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Research Article

Assessment of Arbuscular Mycorrhizal (AM) fungi of *Glyphochloa santapaui*: Vulnerable and endemic grass species of Maharashtra, India.

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Abstract: *Glyphochloa santapaui* (S.K. Jain & Deshp.) Clayton *santapaui* is endemic to Maharashtra state restricted to Ferricrete-Lateritic rocky plateaus at Sindhudurg and Ratnagiri district. Due to ongoing anthropogenic threats this species is rated as Vulnerable under IUCN Red List of Threatened Species and hence attention is needed toward its conservation. The lateritic plant-soil interactions in different taxa is dependent to their microbial or mycorrhizal associates. So far endemic grass species - Arbuscular mycorrhizal associations on Lateritic rocky plateaus are poorly investigated. In present paper critical assessment of AM fungal colonization in roots of vulnerable- endemic grass *Glyphochloa santapaui* is interpreted. Overall colonization percentage was 71.80%. Moreover, root segments of some samples were commonly co-colonized by dark septate hyphae *(dsh)* of other fungal endophytes (*Ofe*) and AM fungi. In present assessment, four Glomeromycota families *viz.,* Acaulosporaceae, Diversisporaceae, Gigasporaceae and Glomeraceae were recorded comprising 18 species under 6 genera. These AM fungal species are *viz., Acaulospora elegans*, *A. rehmii*, *A. scrobiculata*, *A. tuberculata*, *A. appendicular*, *Diversispora epigaea*, *Gigaspora albida*, *G. gigantea*, *G. margarita*, *G. rosea*; *Scutellospora calospora, S. dipurpusescrns*; *Glomus gerdemanni*, *G. hoi*, *G. occultum*, *G. versiforme*, *G. warcuppi* and *Sclerocystis sinuosa*. On the basis of analysis of spore density and relative abundance, two dominanting species of AM fungi viz., *Diversispora epigaea* and *Gigaspora gigantea* were recognized.

Key words: Arbuscular mycorrhizal Fungi, AM fungi, *Diversispora epigaea, Gigaspora gigantea,* Glomeromycota, *Glyphochloa santapaui*, vulnerable- endemic grass.

Introduction

India has about 49 endemic genera, *Glyphochloa* Clayton (Poaceae) is one of the 13 grass genera endemic to Western Ghats (Irwin &Narasimhan, 2011). The genus is restricted to peninsular India which comprises 11 species and four varieties (Royal Botanic Gardens, Kew, 2018). *Glyphochloa santapaui* (S.K. Jain & Deshp.) Clayton show very narrow distribution and it found only in Sindhudurg and Ratnagiri district of Maharasthra. Thus, *G. santapaui* is endemic to Maharashtra state which is restricted and adapted to lateritic plateaus of lower altitude (up to 300 m coastal plains) (Gosavi *et al.,* 2015). Natural habitat of *G. santapaui* are highly fragmented may be converted to Areca and Coconut plantations (Romand-Monnier 2013). Besides, there are additional threats such as overgrazing and trampling by livestock, human settlements and fires (Rawat *et al.,* 2001). Hence, this species is assessed in IUCN Red List of Threatened Species and placed at Category: Vulnerable D2 ver 3.1(Romand-Monnier 2013).

Arbuscular mycorrhizal (AM) fungi are beneficial and ubiquitous fungi in natural and agricultural ecosystems (Smith and Read, 2008). About 90% of vascular plants are estimated to normally establish mutualistic relationships with AM fungi and exhibits widest host association range (Arora, 1991). They plays a significant role in phosphorus acquisition of many terrestrial plants. The function of the AM fungal symbiosis in phosphorus cycling is more significant in grassland ecosystems than in more intensively managed agricultural systems (Murakoshi *et al.,* 1998). Additionally, AM fungi contribute to the grassland plant community through their hyphal network connecting different plant species (Grime *et al.,* 1987). AM fungi also help in tolerance to toxic metals, high soil temperature, adverse pH etc. and also stimulate rooting in plants (Barrow *et al.,* 1977). Schultz *et al.,* (2001) have demonstrated that adaptation of *Andropogon genardii* (Poaceae) to the nutrient levels of their local soils was at least in part due to their dependence on mycorrhizal fungi. Grime *et al.,* (1987), demonstrated that many plants are unable to grow without AM fungal association leading to limited growth and suggested beneficial role of AM fungal symbiosis in nutrient limiting conditions. Ringwall and Dickinson (1997) stated that, "there is no need to establish mycorrhizal fungi in the root systems of most native grasses. However, there is a need to evaluate how various factors may be used to manipulate AM fungi colonization levels,

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so that these levels are most beneficial to the host grass".

The majority of Ferricrete-Lateritic rocky plateau plants have not been focused for mycorrhizal assessment purpose in general. "So far there are no techniques or seed banks established for propagation of Ferricrete-Lateritic rocky plateau plants nor mycorrhizal associations and dispersal vectors fully known. Hence, restoration has to rely on existing seed banks or natural colonization process automatically excluding many species" (Thorpe and Watve 2015). The lateritic plant-soil interactions in different taxa bears ingeniously different mechanisms for phosphorous and micro-nutrient uptake (Verboom *et al.,* 2004). It is dependent to their microbial or mycorrhizal associates, the types of organic anions secreted and whether or not protons accompany such secretion (Lambers *et al.,* 1998; Roelofs *et al.,* 2001; Jones *et al.,* 2003 and Hinsinger *et al.,* 2003).

Although Fonseca (2003), attempted first time to investigate mycorrhizal infection in root*s* of *G. santapaui* from lateritic soil. But failed to explain the details of other structures of fungal components and AM fungal species inhabitant to soil. Hence, *i*n present study an attempt has been made to understand details of roots colonization pattern, AM fungal species and their relative abundance in *G. santapaui* inhabitant soil. Based on critical assessment this paper is proposed as first report on AM fungi of *G. santapaui* vulnerable and endemic grass species of Maharashtra.

Materials and Methods

Site description

The study area is geographically Low level Ferricrete (LLF) - Lateritic rocky plateau of Hativale (Vikhare Gothane), 12 km from Rajapur, falls under Ratnagiri district of Konkan region Maharashtra situated at geographical coordinates of about 16.6572^0 North and 73.5211^0 East. The *G. santapaui*, samples were collected during September 2016 (MMK & SSL) and valid identification was made by consulting with expert Dr. Chandore A. N. (Dept of Botany Abasaheb Marathe ASC College Rajapur, Ratanagiri district, Maharashtra). Authentically identified plant specimens were dry preserved in herbarium and deposited in department.

Sample collection

As *G. santapaui*, is a small, caespitose, annual grass, grows on exposed marshy lateritic hill tops, and in open places locally known as 'sada' in association with other grasses like *Ischaemum indicum* and *Pulicaria angustifolia.* These plateaus have very thin layer of a soil. Therefore, soil sampling was done from about 20-30 selected caespitose habits to make a figure of around 250g soil. The roots excavation was done very carefully because of less availability of soil on rocks. The soil sample were collected for AM fungal spore extraction and for estimation of physico-chemical properties. For this purpose, plants which were sparingly grown and not overcrowded with other associate plants were carefully chosen to avoid AM fungal flora of unwanted vegetation. It helps to prevents misleading results of AM fungal spore's extraction.

The plants along with the soil samples and roots were collected in different collection bags. and transported from field to laboratory which immediately refrigerated at 4°C subsequent to arrival. The roots were processed immediately. All the rhizosphere soil samples were homogenized prior to remove coarse roots segments, stones and adhered particles through sieving procedure (2 mm mesh size). Subsamples of soil were air dried and used for estimation of physico-chemical properties.

Physicochemical parameters of soil

Soil texture and moisture was estimated gravimetrically (Jackson, 1967). Soil pH was analysed on 1:2.5, soil: water suspension (van Reeuwijk, 2002). Organic carbon was analyzed by WB rapid titration method (Walkley and Black, 1938). Carbonate was estimated by Piper's rapid titration method (Piper, 1966) and available Olsen's phosphorus in soils was determined by extraction with 0.5M sodium bicarbonate for 30 min (Olsen *et al.,* 1954).

Status of AM fungal colonization in roots

It was determined by assessing roots for percentage of colonization and occurrence intensity of three mycorrhizal components as given below:

Percentage colonization

The intercept method (Brundrett, *et al.,* 1996) was followed for microscopic observations of stained root segments of *G. santapaui* under a Magnus light microscope. The mycorrhizal colonization percentage, was determined by following rapid method of Phillips and Hayman (1970). A root piece was considered for counting as colonized by AM fungi where any mycorrhizal components such as hyphae, vesicles or arbuscles was observed. The overall colonization percentage (*OCp)* was calculated on the basis of observed values for mean colonization percentage (*MCp)* associated with vesicles, arbuscules and hyphae etc**.** The occurrence of other fungal endophytes in roots, such as dark septate endophytes (DSE) were also recorded by means of a Magnus light microscope, using an objective of 40×.

Occurrence Intensity

All the three components of AM fungi were interpreted for occurrence intensity *viz., poor* (1-25%), *moderate* (25-50%), *good* (50-75%) and *excellent* (>75%) which was denoted as '*p*, *m*, *g* and *e'* respectively. To interpret occurrence intensity (*OI*) of fungal structures, mean colonization percentage (*MCp)* for

each fungal structure (*V*: vesicles, *A*: arbuscules and *H*: hyphae) was determined separately. Based on microscopic observations of randomly selected 100 root segments pattern of AM fungal colonization for *G. santapaui* was determined. Any other special structures of mycorrhizal colonization (*Smc*) if present in root piece was also recorded.

Assessement of AM fungal species

It was performed in two steps, spore extraction followed by species identification as explained below:

AM fungal spore extraction

Spores were extracted from the10g of rhizosphere soil following the sieving and decanting technique (Gerdemann and Nicolson, 1963). Total spore numbers of AM fungi in the soil sample were estimated following Gaur and Adholeya (1994). The spores isolated were mounted in a polyvinyl-lactoglycerol (PVLG) and PVLG solution mixed with Melzer's reagent 1:1 (v/v) ratio (Morton, 1988). Only spores that appeared to be healthy were recorded, counted examined under (Olympus 003421) stereomicroscope and photo-micrographically documented with the help of Canon IXUS 155 digital Camera.

AM fungal species identification

Taxonomic placements of AM fungal spores and sporocarps up to species level was done using bibliographies by Schenk and Perez (1990). The identification is purely based on the synaptic keys (Hall and Fish, 1979; Hall, 1980; Pacioni, 1992) and also after consultation with descriptions of AMF species provided by International Culture Collection of Vesicular and Arbuscular Endomycorrhizal Fungi [http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm]. The species codes were followed after Schenk and Perez (1990).

The voucher slides containing the isolated spore specimens were assigned accession codes '*BCA:MHMMK*n' [where, *BCA:MH* is Bhavan's College Andheri: Mycological Herbarium; *MMK:* initials of second Author and *'n'* is number assigned] have been deposited in the slide collection of the Mycorrhizal Research Laboratory of Department.

Spore density (S) was considered as the number of spores in 10g soil. Relative abundance (RA) of species (*RAspp*) was defined as the percentage of spore numbers of a species divided by the total spores observed (Dandan and Zhiwei, 2007). Similarly, RA of AM fungal family (*RAfam*) was defined as the percentage of spore numbers of a family divided by the total spores observed.

The dominant AM fungal species was determined according to relative abundance (RA > 6%) and spore density (S ≥ 15 spores). Statistical data processing for percentage colonization in roots, spore density and relative abundance of AMF species was performed for standard errors of means by using Microsoft excel 2010.

Results and Discussion

Physico-chemical parameters of soil of *G. santapaui*

Soil requirement for every plant and the microbial strains varies from species to species and thus while cultivation or conservation the soil components should be taken into consideration. The soil of Hativale plateue is lateritic and reddish due to natural iron content. Soil sampled from spots where there is of *G. santapaui* has a relatively loose texture and its characteristics are shown in Table 1. These soils often have up to 40% gravel and slightly acidic with pH 6.2. Organic Carbon (5.88%) and Organic matter (10.11%) calculated is higher in the topsoil of *G. santapaui.* Whereas, Carbonate content recorded is 3.58±0.01mg.kg^{-1}*;* Phosphorus content is 5.89±0.02mg.kg^{-1}

Table 1. Physicochemical properties of *G. santapaui* soil

Sr.No.	Parameters	Status
1.	Colour	Red
2.	Soil texture	≈ 40% gravel,
3.	pH	6.23 ± 0.02
4.	Organic Carbon	5.88 %
5.	Organic Matter	10.11 %
6.	Carbonate	3.58 ± 0.01 mg.kg^{-1}
7.	Phosphorus	5.89 ± 0.02 mg.kg^{-1}

(±) Standard error of mean

Percentage colonization and Occurrence Intensity of AM fungi in roots of *G. santapaui*

In present assessment, roots of *G. santapaui* are assessed for presence of AM fungal colonization (Table 2) and denoted by *MCp i. e.* mean colonization percentage for individual components. The study revealed that the roots of all the plants are colonized in the range of 30 - 92% by by AM fungal components. There is variation in colonization percentage all the root segments. However, overall colonization percentage (*OCp*) in *G. santapaui* is 71.80.

The occurrence intensity (*OI*) observed is qualitative expression of corresponding *MCp* values which are also presented in Table 2. Analysis of mychorrhizal roots suggests no significant variation found in *OI* expression for vesicles and hyphae (*MCp 92.31*); but arbuscles *OI* is comparatively low (*MCp30.77).*

Recently, D`Souza and Fonseca (2015), showed AM fungal association in endemic *Glyphochloa* species found in Goa only on the basis of root colonization and not up to spore identification level. This colonization was variedly ranging (22-86%) in *Glyphochloa species viz., G. acuminata* (Hack.) W.D. Clayton; *G. goaensis* (Rao &Hemadri) W.D. Clayton; *G. talboti* (Hook.f.) W.D. Clayton; *G. henryi* (Janarth et. al) and *G. Veldkampii* (Fonseca &Janarth). In earlier studies, Fonseca (2003), reported 34%

mycorrhizal infection in roots of *G. santapaui;* hyphae and vesicles occurrence was good but arbuscles were absent. Our findings shows almost two-fold colonization (Table 2).

Table 2. Status of AM fungal colonization in roots of *G. santapaui*

Particulars	AM fungal colonization in plant roots		
Fungal structures	**Vesicles**	**Arbuscules**	**Hyphae**
MCp (%)	92.31^{e}	30.77^{m}	92.31^{e}
OCp (%)		71.80^{g}	
OI	Excellent	Moderate	Excellent
OCI		Good	
Smc Features	Formation of *Av, Ch, ERs IRs* & *Mv,* **(Fig. 1B-D)**		
Pmc	VAH		
Other fungal endophytes	*Ofe*-Present dsh & Sc **(Fig. 1D)**		

(MCp) mean colonization percentage*;* (*OI*)Occurrence intensity [*(p) 1-25%, (m) 25-50%, (g) 50-75%, (e)>75%*]; (*Smc*)Structures of Mycorrhizal colonization [*(Av)*Aggregated vesicles, *(ch)* Chlamydospore, *(ERs)* Extraradial spore, *(IRs)*Intraradial spore and *(mV)*moniliform vesicles]; (*OCI*)Overall colonization intensity[range of values is same as *OI*]; (*OCp*)Overall colonization percentage*;* (*Pmc*)Pattern of Mycorrhizal colonization; (*VAH) Vesicular- Arbuscular-hyphal type; (ofe)Other fungal endophytes,,* [*(dsh)* dark septate melanised hyphae and *(sc)*sclerotia.

It is evident that, variation in the occurrence of fungal structural formations arbuscules, vesicles or hyphae may reveal functional differences in the AM symbiosis between plant species or growth conditions. Generally, arbuscules, are formed in the root cortex and known as the sites where carbon and nutrients are transferred between the plant and the fungal symbiont (Smith and Read, 2008). Highest N and P concentrations in plant tissue coincided with highest arbuscular colonization in temperate grasslands where P limited plant grows (Garcia and Mendoza, 2008). Nevertheless, during present investigation at tropical region of study area *G. santapaui* roots proliferates 30.77% arbuscular colonization on lateritic soil condition.

According to Smith and Read (2008), vesicles are assumed to be storage organs for energy reserves within the fungus. Treseder and Allen (2002) reported, increased vesicle colonization at nitrogen-limited sites reflecting higher investment of carbohydrates from the plant to the fungus thereby maintaining nutrient supply under sub-optimal conditions. In present investigation, although the arbuscular colonization is low our results shows highly equilibrated hyphal-vesiclular colonization (92.31%). Thus, it makes general agreement with Jalonen *et al.,* 2013, stated that, 'total AM colonization is often dominated by hyphal colonisation and may be remain unaffected'.

Besides the regular components of mycorrhiza like vesicles, arbuscules and hyphae, other structures such as chlamydospore (*Ch*), moniliform vesicles (*Mv*), extrararadial spore (*ERs*) and intrararadial spore (*IRs*) etc are also recorded in *G. santapaui* roots (Figure 1B-D). Furthermore, root segments are found colonized with other fungal endophyte (*Ofe*) components such as, dark septate melanised hyphae (*dsh*) and sclerotia (*Sc*) (Figure 1 D).

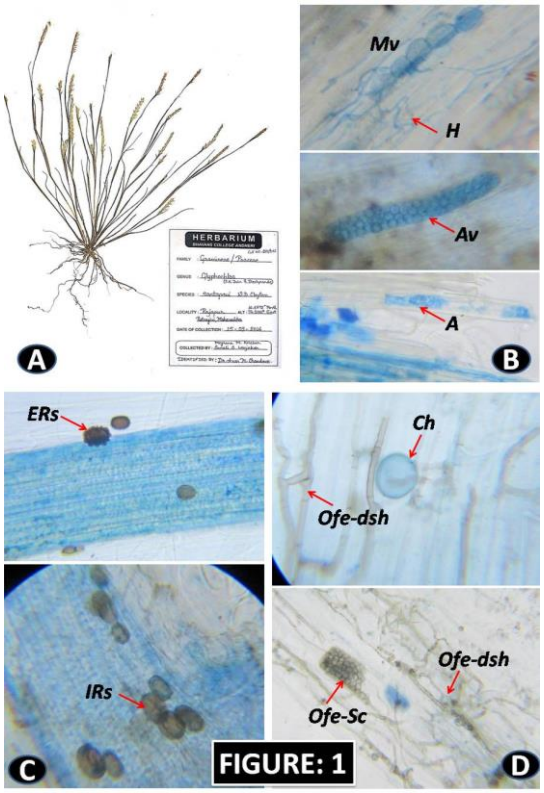

Fig 1 (A). *Glyphochloa santapaui* (Jain & Deshpande) W. D. Clayton : Specimen; Fig 1 (A-D) Root colonization showing mycorrhizal components *viz.* Arbuscules [*A*], Aggregated vesicles [*Av*]; Chlamydospore [*Ch*]; Hyphae [H], Moniliform Vesicles [*Mv*], Extrararadial spore [*ERs*]; Intrararadial spore [*IRs*] etc. and Fig 1 (D) Root colonization with other fungal endophyte [*Ofe*] components *viz.,* dark septate melanised hyphae [*dsh*] and Sclerotia [*Sc*].

In some studies, Dark Septate Endophytes (DSE) were reported in the roots of some plant species colonized by AM fungi (Jumpponen and Trappe 1998; Horton *et al.,* 1998; Muthukumar and Udaiyan 2002, Rains *et al.,* 2003). During present study, it was noteworthy that, some root samples were co-colonized by other fungal endophytes (*Ofe*) in general, particularly dark septate hyphae *(dsh),* sclerotia *(Sc)* and AM fungi (Figure 1 D). Barrow and Aaltonen (2001) suggested that DSE were better adapted to plants than aseptate fungi under certain conditions.

According to Jumpponen and Trappe (1998), and Jumpponen (2001) the role of DSE in roots remains unclear; they may function as pathogens or saprophytes, as well as mutualistic association similar to mycorrhiza. However, there is growing evidence that DSE may play roles similar to those of AMF in enhancing host growth and nutrition uptake (Barrow and Aaltonen 2001). Therefore, co-colonization of

Ofe and AM fungal components of *G. santapaui* root sytem needs further attention to access the level significance for this grass on community level.

Assessement of AM fungal species

In present study total 18 species of AM fungi under four families of Glomeromycetes such as: Acaulosporaceae, *Diversisporaceae,* Gigasporaceae and Glomeraceae are identified from the soil samples of *G. santapaui* distributed over 6 genera *viz., Acaulospora, Diversispora, Gigaspora, Scutellospora, Glomus* and *Sclerocystis*. The spores of all 18 species are presented in Table 3. Amongst the eighteen species, genus *Acaulospora* and *Glomus* represented five species (27.77%); *Gigaspora* represented four species (22.22%) and *Scutellospora* represented two species (11.11%). Whereas, remaining two genera *viz., Diversispora* and *Sclerocystis* represented only one species (5.55%).

Table 3. Identified AM fungi with their spore density (S) & relative abundance (RA) in soil sample of *G. santapaui*

Specimen Accession Code	AM fungal species	S	Relative abundance RA	
			RAspp	***RAfam***
	Family: Acaulosporaceae			
BCA:MH$_{MMK}$01	*Acaulospora elegans* Trappe & Gerd.	4	4.761	26.190
BCA:MH$_{MMK}$02	*Acaulospora rehmii* H. Magn.	12	14.285	
BCA:MH$_{MMK}$03	*Acaulospora scrobiculata* Trappe	2	2.380	
BCA:MH$_{MMK}$04	*Acaulospora tuberculata* Janos &Trappe	3	3.571	
BCA:MH$_{MMK}$05	*Aculospora appendicula* Spain, Sieverding & Schenk	1	1.190	
	Family: Diversisporaceae			
BCA:MH$_{MMK}$06	*Diversispora epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüßler	19	22.619	22.619
	Family: Gigasporaceae			
BCA:MH$_{MMK}$07	*Gigaspora albida* Schenck & Smith	2	2.380	35.714
BCA:MH$_{MMK}$08	*Gigaspora gigantea* (Nicol. & Gerd.) Ger. & Trappe	17	20.238	
BCA:MH$_{MMK}$09	*Gigaspora margarita* (Becker & Hall), Bentivenga & Morton	2	2.380	
BCA:MH$_{MMK}$10	*Gigaspora rosea* (Nicole & Schenck) Bentivenga & Morton	3	3.571	
BCA:MH$_{MMK}$18	*Scutellospora calospora* (Nicol and Gerd) Walker & Sanders.	2	2.380	
BCA:MH$_{MMK}$19	*Scutellospora dipurpusescrns* Mortan & Koske.	4	4.761	
	Family: Glomeraceae			
BCA:MH$_{MMK}$11	*Glomus gerdemanni* Rose, Daniels & Trappe.	3	3.571	15.476
BCA:MH$_{MMK}$12	*Glomus hoi* Berch & Trappe	1	1.190	
BCA:MH$_{MMK}$13	*Glomus occultum* Walker	1	1.190	
BCA:MH$_{MMK}$14	*Glomus versiforme* (P. Karst.) S.M. Berch	4	4.761	
BCA:MH$_{MMK}$15	*Glomus warcuppi* Mc Gee.	2	2.380	
BCA:MH$_{MMK}$16&17	*Sclerocystis sinuosa* Gerdemann & Bakshi	1+1*	1.190	
Total	***18 AM fungal species***	**84 (+1*sporocarp)**	**100**	

Earlier studies (Fonseca, 2003) attempted for mycorrhizal infection in 10 species comprising 3 varieties of *Glyphochloa* including *G santapaui*. Out of which 5 species and 2 varieties *viz., G. acuminata* var. *acuminata*; *G. acuminata* var. *woodrowii*; *G. forficulata; G henryi; G.ratnagirica;* and *G. talbotii* were explored only up to generic level identification of spores. These AM fungal spores associated with in different species of *Glyphochloa* were *viz.*, (i) *Glomus* and *Acaulospora* (in *G. acuminata* var. *acuminata* and *G.ratnagirica*); (ii) *Glomus* and *Scutellospora* (in *G henryi*); (iii) *Acaulospora, Glomus* and *Scutellospora* (in *G. acuminata* var. *woodrowii* and *G talbotii*). Recently (D'Souza and Fonseca 2015), first report on AM fungal association in five endemic *Glyphochloa* species such as *G. acuminata* (Hack.) W.D. Clayton; *G. goaensis* (Rao & Hemadri) W.D. Clayton; *G. talboti* (Hook.f.) W.D. Clayton; *G. henryi* (Janarth *et. al*) and *G. veldkampii* (Fonseca & Janarth) found in Goa is published. But the scope of report is limited to root colonization discussion only, while information about species of AM fungi is not produced.

During present work, AM fungal species associated with *G. santapaui* are identified up to species level (Table 3). These species are *viz., Acaulospora elegans* Trappe & Gerd., *Acaulospora rehmii* H. Magn, *A. scrobiculata* Trappe, *A. tuberculata* Janos & Trappe, *A. appendicula* Spain, Sieverding & Schenk; *Diversispora epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüßler; *Gigaspora albida* Schenck & Smith, *G. gigantea* (Nicol. & Gerd.) Ger. & Trappe, *G. margarita* (Becker & Hall), Bentivenga & Morton, *G. rosea* (Nicole & Schenck) Bentivenga & Morton; *Scutellospora calospora* (Nicol and Gerd) Walker & Sanders.; *S. dipurpusescrns* Mortan & Koske.; *Glomus gerdemanni* Rose, Daniels & Trappe., *G. hoi* Berch & Trappe, *G. occultum* Walker, *G. versiforme* (P.Karst.) S.M. Berch, *G. warcuppi* Mc Gee. and *Sclerocystis sinuosa* Gerdemann & Bakshi. Thus, G. *santapaui* is the first representative of genus *Glyphochloa* ever studied by any Indian mycologist with reference to root colonization followed by spore's identification of AM fungi at species level.

Spore density and and relative abundance

The total number of AM fungal spores recovered from soil samples of *G. santapaui* are 84 including one sporocarp and encountered at the rate of 1-19 spores 10^{-1} g soil as shown in Table 3. The spore density (S) of all 18 AM fungi is determined and expressed as number of spores per 10g of soil of *G. santapaui*

which is presented in Table 3. Among the 18 species following three species *viz., Diversispora epigaea* (S=19), *Gigaspora gigantea* (S=17) and *Acaospora rehmii* (S=12) are apparently dominating the soil sample. However, based on spore density and relative abundance, two species are found dominating the soil (S ≥ 15 spores 10 g^{-1} soil, *RAspp* > 6%) i.e. *Diversispora epigaea* (*S: 19 & RAspp:* 22.619) and *Gigaspora gigantea* (*S: 17 & RAspp:* 20.238).

Thus in present investigation more number of AM fungal species are recovered from soil associated with *G. santapaui.* More number of AM fungi are belonging to Acaulosporaceae (44.44%) followed by Gigasporaceae (33. 33%). Hence, it can be concluded that, all the soil samples of *G. santapaui* shows establishment of multi-sporic pattern of AM fungal colonization.

Based on Relative abundance of spores in Glomeromycota family, we suggest that, Gigasporaceae (*RAfam*=35.714) and Acaulosporaceae (*RAfam*=26.190) contributes more propogules as compared to Diversisporaceae (*RAfam*=22.619) and Glomeraceae (*RAfam*=15.476). Hence, it can be concluded that, family Gigasporaceae is reported as dominating family associated with *G. santapaui.*

Conclusion

Increased human anthropogenic activities on Ferricrete-Lateritic rocky plateaus of Konkan region of Maharshtra are imposing great threat to many endemic taxa. The conservation efforts for these plants are rare and furthermore there are no assessments of AM fungal association with endemic grass species. Hence, there is need of conservational studies by developing effective biological methods, so that to propagate and protect these endemic species. In recent years, AM fungal potential to support plant growth and their adaptability under natural habitat is considered on priority basis in many conservation projects for rare, endemic and endangered taxa. Present data perceptibly facilitate the potential use of identified AM fungi associated with *G. santapaui* and in near future accomplishments aimed at their conservation. However, it needs extension of work to isolate these native AM fungi in pure state of axenic culture followed by mass multiplication so that consortium may be produced for its application in conservation program of vulnerable and endemic grass *G. santapaui.*

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References

1. Arora DK. Handbook of Applied Mycology. Vol. 1: Soil and Plants. New York, NY: Marcel Dekker, Inc. 1991, 1-736.

2. Barrow JE and Roncaderi WR. Mycorrhizal benefit survival and growth of sweet gram in the nursery. *J. Appl. Forest*, 1977, 1, 21-23.

3. Barrow JR and Aaltonen RE. Evaluation of the internal colonization of Atriplex canescens (Pursh) Nutt. roots by dark septate fungi and the influence of host physiological activity. *Mycorrhiza*, 2001. *11,* 199–205.

4. Brundrett MC and Kendrick WB. A developmental study of early stages in vesicular arbuscularmycorrhiza formation. *Canadian Journal of Botany,* 1996, *66,* 184-194.

5. D`Souza J, Fonseca MA. *Arbuscular mycorrhizal* (AM) Fungal Status in Endemic Genus *Glyphochloa. The Journal of Biodiversity. Photon,* 2015, 115, 461-464.

6. Dandan Z and Zhiwei Z. Biodiversity of arbuscular mycorrhizal fungi in the hotdry valley of the Jinsha River, southwest China. *Appl. Soil Ecol.,* 2007, *37*, 118–128.

7. Fonseca MA Systematic studies on the genus *Glyphochloa* W D Clayton. PhD Thesis. 2003. Goa University. http://shodhganga.inflibnet.ac.in/handle/10603/35627.

8. Garcia IV, Mendoza RE. Relationships among soil properties, plant nutrition and arbuscular mycorrhizal fungi-plant symbioses in a temperate grassland along hydrologic, saline and sodic gradients. *FEMS Microbiol Ecol*, 2008. 63:359–371.

9. Gaur A and Adholeya A. Estimation of VAMF spores in soil: a modified method, *Mycorrhiza News, 6,* 1994. 10-11.

10. Gerdemann JW, and Nicolson TH. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.,* 1963, 46, 235-244.

11. Gosavi KVC, Yadav SR, Karanth KP, and Surveswaran S. Molecular phylogeny of *Glyphochloa* (Poaceae, Panicoideae), an endemic grass genus from the Western Ghats, India. *J. Syst. Evol. 9999 (9999):* 1–13, 2015. doi: 10.1111/jse.12185.

12. Grime JP, Mackey JML, Hillier SH and Read DJ. Floristic diversity in a model system using

experimental microcosms. *Nature,* 1987, 328, 420-422.

13. Hall IR and Fish BJ. A key to the Endogonaceae. *Trans. Br. Mycol. Soc.*, 1979, *73,* 261-270.

14. Hall IR. Growth of *Lotus pedunculatus* Cav. in an eroded soil containing soil pellets infected with endmycorrhizal fungi *N. Z. J. Agri. Res.*, 1980, *23*, 103-105.

15. Hingsinger P, Plassard C, Tang C, and Jaillard, B. Origins of root mediated pH changes in the rhizosphere and their responses to environmental constraints: *A review. Plant and Soil* 2003, 248, 43-59.

16. Horton TR, Ca´zares E and Bruns TD. Ectomycorrhizal, vesicular–arbuscular and dark septate fungal colonization of bishop pine (Pinus muricata) seedlings in the first 5 months of growth after wildfire. *Mycorrhiza*, 1998, 8, 11–18.

17. Irwin SJ and Narasimhan D. Endemic genera of angiosperms in India: A review. *Rheedea,* 2011, 21: 87–105.

18. Jackson ML. Soil chemical analysis, Prentice Hall of Indian Private Limited, New Delhi. 1967.1-498.

19. Jalonen R, Timonen S, Sierra J and Nygren P. Arbuscular mycorrhizal symbioses in a cut-and-carry forage production system of legume tree *Gliricidia sepium* and fodder grass *Dichanthium aristatum Agroforest Syst* 2013, 87:319–330. DOI 10.1007/s10457-012-9553-1.

20. Jones DL, Dennis, PG, Owen AG, and van Hees PAW. Organic acid behaviour in soils-misconceptions and knowledge gaps. *Plant and Soil* 2003, 248, 31-41.

21. Jumpponen A and Trappe JM. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol. 1998. 140, 295–310.

22. Jumpponen A. Dark septate endophytes – are they mycorrhizal? *Mycorrhiza* 2001. *11,* 207–211.

23. Lambers H, Chapin III FS and Pons TL. Plant Physiological Ecology. Springer-Verlag, New York. 1998, 1-605.

24. Morton JB. Taxonomy of mycorrhizal fungi: classification, nomenclature, and identification. *Mycotaxon.* 1988, *32,* 267 –324.

25. Murakoshi T, Tojo M, Walker C and Saito M. Arbuscular mycorrhizal fungi on adjacent semi-natural grasslands with different vegetation in Japan *Mycoscience,* 1998, 39, 455-462.

26. Muthukumar T and Udaiyan K. Seasonality of vesiculararbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta Oecologica,* 2002. 23, 337–347.

27. Olsen SR, Cole CV, Cole FS, Watanabe and LA Dean. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circ. No. 939.* U.S. Dept. Agric. Washington, D.C. 1954, 1-19.

28. Pacioni G. Wet - sieving and decanting technique for the extraction of spores of vesicular arbuscularmycorrhizal fungi, *Meth. Microbiol,* 1992, *22,* 317-322.

29. Phillips JM and Hayman DS. Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection, *Trans. Br. Mycol. Soc.* 1970. *55,* 158 – 161.

30. Piper CS *Soil and Plant Analysis*, Hans Publishers, Bombay. 1966. 1-368.

31. Rains KC, Nadkarni NM and Bledsoe CS. Epiphytic and terrestrial mycorrhizas in a lower montane Costa Rican cloud forest. *Mycorrhiza*, 2003 *13,* 257–264.

32. Rawat, GS, Desai A, Somanathan H and Wikramanayake ED. Malabar Coast moist forests (IM0124). Available at: http://worldwildlife.org/ecoregions/im0124. 2001.

33. Ringwall KD and Dickinson ND. Vesicular-Arbuscular Mycorrhizae Fungi Colonization in Native Grasses *Rangelands* 1997, 19(1), 15-18.

34. Roelofs R, Rengel Z, Cawthray GR, Dixon KW and Lambers H. Exudation of carboxylates in Australian Proteaceae: Chemical composition. *Plant Cell and Envir,* 2001, 24, 891-903.

35. Romand-Monnier F. *Glyphochloa santapaui. The IUCN Red List of Threatened Species* 2013: e.T44393261A44522418.http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T44393261A44522418.en

36. Royal Botanic Gardens, Kew. Electronic Plant Information Center [online]. Available from http://epic.kew.org/epic/ [accessed 16 March 2018].

37. Schenk NC and Perez Y. Manual for the identification of VA -Mycorrhizal fungi, third edition. University of Florida, Gainesville, Florida, 1990.

38. Schultz PA, Miller RM, Jastrow JD, Rivetta CV and Bever JD. Evidence of mycorrhizal mechanism for the adaptation of Andropogon gerardii (Poaceae) to- High and - Low nutient Prairies. *American journal of botany*, 2001, 88(9),1650-1656.

39. Smith SE, Read DJ. Mycorrhizal Symbiosis, 3rd edn. Academic Press, London 2008.

40. Smith SE, Read DJ. Mycorrhizal symbiosis, 3rd edn. Academic, UK 2008.

41. Thorpe CJ and Watve A. Lateritic Plateaus in the Northern Western Ghats, India; a Review of Bauxite Mining Restoration Practices. *Journal of Ecological Society*. 2015, 28, 25-44.

42. Treseder KK, Allen MF. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytol,* 2002. 155: 507–515.

43. Van Reeuwijk LP. Procedures for soil analysis: Technical paper-9; International *Soil Reference and Information Centre*, P.O. Box 353, 6700 AJ Wageningen, The Netherlands; 6th ed. 2002. 1-120.

44. Verboom WH, Galloway PD. National Landcare Program (Australia), and Natural Heritage Trust (Australia). *Corrigin area land resources survey: Report 20.* Department of Agriculture and Food, Western Australia, Perth. 2004, 1-65.

45. Walkley A and Black IA. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci., 37*, 1938. 29-37.

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