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SEASONALITY OF ARBUSCULAR MYCORRHIZA AND DARK SEPTATE ENDOPHYTES IN SOME GRASSES UNDER ARID CLIMATIC CONDITIONS

Saima Adil [1]***, M. Atif Muneer**** [2]***, Muhammad Imran*** [3]***, M. Zeeshan Munir*** [4]***, Zaib-un-Nisa*** [5]***, Humaira Elahi*** [6]***, Syed M. Naeem Gillani*** [1]***, Ping Wang*** [2]***, Noor-ul-Ain Saifullah*** [7] ***and M. Shafiq Chaudhry*** [1]

[1] Department of Life Sciences, Islamia University, Bahawalpur, Pakistan. [2] College of Forestry, Beijing Forestry University, Beijing, 100083, [3] Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, [4] School of Biological Sciences and Technology, Beijing Forestry University, Beijing, China. [5] Sorghum Research Sub-station, Dera Ghazi Khan, [6] Department of Botany, University of the Punjab, Lahore, [7] Department of Soil Sciences, Bahauddin Zakariya University, Multan, Pakistan.
*Corresponding author e-mail: m_atifmuneer@yahoo.com

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ABSTRACT

A study was conducted in the Department of Life Sciences, Islamia University, Bahawalpur Pakistan. In this study, seasonality of Arbuscular mycorrhiza (AM) and dark septate endophytes (DSE) fungus seasonality was investigated in terms of fungal colonization and spore abundance. For this purpose, the collection of roots and rhizosphere soil samples of six targeted plants i.e. *Cenchrus ciliaris, C .biflorus, Lasiurus scindicus, Ochthochloa compressa, Panicum antidotale,* and *Saccharum munja* were made from Baghdad-ul-Jadeed (BJ) Campus of Islamia University, Bahawalpur, Pakistan. All the plants were found in mycorrhizal association and extent of mycorrhization disclosed variations with the season. The hyphal colonization rate ranged from 27.2 to 90% and vesicular colonization from 0.8 to 73.6%. DSE co-colonized the three host plants among six studied plants. DSE ranged from 4 to 22%. Unlike Arbuscular mycorrhiza, DSE fungus showed peak colonization in June and became negligible in November, illustrating the direct effect of low temperature. A number of mycorrhizal spores were highest in *Saccharum munja* (155/100gm soil) in September. Colonization levels and spore abundance were correlated with edaphic and climatic factors. The high temperature supported the DSE formation, whereas high humidity supported the spore formation. In conclusion, results suggested that extent of mycorrhization, DSE colonization and spore abundance are under the direct effect of season and host specificity.

KEYWORDS: Arbuscular mycorrhiza; dark septate endophytes; grasses; seasonality; colonization; spore abundance; climatic factors; Pakistan.

INTRODUCTION

Arbuscular mycorrhiza (AM) is universally important from the ecological and evolutionary point of view (Allen *et al.,* 2003). AM forms mycorrhizal associations (a common symbiotic relationship between plant roots and fungus) with 80 percent known plant species (Mandyam and Jumpponen, 2005). It is an important component of almost all terrestrial ecosystems. The association is of mutualistic nature that delivers excellent benefits to both symbionts through their interaction with soil and host (Mosse, 1981). The fungus gets photosynthates from the plant and in turn feeds the host with increased uptake of nutrients especially nitrogen and phosphorus through its extended network of external mycelia (Udaiyan *et al.,* 1996).

Mycorrhizal associations improve host plant performance by enhancing nutrient and water acquisition, pathogenic resistance and defense against root grazing (Dighton, 2009). Further, mycorrhizal symbiosis is important in protecting plants against multiple stresses like environmental, cultivational, drought, salinity and acidity stresses (Bohrer *et al.,* 2004; Cho *et al.,* 2006). The researchers studied morphological diversity in two kinds of grass of cholistan (*Cymbopogon jwarancusa* and *Vetiveria zizanioides*) and concluded that both species are obligate mycotrophs (Chaudhry *et al.,* 2012).

In addition, vegetation of arid areas accommodates a variety of fungal endophytes which are important in regulating plant community structure and production (Porras-Alfaro *et al.,* 2008). Previous

work documented the fact that some host plants are co-colonized by AM and dark septate endophytes (DSE) (Lingfei *et al.,* 2005). Similar to AM, DSE is also recognized as a good candidate in providing drought tolerance, better nutrient supply, and maintenance of plant communities (Mandyam and Jumpponen, 2005; Porras-Alfaro *et al.,* 2008). However, physico-chemical properties of soil, metrological characteristics, above and below ground biodiversity may substantially regulate the basic biology of association influencing AM community, rate of colonization and spore production (Miller and Jastrow, 1992; Escudero and Mendoza, 2005). Many researchers reported that seasonal variations could be directly correlated with developmental or physiological stage of the plant, controlling the level of colonization (Titus *et al.,* 2002). In the same way Mandyam and Jumpponen (2008) correlated the ups and downs in colonization rate with plant phenology while investigating C4 tall grass prairie ecosystem.

The degree of association of Poaceae with AM varied with the season (Allen *et al.,* 2003). Family Poaceae being one of the largest plant families with approximately 10,000 plant species and members is cosmopolitan in its occurrence inhibiting a wide variety of edaphic and climatic conditions. Few important crop plants also belong to this family fulfilling 80% world's nutrient demand. Despite the large size of family, less than 500 members have been investigated for their mycorrhization (Sathiyadash *et al.,* 2010). The factors responsible for seasonal variation in AM are also poorly documented. Such information is mandatory to identify and exploit most suitable conditions for mycorrhizae development and utilization of fungal inoculum.

Regarding the value of AM and DSE as a Panacea, it was felt necessary to determine (1) seasonal pattern of DSE and AM colonization in roots, (2) spore populations in the rhizosphere of studied plants; and (3) correlation between fungal colonization percentages and environmental (climatic and edaphic) factors.

MATERIALS AND METHODS

This study was conducted in the Department of Life Sciences, Islamia University, Southern Punjab, Bahawalpur, Pakistan. The sampling site was located near the Cholistan Institute of Desert Studies (CIDS) in Baghdad-ul-Jadeed (BJ) Campus of Islamia University (longitude 29°4 latitude 71°7, Altitude 113). The soil was sandy loam texturally and alkaline with low organic matter. Annual average maximum and minimum temperature in 2012 was 32.3°C and 18.5°C, respectively.

Six grassis i.e. *Cenchrus ciliaris, C.biflorus, Lasiurus scindicus, Ochthochloa compressa, Panicum antidotale,* and *Saccharum munja were selected* to study seasonality of AM. All the selected plants were important in terms of productivity, palatability, easy to grow in desolate areas and also important ethnobotanically. At study site plant cover is poor and vegetation is typically xeric. The common herbaceous plant species at this site are *Cenchrus ciliaris, C. biflorus, Ochthochloa compressa, Panicum antidotale* and *Cymbopogon jwarancusa.* In this area dominant shrubs are *Capparis decidua, Calligonum polygonide,* and *Calotropis procera,* while *Acacia nilotica* and *Prosopis cineraria* are common tree species.

Sampling strategies

Soil and root samples were collected from the rhizosphere of selected grasses at the depth of 3-10cm. For each plant, five replicates were sampled randomly that appeared within a 30cm x 30cm quadrate. Sampling was made for consecutive six months from June - November 2012 and a total 60 rhizospheric samples were gathered in each month. All samples were put into polythene bags, labelled with the name of plant species, date, and location. Then bags were sealed and brought to the laboratory. In the lab, sampled roots were thoroughly rinsed with tap water to remove adhered soil and fine tertiary roots were carefully separated from the whole mass of roots. Fine roots were chopped into fragments of 1cm length and immersed in FAA (formalin: acetic acid: 90% ethanol in 5:5:90 v/v ratio) in glass jars and stored at 4°C for further analysis (Quilliam and Jones, 2010).

Quantification of mycorrhization

A suitable quantity (5-10g) was separated from the roots preserved before, thoroughly rinsed and divided into three sub-samples to ensure

smooth and uniform clearing and staining. Each and every sub-sample was treated by omitting phenol (Phillips and Hayman, 1970). Magnified line intersection method was used to record percentage of colonization presence or absence of intra-radicle structures i.e. hyphae, vesicles, arbuscules and DSE (McGonigle *et al.,* 1990) using XSZ 107- BN compound microscope. One hundred root segments were assessed to record percent values.

Soil analysis and AM spore isolation

The soil samples were subjected to physico-chemical analysis according to standard procedures. The spores were collected with the help of Luxeo 2S stereoscope, mounted in PVLG (polyvinyl alcohol-lactic acid-glycerol) and PVLG + Melzer's reagent, identified and enumerated (Gerdemann and Nicolson, 1963). Identification of spores was made possible with the help of synoptic keys given in INVAM website and in the manual (Schenck and Perez, 1990).

Statistical analysis

Statistical analysis was performed with MSTAT-C and Microsoft Excel 2007. The mean of percent values of colonization and rhizosphere spore expressed alongwith standard error (Mean ± SE). Analysis of variance (ANOVA) followed by DMRT (Duncan multiple range test) at $p \leq 0.05$ was used to examine the effect of time and plant species on AM and DSE colonization and spore count. Pearson's correlative analysis was carried out to estimate the relationship between mean values of physico-chemical properties of soil, temperature, colonization levels and spore number.

RESULTS AND DISCUSSION

Edaphic characteristics

Soil at each sampling site was alkaline. Mean values of temperature and soil moisture showed fluctuation on a monthly basis and ranged 19°C-35°C and 45-60%, respectively (Fig. 1). An overview of edaphic properties (Table 1) indicated that all host plants under study were growing in nutrient-poor conditions.

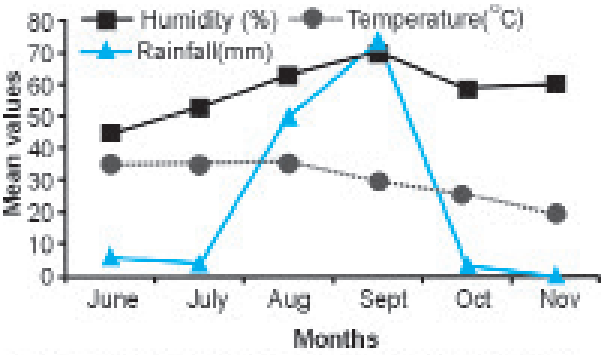

Fig. 1. Seasonal changes in characteristics of climate from June to November 2012

Seasonal variations in colonization and spore abundance

All the tested plants were colonized by AMF (Fig. 2, 3, 4 & 5). Half of the plants species under investigation were found to be co-colonized by dark septate endophytes (Fig. 6). During the sampling period, hyphal colonization ranged from 27.2% in *Cenchrus ciliaris* during September to 90% in *Saccharum munja* during August. Vesicular colonization was recorded to be the lowest in *Cenchrus ciliaris* (0.8%) in August and highest in *Lasiurus scindicus,* (73.6%) in November. As compared to vesicular and

Table 1. Edaphic characteristics of different grasses during studied period.

Name of grass	OC	P	K	N	Mg	Na	CO_3^-	HCO_3^-	Ca
Cenchrus biflorus	0.26±0.07	1.7±0.31	170±7.07	0.008±0.001	0.23±0.07	0.38±0.03	0.2±0.01	0.74±0.07	0.64±0.02
Cenchrus ciliaris	0.21±0.05	2.14±0.56	164±12.9	0.006±0.001	0.38±0.09	0.68±0.04	0.4±0.05	0.92±0.09	0.92±0.09
Saccharum munja	0.18±0.05	3.16±0.32	272±28.2	0.005±0.001	0.42±0.04	0.48±0.05	0.2±0.01	0.8±0.05	0.69±0.05
Ochthocloa compressa	0.11±0.04	3.64±1.35	184±17.8	0.003±0.001	0.35±0.05	0.44±0.04	0.2±0.01	0.8±0.08	0.37±0.03
Panicum antidotale	0.53±0.09	4.96±0.88	214±28.0	0.015±0.002	0.27±0.07	0.52±0.03	0.2±0.01	0.74±0.07	0.52±0.02
Lasiurusas cindicus	0.19±0.05	2.7±0.61	202±30.4	0.005±0.001	0.21±0.03	0.69±0.04	0±0.00	1.12±0.05	0.59±0.05

OC= Organic carbon

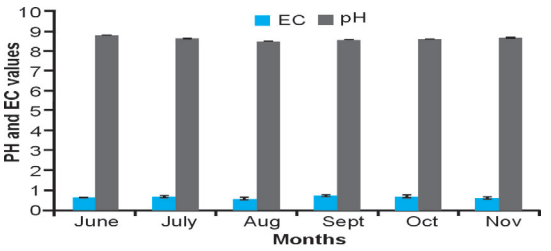

Fig. 2. Seasonal changes in the pH and EC of soil collected from various sites from June to November 2012.

hyphal colonization, arbuscules were low during all months in all surveyed plants. AM fungal exchange sites (arbuscules) were frequent in November, though fluctuations in arbuscular percentage were incredible in all plants. *Panicum antidotale* harbored maximum arbuscules (13.2%) while *Lasiurus scindicus* did not show arbuscular colonization in any month.

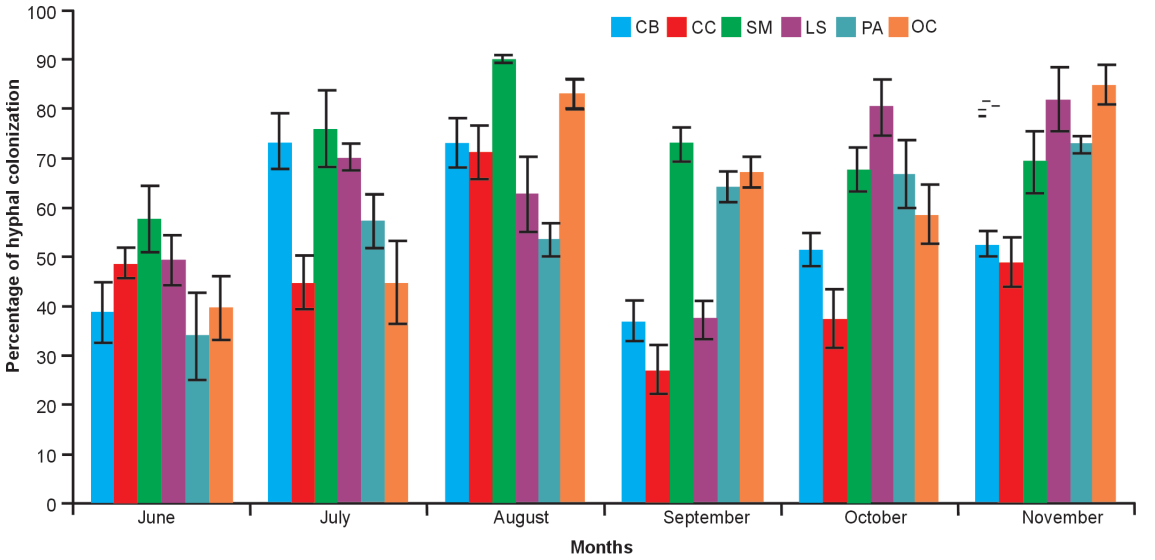

Fig. 3. Seasonality of hyphal colonization in the roots of selected grass species

CB = *Cenchrus biflorus*, CC = *Cenchrus ciliaris*, SM = *Saccharum munja*,
LS = *Lasiurus scindicus*, PA = *Panicum antidotale*,
OC = *Ochthochloa compressa*,
Values ± SE, DF = Degree of freedom = 4, Error bars represent standard error

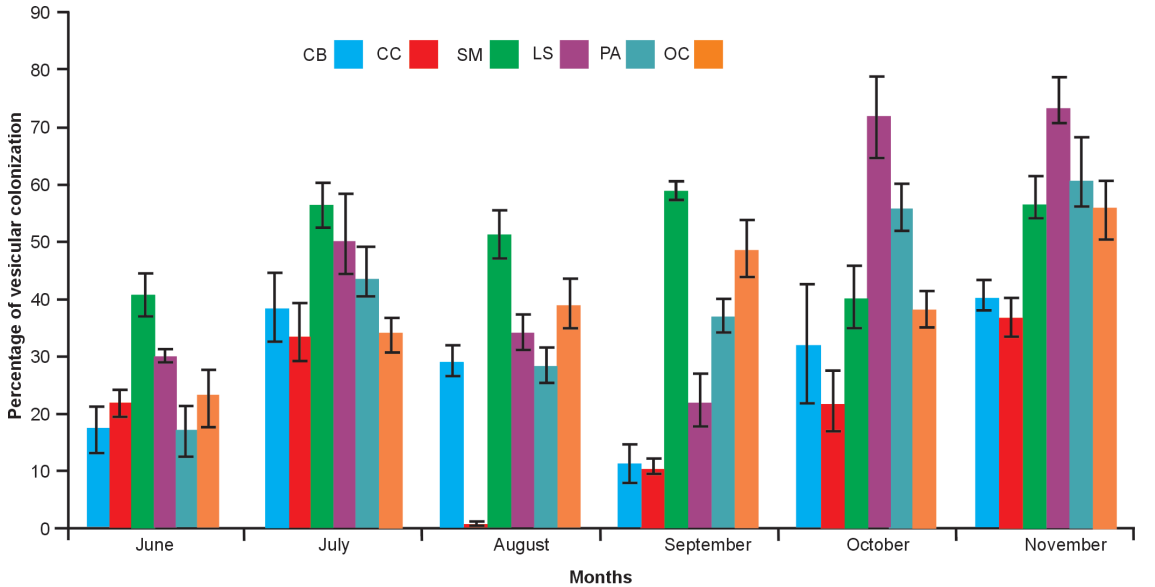

Fig. 4. Seasonality of vesicular colonization in the roots of selected grass species.

CB = *Cenchrus biflorus*, CC = *Cenchrus ciliaris*, SM = *Saccharum munja*, LS = *Lasiurus scindicus*,
PA = *Panicum antidotale*, OC = *Ochthochloa compressa*, Values ± SE, DF = Degree of freedom = 4

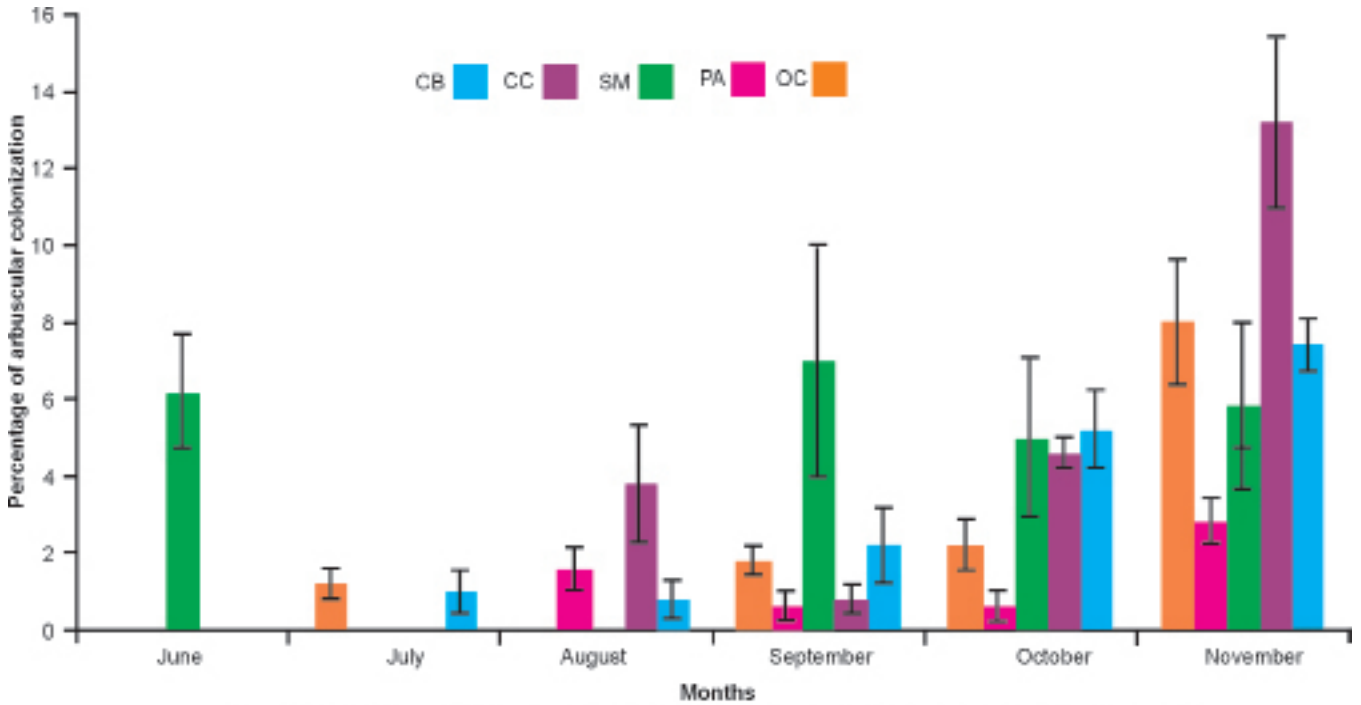

Fig. 5. Seasonality of Arbuscular colonization in the roots of selected grass species.

CB = *Cenchrus biflorus*, CC = *Cenchrus ciliaris*, SM = *Saccharum munja*, LS = *Lasiurus scindicus*, PA = *Panicum antidotale*, OC = *Ochthochloa compressa*, Values + SE, DF = Degree of freedom = 4

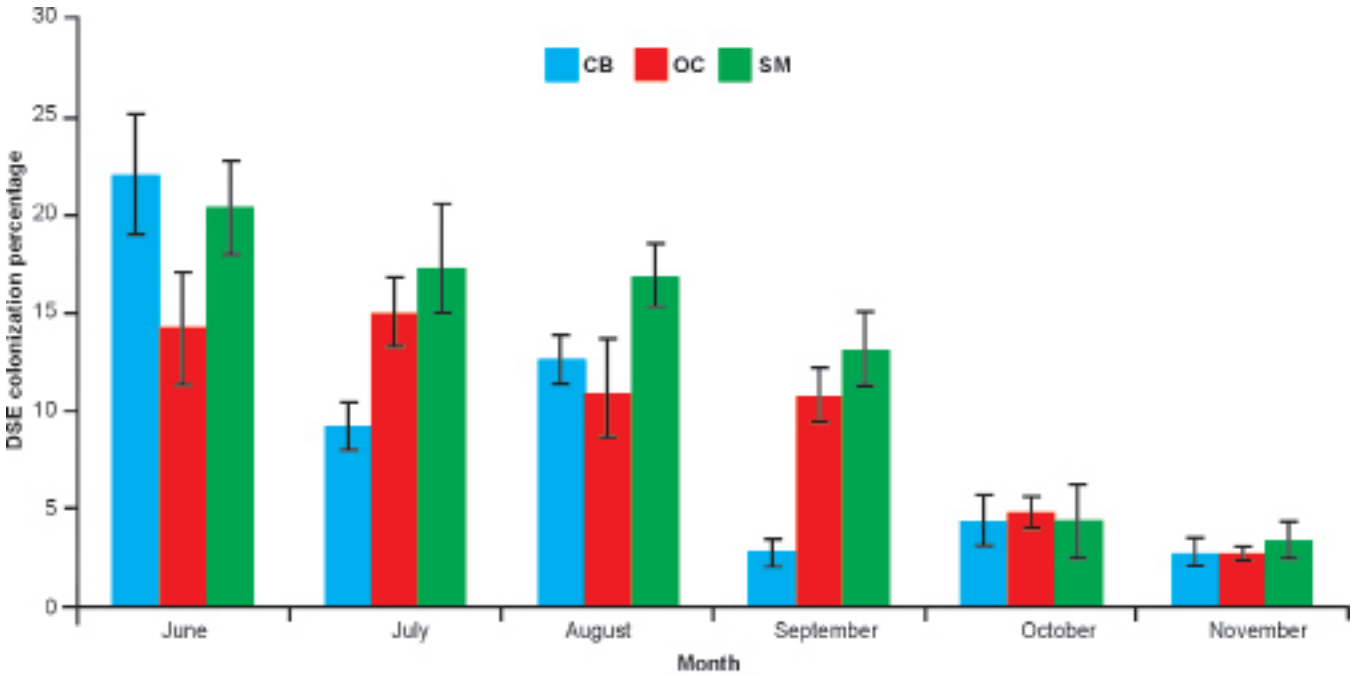

Fig. 6. Seasonality of DSE colonization in the roots of selected grass species.

CB = *Cenchrus biflorus*, OC = *Ochthochloa compressa*, SM = *Saccharum munja*, Value ± SE, DF = Degree of freedom = 4

Absolute abundance of rhizosphere spores fluctuated through time (Fig. 7). The spores were highest in September (155/100g soil) in *Saccharum munja* following a wet season, whereas lowest number was recorded in *Ochthochloa compressa* in July (28.6/100g soil).

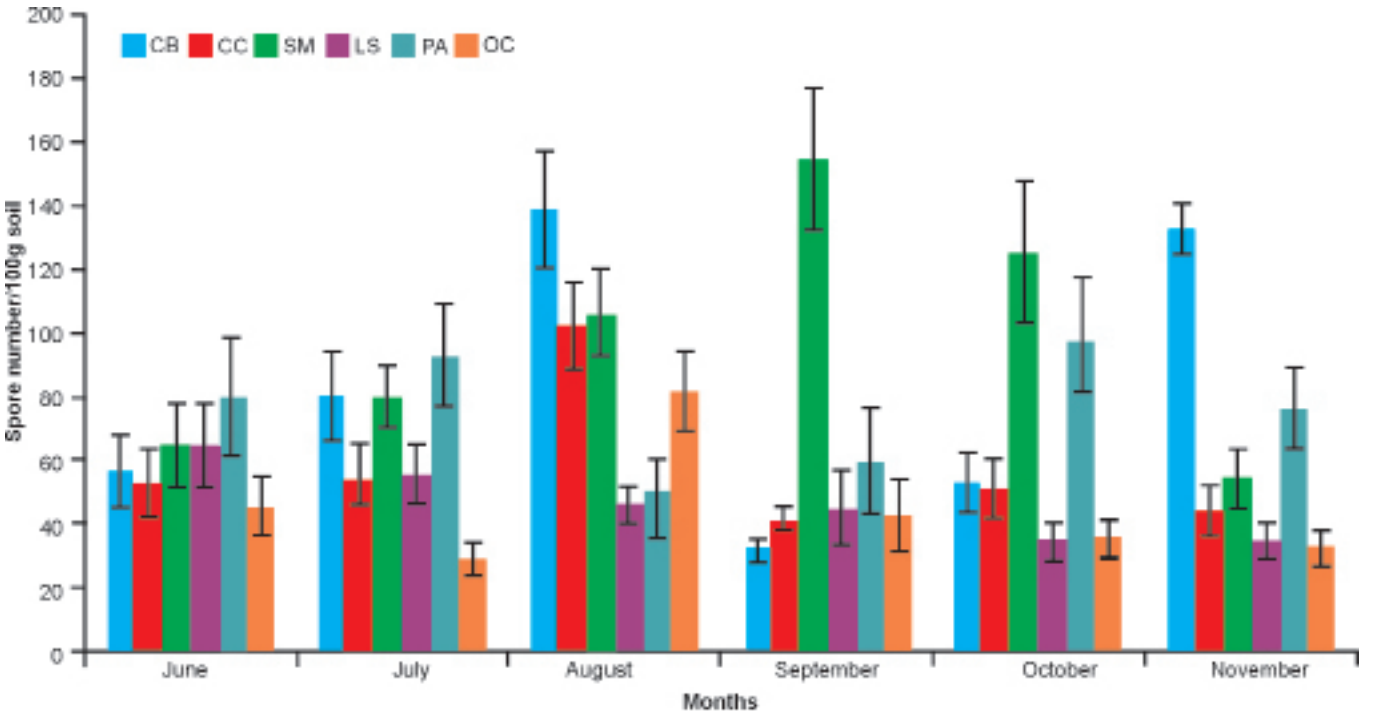

Fig. 7. Seasonality of spore number in the roots of selected grass species.

CB = *Cenchrus biflorus*, CC = *Cenchrus ciliaris*, SM = *Saccharum munja*, LS = *Lasiuruss scindicus*, PA = *Panicum antidotale*, OC = *Ochthochloa compressa*, Values ± SE, DF = Degree of fredom = 4

From F-values of ANOVA (Table 2), it is clear that all colonization parameters except hyphal colonization differed with host species and time. Moreover, the mutual effect of plants and time (months) upon colonization was insignificant except for DSE and spore count. DMRT indicated significant differences in hyphal vesicular, arbuscular, DSE colonization and spores over time in all studied host plants (Table 3).

Table 2. F values of ANOVA for mycorrhization, DSE colonization and spore number in six surveyed plant species.

	Hyphae	Vesicles	Arbus-cules	DSE	Spores
Months (M)	1.6NS	24.4**	7.60**	39 **	3.2*
Plants (P)	0.4 NS	8.70**	9.93**	91.4**	3.5**
M+P	1.0 NS	1.20 NS	1.0 NS	6.90*	2.3***

NS = Non-significant,*Significant at 0.05 level, **Significant at 0.01 level, ***Significant at 0.001 level.

Table 3. Comparison of colonization levels across time by DMRT.

	Hyphae	Vesicles	Arbuscules	DSE	Spores
June	B	D	B	A	B
July	AB	B	B	BC	B
August	AB	CD	B	AB	AB
September	AB	C	B	C	AB
October	A	B	A	C	B
November	AB	A	A	C	B

DSE = Dark septate endophytes, Same letter(s) indicated that the values do not differ significantly as determined by ANOVA and DMRT at P=0.05 (n=5)

The presence of AM in all surveyed grass species confirms widespread occurrence of mycorrhizae in family Poaceae. These results are in accordance with Ahulu *et al.* (2005) who found that all examined members of Poaceae from sand dune series of Niigata were mycorrhizal. To the

best of our knowledge, these selected plants have not been examined earlier for the assessment of seasonality of AM colonization. It was clear from our results that time factor played an important role in causing variations in fungal colonization and number of spores in all surveyed plant species. But the rise and fall did not follow any consecutive pattern in any of the studied hosts. There was also no consistent seasonal pattern while investigating sedges of semi-arid tropical grasslands (Muthukumar and Udaiyan, 2002).

In present study, hyphae and vesicles were consistently present throughout the sampling period though their percentages showed peak and troughs at different times in different species. Present results are in line with findings of Udaiyan *et al.,* (1996). Vesicular colonizations were more frequent (0.8%-73.6%) than arbuscular colonization (0.4%-13.4%) in all surveyed plants as supported by Chen *et al.* (2008).

The researchers delineated seasonal dynamics in mycorrhization and spore population in the rhizosphere of orchard grass and white clover (Xin *et al.,* 2012). According to them, the first peak of total colonization was in June (above 30%), while the second peak occurred in September (white clover) and November (orchard grass). Likewise, in present work, peak colonization appeared twice in each plant species during the sampling period. During first phase maximum hyphal colonization appeared in July (*L. scindicus, P. antidotale)* and August (*S. munja, C. ciliaris, C. biflorus, O. compressa*), but frequent vesicular colonization was observed in July. Arbuscular colonizations were very low and fluctuated incredibly. With few exceptions, the second phase of peak colonizations (hyphal, vesicular, arbuscular) appeared in starting winter season (November).

Different researchers working with the seasonality of AM reported ups and downs in different times of the year. Some of them observed maximum AM infection in summer (Sigüenza *et al.,* 1996; Escudero and Mendoza, 2005) while others in spring (Lugo *et al.,* 2003; Bohrer *et al.,* 2004) or winter (Xin *et al.,* 2012). In this study a high incidence of AM infection was observed both in summer (July) and winter season (November). So no clear-cut pattern of mycorrhization could be established regarding seasonal variation.

Absolute abundance of spores was significantly influenced by season. Maximum number of spores was recorded in September when the mean monthly temperature was 29.8°C and soil moisture 70%. However, Lingfei *et al.,* (2005) reported maximum spore population from July to October indicating that autumn may be the best time for sporulation. One school of thought hypothesized that increase in spore number may be the result of root growth supporting microflora (Udaiyan *et al.,* 1996).

Correlative analysis

Correlative analysis (Table 4) depicted that temperature had significant negative correlation with vesicular and arbuscular colonization. DSE colonization showed significant positive correlation with temperature while a number of spores had significant positive correlation with soil moisture.

Table 4. Correlation between factors (climatic and edaphic) and colonization percentages and rhizosphere spore count.

	Hyphae	Vesicles	Arbuscules	DSE	Spore	Temperature	Humidity	EC
Vesicles	0.550							
Arbuscules	0.401	**0.868***						
DSE	-0.474	**-0.878***	**-0.825***					
Spore	-0.005	-0.277	-0.152	-0.312				
Temperature	-0.401	**-0.896***	**-.893***	**0.885***	0.006			
Humidity	0.376	0.160	0.155	-0.634	**0.867***	-0.392		
EC	-0.357	-0.053	-0.442	-0.027	0.330	0.051	0.346	
pH	-0.613	-0.079	0.189	0.274	**-0.805***	0.081	-0.795	-0.356

*Significant at 0.05 level.

Edapho-climatic properties influenced temporal and spatial dynamics of AM infection and spore population (Lingfei *et al.,* 2005). We also found that seasonal changes in temperature and soil moisture markedly affected the extent of mycorrhization and number of AM spores. Generally, it is considered that temperature and light hours had a positive correlation with AM population due to their ability to increase photosynthetic efficiency (Koide and Mosse, 2004). However, in present study a negative correlation was observed between temperature and vesicular colonization which is in accordance with the findings of Muthukumar and Udaiyan (2002). Soil moisture had a positive correlation with hyphal, vesicular and arbuscular colonization and number of spores also observed the same trend (Muthukumar and Udaiyan, 2002). Significant negative correlation of temperature with vesicular and arbuscular colonization indicated that temperature had a striking effect on AM (Saito *et al.,* 1994). Spore production and root colonization could not be correlated to one another because numerous factors i.e host fungi and environment influenced the two phenomena which are in agreement with observations of Mathur *et al.* (2007) but inconsistent with the result of Mutabaruka *et al.,* (2002).

Previous reports documented that some plant species were co-colonized by DSE and AM (Lingfei *et al.,* 2005). In present study, DSE were found colonizing half of the studied plants (three out of six i.e. *O. compressa, C. biflorus* and *S. munja*) together with AM. DSE colonization showed significant positive correlation with temperature and followed a steadily declining pattern from June to November. Contrarily, Lingfei *et al.* (2005) did not find a significant correlation between DSE and climatic factors except for the humidity. The role of DSE in the ecosystem is equivocal. A study suggested that DSE may play a role in improving nutrient uptake and plant growth (Barrow, 2003) while some other state that DSE may behave as a saprophyte, pathogen or mutualistic in nature like AM (Jumpponen and Trappe, 1998).

CONCLUSION

The results conclude that pattern, extent and timing of mycorrhizae development are influenced by plant phenology (Bohrer *et al.,* 2004), fungal community composition (Lugo *et al.,* 2003), edaphoclimatic features (Saito *et al.,* 1994; Muthukumar and Udaiyan, 2002). So, it is bit difficult to pinpoint single factor to be responsible for increase or decrease in colonization and spore count over time. Present work should be considered a prelude to further investigate a very important aspect of the seasonality of AM and DSE in order to get a comprehensive understanding of factors causing variations.

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CONTRIBUTION OF AUTHORS

Saima Adil	**Principal author, conducted research and prepared write-up**
Muhammad Atif Muneer	**Assisted in the experimentation and write-up preparation**
Muhammad Imran	**Reviewed the first draft and made helpful changes**
Muhammad Zeeshan Munir	**Scientifically and finally improved the draft**
Zaib-un-Nisa	**Performed statistical analysis**
Humaira Elahi	**Conducted the part of study on AMF colonization**
Syed Muhammad Naeem Gillani	**Collection, isolation and indentification of spores from the soil**
Ping Wang	**Analyzed the AMF colonization and spores identification**
Noor-ul-Ain Saifullah	**Helped in soil analysis**
Muhammad Shafiq Chaudhry	**Supervisor**