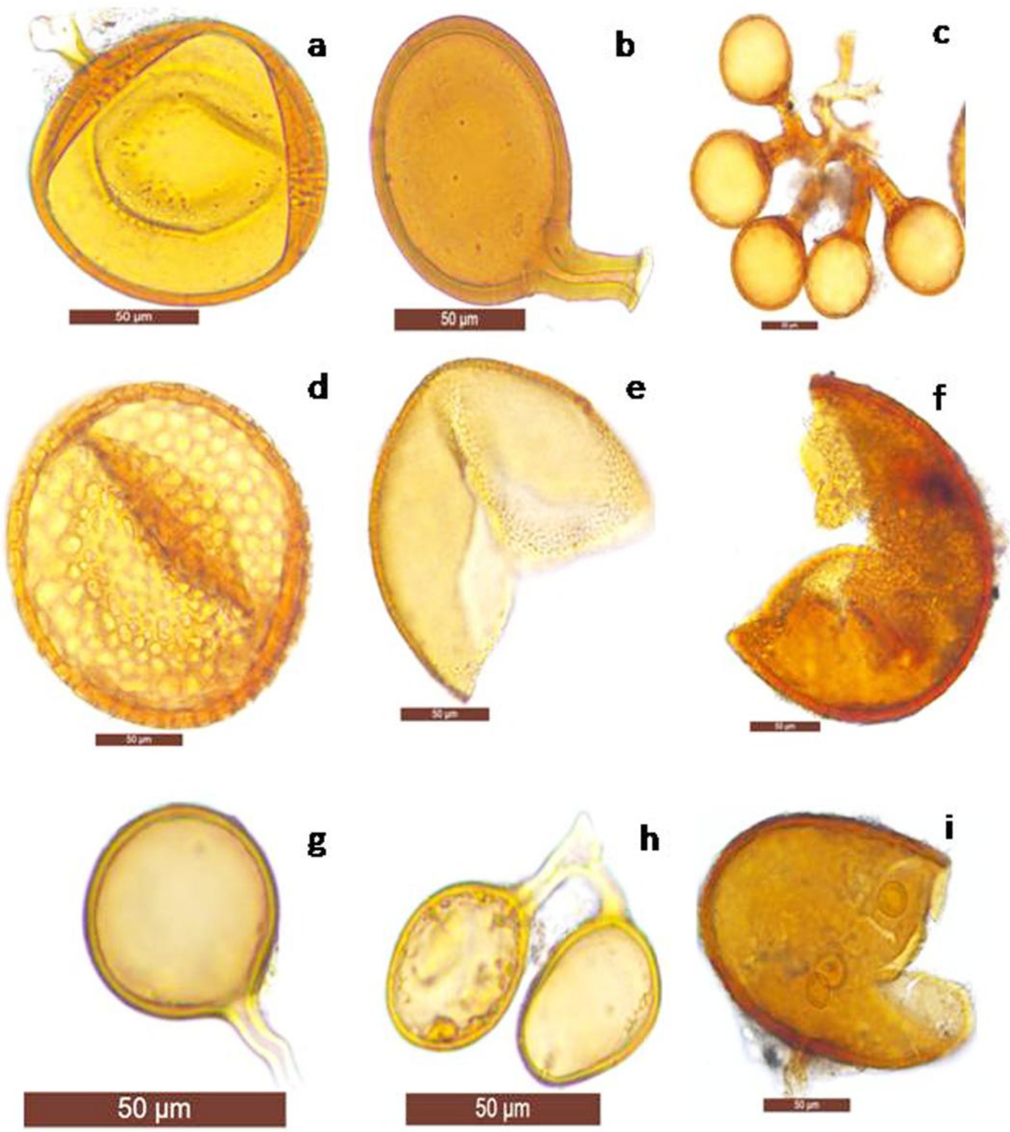

Fig. 2. Some AMF spores isolated from the rhizospheres of *Panax pseudoginseng* (a-e) and *Solanum khasianum* (f-i) *Glomus* spp. (a-c), *Acaulospora* spp. (d-f), *Glomus* spp. (g-h), *Gigaspora* spp. (i).

with 28 species at each site. Tables 1 and 2 show the spore density, species richness of AMF from rhizosphere soil samples of *Panax psuedoginseng* and *S. khasianum*. Photographs of some of the isolated AMF spores are provided in Fig. 2.

Discussion

The present study provides information on the status of arbuscular mycorrhizal fungal diversity in the rhizospheres of *Panax psuedoginseng and Solanum khasianum.* Our results revealed low AMF species diversity in the rhizosphere of both the plants. Our findings are concurrent with earlier reports (Guadarrama and Alvarez-Sanchez, 1999; Muthukumar and Udaiyan, 2000; Zhao *et al.,* 2003; Yang *et al.,* 2011). This may be due to the fact that AMF growth rates are highly host specific when fungi are examined as a community (Bever *et al.,* 2001). This fungal specificity could be a major factor responsible for maintaining diversity of AMF within the community (Bever *et al.,* 2001). In addition, it is also known that sporulation of AMF species is influenced by host plant species (Yang *et al.,* 2011). Therefore, different AMF species compositions might play distinct roles in associating preferentially with particular plant community (Daniell *et al.,*

Table 1. Spore density, species richness of arbuscular mycorrhizal fungi from rhizosphere soil samples of *Panax psuedoginseng* of Ziro.

Sl No.	Sites	Spore density (10gm soil)	AM fungal genera found	Species richness
1	1st	53	a) *Glomus*	9
			b) *Acaulospora*	9
			c) *Gigaspora*	1
			d) Unidentified species	8
2	2nd	39	a) *Glomus*	22
			b) *Acaulospora*	15
			c) *Entrophospora*	3
			d) Unidentified species	9
3	3rd	19	a) *Glomus*	9
			b) *Acaulospora*	8
4	4th	36	a) *Glomus*	9
			b) *Acaulospora*	9
			c) *Gigaspora*	1
			d) Unidentified species	1
5	5th	60	a) *Glomus*	15
			b) *Acaulospora*	8
			c) Unidentified species	2

Table 2. Spore density, species richness of arbuscular mycorrhizal fungi from rhizosphere soil samples of *Solanum khasianum* of Ziro.

Sl No.	Sites	Spore density (10 gm soil)	AM fungal genera found	Species richness
1	1st	48	*a) Glomus*	14
			b) Acaulospora	18
			c) Entrophospora	1
			d) Unidentified species	5
2	2nd	58	a) *Glomus*	13
			b) *Acaulospora*	9
			c) *Entrophospora*	2
			d) Unidentified species	4
3	3rd	76	a) *Glomus*	26
			b) *Acaulospora*	9
			c) *Gigaspora*	2
			d) Unidentified species	4
4	4th	106	a) *Glomus*	21
			b) *Acaulospora*	13
			c) Unidentified species	2
5	5th	71	*a) Glomus*	19
			b) Acaulospora	17
			c) Unidentified species	5

1999). Thus, our findings suggest that even though AMF species diversity in association with *P. pseudoginseng* and *S. khasianum* are low, the various combinations of AMF species may be beneficial for both the plants.

Our study also reveals that *Glomus* and *Acaulospora* were the most dominant AMF species in the rhizospheres of the plants, *P. psuedoginseng and S. khasianum.* This may be explained by the sporogenous characteristics of these two species. As *Glomus* and *Acaulospora* have the smallest size spores in AMF taxa, a large number of spores are produced in a short time and are easily distributed (Hepper, 1984; Dhar and Mridha, 2006; Yang *et al.,* 2011). Another reason could be the wider adaptation of these taxa in varied soil conditions (Das and Kayang, 2009). Other genera like *Gigaspora*, and *Entrophspospora* were also found; however, their number was extremely less. In natural ecosystems, such disproportionate distribution of AMF is quite prevalent (Yang *et al.,* 2011). There are also reports of huge disparity in spore density and species richness of AMF in different rhizosphere in many sites (Azcon-Aguilar *et al.,* 2003; Zhao *et al.,* 2003; Pande and Tarafdar, 2004; Wang *et al.,* 2004; Radhika and Rodrigues, 2010). The difference in diversity of spore observed in the study may be attributed to many factors that disturb spore production in a rhizosphere soil of host plant. Spore population is affected by a wide range of soil, climatic, fungal and host factors such as plant phenology and root production (Anderson *et al.,* 1983; Howeler *et al.,* 1987).

The significance of AMF in ecosystem and their beneficial effects of medicinal plants are being increasingly recognized. This study provides information of the diversity of AMF in *Panax psuedogensing* and *Solanum khasianum*. Since these plant species are of great medicinal importance, our findings may help in selection of appropriate AMF which may improve their growth and productivity.

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