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OCCURRENCE OF MYCORRHIZAE IN SOME SPECIES OF *CAREX* (CYPERACEAE) OF THE DARJEELING HIMALAYAS, INDIA

***ASOK GHOSH[1], SHEKHAR BHUJEL[2] AND GAUR GOPAL MAITI[3]**

***[1]Department of Botany, Krishnagar Govt. College, Nadia, West Bengal, India**
[2]Department of Botany, Darjeeling Govt. College, Darjeeling, West Bengal, India
(Presently DTR&DC, Tea Board, A.B. Path, Kurseong, West Bengal, India)
[3]Department of Botany, University of Kalyani, Kalyani, West Bengal, India

ABSTRACT

The Cyperaceae (sedge family) have generally been considered non-mycorrhizal, although recent evidences suggest that mycotrophy may be considerably more widespread among sedges than was previously realized. In this study, 28 in situ populations of 12 species of *Carex* L.(*C. myosurus* Nees, *C. composita* Boott, *C. cruciata* Wahl., *C. filicina* Nees, *C. inanis* Kunth, *C. setigera* D. Don, *C. insignis* Boott, *C. finitima* var. *finitima; C. fusiformis* Nees subsp. *finitima* (Boott) Noltie, *C. teres* Boott, *C. longipes* D. Don ex Tilloch and Taylor, *C. nubigena* D. Don ex Tilloch and Taylor and *C. rochebrunii* subsp. *rochebrunii; C. remota* Linnaeus subsp. *rochebrunii* (Franchet and Savatier) Kükenthal) occurring in the Darjeeling Himalayas were surveyed. Mycorrhizal infection by VAM (Vesicular Arbuscular Mycorrhiza) fungi was found in roots of 9 species (*C. myosurus, C. composita, C. cruciata, C. filicina, C. inanis, C. setigera, C. finitima, C. teres,* and *C. nubigena*) and appears to occur in response to many factors, both environmental and phylogenetic. In non-mycorrhizal species, a novel root character, the presence of bulbous-based root hairs, was identified.

Key words: *Carex,* Cyperaceae, vesicular arbuscular mycorrhiza (VAM), mycotrophy, root hairs

1. INTRODUCTION

Mycorrhizal association has been reported in most of the families of the modern day taxa, but there have also been exceptions as Brassicaceae, Cyperaceae, Juncaceae, which are assumed to be non-mycorrhizal (Hirsch and Kapulnik, 1998). Sedges, due to their disturbed habitat condition were long considered as non-mycorrhizal (Newman and Reddell, 1987). Harley and Smith (1983) mentioned Cyperaceae as non-mycotrophs and further confirmation was given by Bagyaraj et al. (1979) when no colonization was reported on *Cyperus eleusinoides* Kunth. Moreover, further works on mycorrhizal association by Koske and Halvorson (1981), Malloch and Malloch (1982), and Brundrett and Kendrick (1998), Louis (1990) also reported the absences of association in this group. The non-mycorrhizal nature of *Carex coriaceae* Hamlin and *Uncinia divaricata* Kük., even under extreme phosphorus deficient condition tempted (Powell, 1975) to conclude sedges among non-mycorrhizal groups. Sedges like *Carex flacca* Schreb*, C. mertensii* Prescott ex Bong. and *Cyperus rotundus* Linn. are not benifitted by mycorrhizal association and have maximum biomass in absence of this association (Muthukumar et al. 1997; Titus and del Moral, 1998; Vander Heijden et al. 1998). Moreover lack in mycorrhizal association enhances the competitive ability of plants (Titus and del Moral,1998). In spite this assumptions that sedges are non-mycorrhizal, there have been several reports on mycorrhizal association in sedges (Harley and Harley, 1987; Tester et al. 1987; Newman and Reddell, 1987; Miller et al. 1999; Muthukumar et al. 1996, 1997; Muthukumar and Udaiyan 2000). Harley and Harley (1987) examined 54 species of Cyperaceae, of which 31% had mycorrhizal infection. In a study in Southern India that sampled 24 species for six genera of Cyperaceae, all the plants sampled had some infection and 42% of them had formed arbuscules (Muthukumar et al. 1996, 1997; Muthukumar and Udaiyan 2000). Miller et al. (1999) have also reported the presence of mycorrhizaae in *Carex* L. along with its simple types. Hence, this variation in mycorrhizal association as noted in sedges in general and the genus *Carex* in particular may be environmental or edaphic rather than phylogenetic constraint (Read et al. 1976; Muthukumar et al. 1996). It has also been speculated that the non-mycorrhizal condition of some sedges might result from their habitat condition, anaerobic soil rather than taxonomic position (Tester et al. 1987). But to complicate the matters even more, the root of the sedges appear to be associated with several kinds of fungi or even dark septate hyphae (Haselwandter and Read, 1980; 1982; Bledsoe et al. 1990; Kohn and Stasovski, 1990). The study of development and seasonal fluctuations in VAM associated plants have been carried out by Sanders and Fitter (1992), Louis and Lim (1987), Brundrett (1991), and have concluded that VAM development depends on edaphic factors, or variation in plant nutrient level (Mullen and Schmidt 1993; Tester et al. 1987). Effects of moisture and nutrient availability on root growth and VAM colonization was reported by (Raab et al. 1999; Rabatin, 1979; Allen, 1983; Muthukumar et al. 1997). As there is negative correlation between mycorrhizal association and soil moisture (Anderson et al. 1984) the non mycorrhizal nature of sedges may be due to their presence in marshy land, anaerobic soil rather than taxonomic position (Haselwandter and Read, 1980; Kohn and Stasovski, 1990). Production of swollen dauciform roots with long hairs when growing in nutrient deficient condition was reported by (Davies et al. 1973; Lamont, 1974; Miller et al. 1999) which increases under low nutrient condition (Fohse and Jungk, 1983; Fohse et al. 1991) and this presence of root hairs were negatively correlated with mycorrhizal association (Baylis, 1975). This fuzziness in root hairs in *Carex* spp. has been used by authors like Reznicek (1986) for description and identification of species. Fungal hyphae and its relation with vesicle formation were reported by Giovannetti and Sbrana (1998), which occurs after arbuscular development. These vesicles are considered as storage organs and often functions as propagules (Biermann and Lindermann, 1983). Uptake of carbon from host cell interface does prevail (Solaiman and Saito 1997; Douds et al. 2000), and hyphal network could be involved in carbon transport (Robinson and Fitter, 1999). Along with the presence of fungal mycelium, vesicles, arbuscular unidentified dark septate fungus was also reportd in several species of *Carex* from Artic and Alpine region (Jumpponen and Trappe, 1998) and by Miller et al. (1999) from the shavanas.

Since 1987, studies and information have been made available for 221 sedges species (including 78 species of *Carex*; of which 27 mycorrhizal, 4 facultative mycorrhizal and 47 non-mycorrhizal) of which 88(40%) are mycorrhizal 24(11%) are facultative and 109(49%) are non-mycorrhizal (Muthukumar et al. 2004). Based on these studies, the genus *Carex* as well as the family Cyperaceae is no longer a non-mycorrhizal, but the mycorrhizal. Even, status is greatly influenced by environmental conditions. Even more, *Carex* spp. appear to have several morphological adaptations to thrive in the absence of mycorrhizal associations. Though mycorrhizal, associations have been noted in many *Carex* species, the ecological role of this association is not well documented. Similarly, the beneficial role of mycorrhiza in *Carex* spp. and other members of sedges are yet to be fully ascertained. Hence, to verify, many reports on the mycorrhizal association in *Carex* and since no report was found to have been reported from this region the study was conducted and there is a comprehensive report of mycorrhizal association in Cyperaceae especially in the genus *Carex*.

2. MATERIALS AND METHODS

The plants worked out for mycorrhizal association were collected from Darjeeling, Senchal, Rangbul, Leebong, and Lava Bazar of the Darjeeling

Himalayas. Same specimens were collected from different location to see the variability among mycorrhizal association. The roots of 28 populations for 12 species of *Carex,* Cyperaceae were sampled in between August, 2006 and November, 2010. From the study site plant specimen were collected along with the roots and the rhizosphere. (Details of the collection information presented in Table 1). Rhizospheric soil was collected for each sample by shaking the soil loose from the roots, as they were collected. This assured that the soil of collected plant materials were packed in a polythene bag along with roots and soils from the study site itself. Upon return to the work place, the core attached with plant shoot was placed in a large polythene bag and refrigerated at 0°C to 4°C. Within 3 days of sampling, the soil was excavated and the root portion was washed out until most of the mud particles were rinsed (after Miller et al. 1999). To assure that roots of appropriate individuals were sampled, rhizomes and stolons, attached to the main root were excavated. Young and newly formed roots were selected and carefully collected. This could be easily distinguished by their pale brown to brown coloration, against the dark brown to black colors of old and dead roots (Muthukumar et al. 1999). Only fibrous roots attached to the crown of the *Carex* were retained. These roots were cut into 1-1.5 cm long segments, cleared in 10% KOH for 16 to 18 hours, acidified with HCl for 40 to 60 minutes and then stained with Trypan Blue (0.5 gm/liter) in 1:2:2 volume of Lactic acid: Glycerol: De-ionised Water (by volume). Finally root segments were then mounted on the glass slide, carefully spreaded with the help of a brush, de-stained with lacto-phenol and then sealed with a cover slip. The prepared slides were examined under various magnifications (10X, 40X and 100X) and the presence or absence of mycorrhizal association was determined. The mycorrhizal status of the plant (that is the presence or absence of colonization by mycorrhizal fungal structures) was determined in the presence of vesicles, arbuscules, hyphae, and intraradical spores. The occurrence of dark septate hyphae was also noted. Notes were taken on root morphology, such as "Fuzziness" and presence or absence of bulbous root hairs was also taken in account. Minimum 20 to 25 intersections were observed for each sample and photographs were taken where any kind of presence was noted. Notes were specially taking account of the presence or absence of same type or different type of mycorrhizal associations in the same species or in a different species, or even the different individual of same species.

2.1. ESTIMATION OF AVAILABLE PHOSPHORUS FROM THE SOIL

Since the availability of phosphorus is inversely related with the presence of mycorrhizal association (Allen et al. 1987; Muthukumar and Udaiyan, 2000; Raab et al. 1999; Rickerl et al. 1994); test for estimation of total phosphorus (Table 2) was carried out with every soil sample collected from the rhizosphere of all the populations studied to find out correlation if any present between the phosphorus availability and mycorrhizal association. The phosphorus present was ascertained through modified methods of Mazumdar and Majumder (2003).

3. OBSERVATION AND DISCUSSION

Out of 28 individual populations for 12 species of *Carex* (*Carex myosurus* Nees, *C. composita* Boott, *C. cruciata* Wahl., *C. filicina* Nees, *C. inanis* Kunth, *C. setigera* D. Don, *C. insignis* Boott, *C. finitima* var. *finitima; C. fusiformis* Nees subsp. *finitima* (Boott) Noltie, *C. teres* Boott, *C. longipes* D. Don ex Tilloch and Taylor, *C. nubigena* D. Don ex Tilloch and Taylor and *C. rochebrunii* subsp. *rochebrunii; C. remota* Linnaeus subsp. *rochebrunii* (Franchet and Savatier) Kükenthal) were assessed for mycorrhizal association, 25 were found infected and 3 populations of 3 different species (*C. insignis*, *C. longipes* and *C. rochebrunii*) were without any colonization. Arbuscular mycorrhizal associations were found in 9 species (*Carex myosurus*, *C. composita*, *C. cruciata*, *C. filicina*, *C. inanis*, *C. setigera, C. finitima*, *C. teres* and *C. nubigena*) and 11 out of 28 populations sampled with the occurrence percentage of 41.37% (Table1).

Table 1

Details of the collection information and mycorrhizal status of the studied specimen

Sl. No	Name of the species	Place of collection	Date of collection	Infection type	Bulbous root hair
01	*Carex myosurus* Nees	1. Grave yard 2. Mall Road	1. 06/06/10 2. 15/07/10	M, V, A(*Paris* type) M, V	(-) (-)
02	*Carex composita* Boott	1. Lava Bazar 2. Lava Bazar	1. 10/08/10 2. 10/08/10	M, S, A M, V, S	(+) (+)
03	*Carex cruciata* Wahlenb.	1. Senchel 2. Mall Road	1. 29/06/10 2. 15/07/10	M, V, S M, V, A D.S.H	(-) (-)
04	*Carex filicina* Nees	1. Senchel 2. Rangbull 3. Rangbull 4. Senchel	1. 14/06/10 2. 30/07/10 3. 30/07/10 4. 06/06/10	M, H, V M, V, A V, S M, V	(-) (-) (-) (-)
05	*Carex inanis* Kunth	1. Senchel 2. Darjeeling Govt.College compound	1. 14/06/10 2. 15/07/10	M, V, S, A M, V, E.F	(-) (-)
06	*Carex setigera* D. Don	1. Senchel 2. Senchel	1. 29/06/10 2. 29/06/10	V, M, A M	(+) (-)
07	*Carex insignis* Boott	1. Lebong 2. Lebong	1. 07/07/10 2. 07/07/10	M M, V	(-) (-)
08	*Carex finitima* var. *finitima Carex fusiformis* Nees subsp. *Finitima* (Boott) Noltie	1. Rangbull 2. Rangbull	1. 30/07/10 2. 30/07/10	M, V, A, S M, V, S, A	(-) (-)
09	*Carex teres* Boott	1. Senchel 2. Senchel	1. 30/05/10 2. 14/06/10	A, V, S, M M, V, S, A	(-) (-)
10	*Carex longipes* D.Donex TillochandTaylor	1. Senchel 2. Grave yard, Darjeeling	1. 14/06/10 2. 15/07/10	M, H, V M, V, S	(-) (+)
11	*Carex nubigena* D. Don ex Tilloch and Taylor	1. Senchel 2. Senchel	1. 14/06/10 2. 29/06/10	M, V, A(*Paris* type) M, V, D.S.F	(-) (-)
12	*Carex rochebrunii* subsp. *rochebrunii Carex remota* Linnaeus subsp. *rochebrunii* (Franchet andSavatier) Kükenthal	1. Senchel 2. Grave yard 3. Grave yard 4. Mall Road, Darjeeling	1. 30/05/10 2. 06/06/10 3. 06/06/10 4. 15/07/10	(-) (-) (-) M	(+) (+) (+) (+)

N.B.; A=arbuscule; M=mycelial; V=vesicle; DSH=dark septate hyphae; E.F=endophytic fungi; S=spore; DSF=Dark septate fungi.

Examinations of the root hairs of different species of *Carex* revealed differences in morphological features and these were correlated with the species mycorrhizal status. The root hairs typically for the majority of species of *Carex* under the study were usually quite long, greater than 1 mm and sparsely distributed. In some species as *C. rocheburnii* the roots were covered with fine layers of comparatively distinct short hairs. The roots are somewhat variable in morphological features and are distinguished by their bulbous swellings at base. These are sometimes topped by filamentous root hairs as also found in *C. rocheburnii* and *C. longipes, C. setigera* and *C. finitima*. They always form mat covering every surface of root. The presence of bulbous-based root hairs was negatively associated with the presence of mycorrhizal association. In fact *C. rocheburnii* with these bulbous swellings were found not to be infected but the other specimen with these structures had many other different types of association. Further study revealed the presence of mycorrhizal association in other sections of the roots. Other species with arbuscules were either with dense root hairs or completely lacking. The species like *C.*

teres, C. nubigena and *C.myosurus* (Figure) did not have any root hairs but *C. finitima* and *C. cruciata* have short dense root hairs. In some specimen presence of fungal spore was also noticed both inside the root cells and outside the root vicinity as found in *C. teres, C. filicina, C. inanis, C. cruciata, C. finitima,* and *C. composita* (Figure). Mycelial type of association was of common occurrence with highest percentage (86.20%) as observed in all the specimens. Even, vesicles were also much observed and also quite in occurrence with the percentage of 75.86%. The hyphae were hyaline and well ramified.

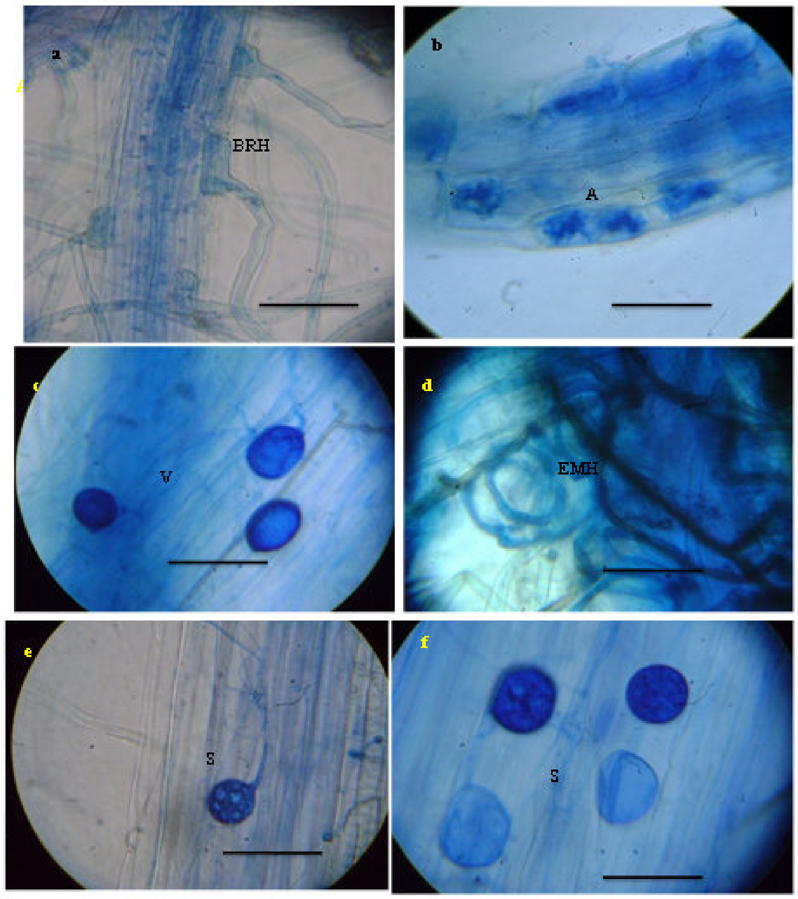

Figure
Microphotographs of root of Carex spp.
a. Bulbous-based root hairs(BRH) of C. rochebrunii;
b. Arbuscle (A) of C. nubigena;
c. Vesicles(V) of C. teres;
d. Extramatrical (EMH) and intraradical hyphae of C. teres;
e. and f. Spores(S) of Paris type endomycorrhizal fungi of C. teres. Scale bars A-F (20µm)

Out of 28 populations sampled, 25 were infected either with arbuscules, vesicles, mycelium, and fungal spores or sometimes with dark septate endophytic fungi. Mycorrhizal association in *Carex* was present in higher amount than accounted before by many workers as Harley and Harley (1987), Tester et al. (1987), Newman and Reddell (1987), Miller et al. (1999), Muthukumar et al. (1996, 1997) and Muthukumar and Udaiyan (2000). Because all *Carex* species examined were not present at all sampled site, thus could not definitely attribute site related effects of differences in environmental variables, since some species were noted from wetlands with running water (*C. teres, C. finitima* and *C. rocheburnii*), whereas some species were from quite dry region among the slopes (*Carex cruciata* and *C. myosurus*) or in the walls or even avobe the rocks (*Carex insignis*).

Table 2
Details of the available phosphorus in soil with mycorrhizal incidence

Sl. No	Names of the species	Date of collection	Concentration (%)
I	*Carex myosurus* Nees	1. 06/06/10 2. 15/07/10	0.0014 0.0013
II	*Carex composita* Boott	1. 10/08/10 2. 10/08/10	0.0015 0.0017
III	*Carex cruciata* Wahl.	1. 29/06/10 2. 15/07/10	0.0015 0.0010
IV	*Carex filicina* Nees	1. 06/06/10 2. 14/06/10 3. 30/07/10 4. 30/07/10	0.0014 0.0018 0.0013 0.0019
V	*Carex inanis* Kunth	1. 14/06/10 2. 15/07/10	0.0011 0.0006
VI	*Carex setigera* D. Don	1. 29/06/10 2. 29/06/10	0.0019 0.0010
VII	*Carex insignis* Boott	1. 07/07/10 2. 07/07/10	0.0032
VIII	*Carex finitima* Boott	1. 30/07/10 2. 30/07/10	0.0011 0.0012
IX	*Carex teres* Boott	1. 30/05/10 2. 14/06/10	0.0008 0.0015
X	*Carex longipes* D.Don ex Tilloch and Taylor	1. 14/06/10 2. 15/07/10	0.0031 0.0015
XI	*Carex nubigena* D.Don ex Tilloch and tylor	1. 14/06/10 2. 29/06/10	0.0012 0.0009
XII	*Carex rochebrunii* Franch. and Savatier	1. 30/05/10 2. 06/06/10 3. 06/06/10 4. 15/07/10	0.0019 0.0039 0.0022 0.0008

The observed data (Presented in Table 2) indicates that where there is less concentration of phosphorus in soil there is maximum intensity and type of mycorrhizal infection. The phosphorus content in soil is quite low and the species *C. teres, C. nubigena, C. filicina, C. inanis* etc. are growing with mycorrhizal association. When the concentration of phosphorus is relatively higher incidence of mycorrhizal appearance in general and arbuscular mycorrhizae in particular is low as found in species of *C. rochebrunii* and *C. insignis*. Even for the same species but different population of *C. rochebrunii* and even in *C. inanis* there seems to have been a different type of association due to variations of habitat conditions. Based on the present study it cannot solely be concluded that phosphorus availability is directly affects the mycorrhizal status in *Carex*. These differences had been reported due to seasonal fluctuations as noted by Muthukumar and Udaiyan (2000). It was even stated by Miller et al. (1999) that mycorrhizal association and phosphorus availability in soil is less or not related. But in this study it was observed that high phosphorus avaibility in soil was negatively correlated with mycorrhizal association as found in *C. rocheburnii* and *C.insignis*, whereas low concentration of phosphorus led to well establishment as in the case of *C. nubigena, C. finitima*. Even when there was low phosphorus content, the association was noticed between plant specimen and mycorrhiza, and on the same species when the phosphorus content was little higher there was no or a very little association (*C. rocheburnii* and *C. inanis*). In the mid range, mycorrhizal association was observed in most of the specimens of *C. filicina, C. composita* and *C.inanis*. By this observation it can be concluded that phosphorus availability in soil is inversely related to mycorrhizal association as stated by Allen et al. (1987), Rickerl et al. (1994), Raab et al. (1999) and Muthukumar and Udaiyan (2000). The observation presented in other studies and the present finding indicates that the extent of mycotrophy in genus *Carex* is much greater than that had been realized before. Harley and Smith (1983) mentioned the family Cyperaceae as “Non mycotrophic”. Bagyaraj et al. (1979) observed that *Cyperus eleusinoides* Kunth (aquatic macrophyte) was not colonized by mycorrhizal fungi. However, they have considered that the establishment of association was not favoured in plants due to aquatic habitat. Studies performed in different areas by Koske and Halvorson (1981), Malloch and Malloch (1982), Brundrett and Kendrick (1988) and Louis (1990) did not find any colonization in the species of this genus. However *Kobresia myosuroides* (Vill.) Fiori and Poal was found to form ectomycorrhiza in the artic area (Kohn and Stasovski, 1990). Bledsoe et al. (1990) have considered that arbuscular mycorrhizal association are rare in high artic area, and probably has a small or no importance in improving growth and nutrition of species in such environment. Presence of arbuscular mycorrhiza and other forms in specimens of *Carex* spp. as seen here confirm the observation of other authors like Harley and Harley (1987), Miller et al. (1999). Muthukumar and Udaiyan (2000) suggested that in genus *Carex*, taxonomic and environmental factors have influenced the mycorrhizal status. Hence, based on these observations, Cyperaceae should not be in the non-mycorrhizal group, since the strong evidence of association in several species of this family does exist. In this study, most of the species were typically mycorrhizal and some depends on environmental factors as in *C. rocheburnii,* 3 out of 4 studied populations were non-mycorrhizal and the remaining one showed only mycelial type of association. *C. rocheburnii* being “flood-tolerant” and the influence of environment may have been the major cause, since its habitat is with high moisture content. Due to this there may have been the fluctuations in the occurrence of mycorrhizae. Morphologically some *Carex* species produce swollen dauciform roots with long hairs when growing in nutrient deficient and poorly drained soil (Davies et al. 1973; Lamont, 1974). It is believed that similar proteoid roots may be morphological adaptation to the non-mycorrhizal condition (Lamont, 1993). The occurrences of root hairs increase the nutrient uptake and its abundance increases under low nutrient condition (Fohse and Jungk, 1983; Fohse et al. 1991). Moreover, root hair abundance and length of root hairs are negatively correlated with mycorrhizal dependency or benefit (Baylis, 1975). In this study, unique root hair morphology was found in some species as *C. rocheburnii, C. longipes, C. setigera, C. composita* where the morphology of root hairs was quite different. The root hairs with bulbous swelling base in *C. rocheburnii* found in non-mycorrhizal condition and in other cases where present mycorrhizal incidence was very low. This characteristic feature gives the unique root hair morphology with bulbous swellings, which is associated with the non-mycorrhizal condition. This morphological feature or the “fuzziness” in roots has used by Reznicek (1986) for description and identification of different species of *Carex*. These root hairs also can be the consequence of adaptation as the hair production of root in *Carex* as has been shown to increase under soil anoxia (Moog and Janiesh, 1990). Such a pre-adaptation to non-mycorrhizal condition might have allowed *Carex* spp. to colonize in wetland. Hence, the association between the bulbous-based root hairs and non-mycorrhizal state may have been coincidental. The fuzziness in roots may have been resulted as the consequence of water-logged condition. Even this character is not consistently present in other species. This study even clearly showed the presence of VAM which were present within the roots of the *Carex* spp. and correlated with other studies in reference to VAM association (Tester et al. 1987; Koske et al. 1992; Muthukumar et al. 1996, 1997; Muthukumar and Udaiyan, 2000). In this study, even most association types are as arbuscules, vesicles, hyphal, spores and dark septate hyphae, endophytic fungi were also found. Moreover, many authors had described the presence of non-mycorrhizal families with intercellular development of fungal hyphae often associated with the formation of *Glomus* type vesicles (Giovannetti and Sbrana, 1998) which usually occur after arbuscules development in VAM host. The vesicles produced by VAM fungi are considered to function as storage organs and when with multi-layered propagules can be isolated from the roots (Biermann and Lindermann, 1983). Studies revealed that intra-radical hyphae could take up carbon from the host cell interface (Solaiman and Saito, 1997; Douds et al. 2000). Further, root systems of adjacent plant species or individuals can be linked through the VAM fungal hyphal network in soil. These connections could be involved in nutrient and carbon transport (Robinson and Fitter, 1999) which could be of major significance for the survival of mycorrhizal species in *Carex* and other sedges dominated plant communities. It was even noted in our study that some species like *C. nubigena, C. cruciata* were colonized by unidentified dark septate fungal hyphae that too was reported in species of *Carex* from artic and alpine sites (Jumpponen and Trappe, 1998). Miller et al. (1999) reported association of dark septate fungi in species of *Carex* from savannas and to our knowledge this is the first report of its presence from this North-Eastern Himalayan Region. Finally, past studies have documented fewer spores in wetland species (Anderson et al. 1984) but here they were frequent in appearance with occurrence percentage of 34.48% in some species as in *C. teres, C. filicina, C. inanis, C. longipes, C. cruciata, C. finitima* and *C. myosurus*. Although presence of mycorrhizal association has been frequently reported by other authors, this is the first report of its presence, association and abundance in the genus *Carex* from Eastern Himalayan Region of India.

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