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Effect of Water Stress Condition on Architecture of Root Colonizing Arbuscular Mycorrhizal Fungi

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Abstract: - Availability of water is one of the important factor which determine the colonization of Arbuscular mycorrhizal fungus (AMF) in the roots of higher plant. *Triticum aestivum* plant is selected to study effect of water stress on root colonization pattern. Monocot plants are known for having 90% AMF colonization. AMF colonization pattern has been studies under drought condition, water logged condition and control condition. Under drought condition and control condition AMF colonization pattern is more or less similar while under drought condition AMF colonization percent is significantly reduced. AMF hyphal network and sporulation both are highly influence under water logged condition. Under drought condition sporulation was more in comparison to water logged and control condition. AMF colonization pattern was more extensive and prominent under drought conditions, while sporulation was comparatively low in waterlogged condition in comparison to drought condition. Various type of hyphal structures were also varied due to availability of water. Hypahl netwrok was more extensive and prominent under drought condition. Absorbing and runner hyphae extended quite long under drought condition in comparison to waterlogged condition. Appressoria formation was also prominent under drought condition. Under water logged condition only spores of *Acaulospora* species was found associated with the roots while under drought and control condition spores of Genus *Glomus*. *Entrophospora* and *Acaulospora* were found to associate with the roots. The results present study revealed that water is one of the important factor which regulated the colonization pattern of AM fungi.

Keywords-Arbuscular mycorrhizal fungi, Hyphae, Acaulospora, Glomus, sporulation

I. INTRODUCTION

An Arbuscular mycorrhizal fungus (AMF) is a type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant. The host plant plays an important role in the intercellular hyphal proliferation and arbuscule formation. Major modifications are required in the plant host cell to accommodate the arbuscules. The plant cell cytoskeleton is reorganized around the arbuscules. There are two other types of hyphae that originate from the colonized host plant root. Once colonization has occurred, short-lived runner hyphae grow from the plant root into the soil. These are the hyphae that take up phosphorus and micronutrients, which are conferred to the plant. AM fungal hyphae have a high surface-to-volume ratio, making their absorptive ability greater than that of plant roots [1]. AMF hyphae are also finer than roots and can enter into pores of the soil that are inaccessible to roots [2]. The third type of AMF hyphae grows from the roots and colonizes other host plant roots [3].

In view of the above importance and needs of AM associations and also the paucity of work done in the tropical countries particularly in crop plants, the present study was undertaken to work out on the affect of water stress on AM colonization in an important crop plant.

II. RELATED WORK

It is well documented that AMF symbiosis can increase plant growth and nutrient uptake, improve fruit quality under various abiotic stress such as low temperature stress, drought, salt stress etc [4, 5, 6]. However the development of AM colonization is greatly influenced by availability of water. Flooded conditions may suppress the mycorrhizal association [7] or have no adverse affect on root colonization [8, 9]. Furthermore, there appears to be no relationship between percentage of root length colonized by AM fungi and plant hydrological category [10, 11].

III. METHODOLOGY

Experimental design

The wheat plant (*Triticum aestivum*) a major crop in central India, selected for the present investigation. Experiment was

conducted in plastic pots (Length 20 cm, diameter 20 cm, 15 seed per pot). To check the structure and function of native AM species soil from garden site were collected for the experiment, it has natural consortium of AM fungi which is used as inoculum. Experiment was performed in triplicate. Seed of wheat was sown and watered regular till germination. After germination of seed three different condition were established.
i) One set of pots were watered regularly to maintain control condition
ii) Second set of pots were watered just after the commencement of wilting to maintain drought stress
iii) Third set of pots were filled with water to maintain water logged condition.

Collection of Root samples
Roots were collected after two month of germination. For the collection of root sample plants were uprooted from the pots carefully with the help of hoe and care was taken to prevent damage to the fine roots. Fine feeder roots were separated and lightly shacked. Root samples were collected in three replicates from each pot and kept in labelled polyethylene.

Processing of root samples
Freshly collected root samples were rinsed with tap water several times to wash the roots thoroughly. Roots were cut into small bits of about 1 cm pieces for clearing and staining procedure.

Clearing and Staining of Root Samples
Since AM root infection consists of intra and intercellular hyphae and vesicles together with finely branched arbuscules within the host cortical tissue, the study of antomical characteristics of AM requires suitable staining. The Phillips and Hayman [12] procedure for root clearing and staining was followed for its quick results and application for wide range of host plants.

In the present study, AM staining and colonization method of Phillips and Hyman [12] modified by Kormanik *et al.* [13] was followed in the present study.

Assessment of colonization
Assessment of colonization of AM fungi was done by slide method of Giovannetti and Mosse [14]. Root segments, each approximately 1 cm long, were selected at random from a stained sample and mounted on microscopic slides in group of 10.

Alternatively presence or absence of infection was recorded in each of the 10 pieces. Minimum 100 root segments were used for this method. The per cent AM colonization was calculated by using following formula.

$$\% \text{ AM colonization} = \frac{\text{Total no. of infected root pieces}}{\text{Total no. of root pieces studied}} \times 100$$

Microscopic Studies and Photomicrographs
Microscopic study of roots and micrographs were taken from Olympus binocular microscope attached with MIPS model no. CH20i.

IV. Results and Discussion

This study has shown that AMF colonization in plants affected by a wide range of water availabilities. Result of the present finding reveal that at three different conditions of water availability play an important role on AM colonization (%) in *Triticum aesivum* (Table 1). There is no significance difference in AM colonization under control condition (48±0.57%,) and drought condition (46±1.15%;) in host plant but waterlogged condition had greater effect on AM colonization (AM colonization-10±1.15%). Reductions in AMF colonization levels with increasing levels of water availability are consistent with previous field and greenhouse/growth-room studies [8, 15]. Ruiz-Lozano [16] reported that transplanted 4-week-old *A. tripolium* plants to field collected soils and imposed three levels of water availability and found that total colonization levels in their continuously flooded treatments conditions were significantly lower than in pulsed or drier treatments.

Different water availability play important role in colonization pattern in both host plant selected for study. Arbuscular mycorrhizal structures i.e. Extraradical hyphae, extra radical spore, intraradical hyphae, vesicles as well as intraradical vesicles and spores were observed however, frequency of occurrence were varied according to water availability. In *T. aestivum* AM colonization was *Arum*- type in all three water availability conditions.

Table 1: Different attributes of AM fungi in differential water stress in Triticum aestivum L

S. No.	Structure of AM fungi present in root	Controlled condition	Drought condition	Water logged condition
1.	AM colonization (%)	48±0.57%	46±1.15%	10±1.15%
2.	Type of AM colonization	Arum type	Arum type	Arum type
3.	Mycelial status	Extensive extra-radical and intra-radical mycelium, frequent appressoria	Extensive extra-radical and intra-radical mycelium,	Extensive extra-radical and intra-radical mycelium,
4.	Vesicles	Present	Present	Not detected
5.	Spore formation	Formation of extra-radical and intra-radical spore presents (*Glomus* and *Acaulospora* species)	Formation of extra-radical and intra-radical spore (*Glomus* and *Acaulospora* species)	Formation of extra-radical and intra-radical spore (*Glomus* and *Acaulospora* species)

In control condition AM colonization pattern more extensive and prominent (Plate 1) than other conditions however, sporulation was quite low in comparison to drought condition (Plate 1). This result indicate that drought condition trigger the sporulation of AM species present in the native soil. Drought has also been considered as a potential factor impacting spore production [17].

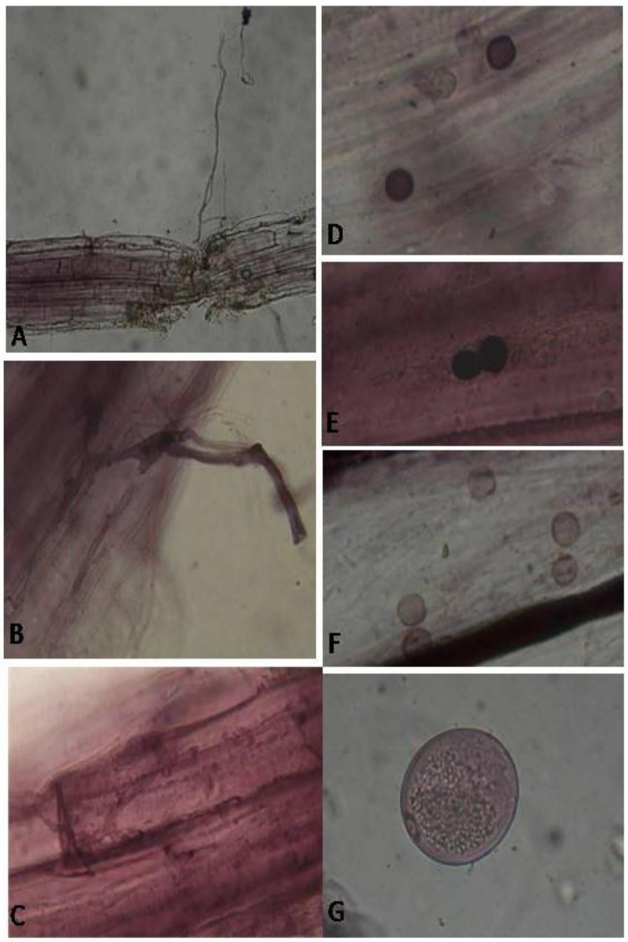

Plate 1: Structures of arbuscular mycorrhizas formed on roots of Triticum aestivum under control condition: (A) Extension of extraradical hypha, (B) Appressoria, (C) Intracellular hyphae, (D), (E) & (F) Intraradical spores, (G) Extraradical spores.

In drought condition AM status was similar to that of control condition but the network of runner hyphae was very extensive. A network of extraradical mycelium of AM fungi was observed which having both runner and absorbing hyphae (Plate 2). Long extension of runner hyphae was found to attach with the root. Appressoria formation was also prominent. Intraradical spore formation was very interesting and genus *Glomus* produces spherical dark brown coloured spore in series and extraradical spores of *Entrophospora* sp. and *Acaulospora* was also identified (Plate 2).

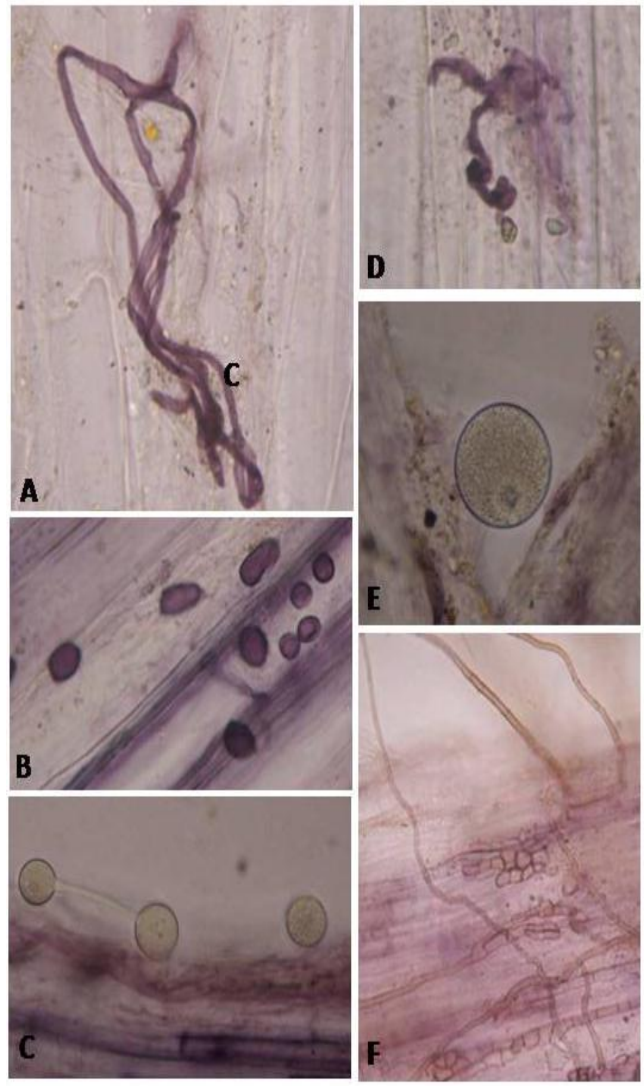

Plate 2: Structures of arbuscular mycorrhizas formed on roots of Triticum aestivum under drought condition: (A) Extraradical mycelium, (B) Intra-radical spores of Glomus sp. (C) Extraradical spores Entrophospora sp. (D) Appressoria and entry point, (E) Extraradical spore of Acaulospora, (F) Dark septate endophyte (DSE) mycelium.

In waterlogged or flooded condition hyphal architecture was quite different, extraradical hyphal extension was not so extensive but intraradical hyphal network was highly extensively branched (Plate 3) and only extraradical and intraradical spores of *Acaulospora* sp. was found associated with the roots of *T. aestivum* (Plate 3).

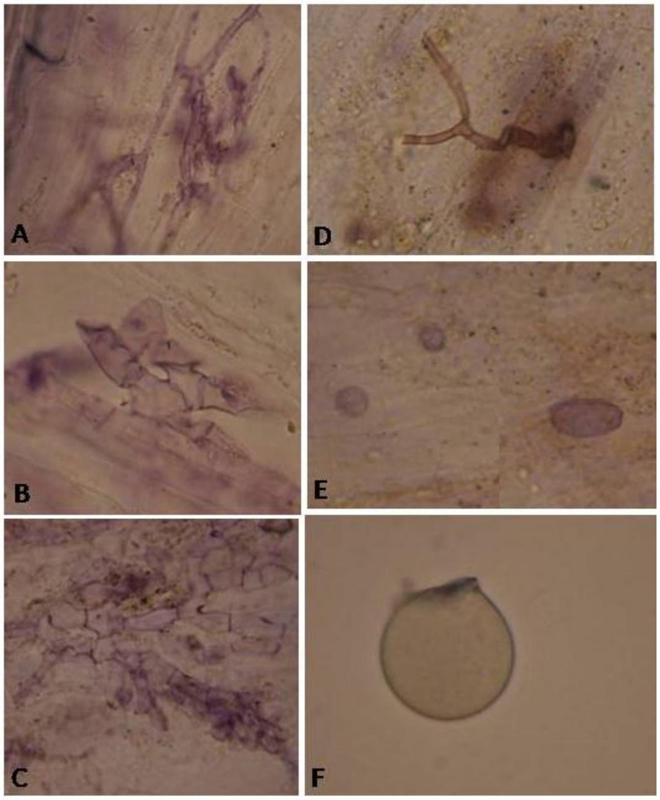

Plate 3: Structure of arbuscular mycorrhizas formed on roots of Triticum aestivum under water logged condition: (A) Intraradical mycelium, (B) Highly branched non septate mycelium, (C) Highly septate mycelium, (D) Extraradical hyphae and appressoria at entry point, (E) Vesicles of various shape and size, (F) Extraradical spore of Acaulospora sp

Miller and Sharitz [18], Carvalho *et al*. [19] and the current study, together they indicate that while soil inundation may inhibit AMF formation in plant species under certain conditions, this is not always the case and AMF associations can establish in inundated soils. This study has shown that AMF can colonize plants at a very early stage in their development across a wide range of water availabilities including flooded and drought condition and have the capacity to affect patterns of root morphology that is species and environment specific. Duan *et al.* [20] have suggested that AM fungi probably increase the ability of the root system to scavenge water in dried soil, resulting in less strain on foliage, and hence higher stomatal conductance and shoot water content at particular soil water potential. Auge *et al.* [21] investigated whether the AM symbiosis influences soil moisture retention properties and found that 7 months of mycorrhization by *G. intraradices* in a sequatchie loam altered the characteristic soil moisture curve relative to “non-mycorrhizal”.

All these results suggest that mycorrhizal protection against water stress caused by drought may be one of the most important mechanisms by which the AM symbiosis increases the tolerance of host plants to drought. These observations also agreed that arbuscular mycorrhizal colonization, pattern of hyphal growth, and sporulation is greatly affected by water availability.

V. CONCLUSION and Future Scope

Results of present investigation shows that arbuscular mycorrhizal fungi are capable of adapting to the abiotic environment and AM fungi may be used as potential bioinoculants for improving plant tolerance to abiotic stress. AM fungi respond to abiotic stress independently of their host plant. Abiotic stresses affect the abundance and community composition of am fungi. In agriculture an AM fungal inoculum that is tailored to an abiotic stress could be a sustainable strategy to help farmers in regions where agriculture is restricted by an abiotic stress. Similarly such AM fungal species could be used in restoration efforts to restored environment.

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