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Effect of arbuscular mycorrhizal fungi on physiological and biochemical activities in *Plectranthus barbatus* Andrews and *Mentha piperita piperita* L.

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The study was conducted to observe the various aspects related to drought tolerance in arbuscular mycorrhizal(AM) inoculated *Plectranthus barbatus* (= *Coleus forskholi iforskholii)* and *Mentha piperitapiperita*plants.Non AM and AM plants were grown under normal and water stressed conditions. Water stress was induced with the help of PEG and *Glomus mosseae* spores were used for AM source. The plants were taken to determine the effects of AM fungi under stress condition on relative water content (RWC), water potential (ψ) and free proline. In this comparative study AM fungi had influence on RWC, water potential and proline accumulation under normal and stress conditions with and without AM fungi. Mycorrhizal plants had higher leaf water potential, RWC than non mycorrhizal plants. However, the proline accumulation was lower in mycorrhizal plants compared to non mycorrhizal plants. Both the plants have shown well adjustment towards water stress.

Key words: AM fungi, RWC, proline, osmolyte, osmotic stress

INTRODUCTION

Plants are exposed to various environmental conditions and stressors. Abiotic stressors, such as drought, salinity, extreme temperature and metal and chemical toxicity are serious threats to agriculture (Audet and Charest, 2009). These stressors lead to a series of morphological, physiological and molecular changes that adversely affect plant growth and productivity (Wang *et al.* 2001).

Stress is defined as an external factor that exerts a disadvantageous influence on the plants. It is measured in relation to the plant survival, crop yield, growth or primary assimilation processes which are related to the overall growth of the plant. The plants response to stress is a complex phenomenon, involving synthesis of polyamines and new proteins of unknown functions and common antioxidant enzymes such as SOD, POX.

Drought stress incurred a large set of parallel changes in the morphological, physiological and biochemical responses where asarbuscular mycorrhizal (AM) fungi symbiosis can protect host plants against the detrimental effects (Abdel Latef and He, 2011).

Arbuscular mycorrhizal fungi are known to improve the ability of plants to uptake and acquire mineral nutrients. AM fungi are particularly important in nutrient and water uptake from the soil and to improve the plants resistance to disease. Mycorrhizal symbiosis is important for plant nutrition as this type of symbiosis is often obligatory to the survival of the host (Ruiz-Lozano *et al.* 1995). Tolerance to osmotic stress in plants is a complex phenomenon and involves many changes at the biochemical and physiological levels (Ingram and Bartels, 1996). However, the mechanisms behind the modulation of tissue water conductivity and osmotic adjustment (which help to maintain tissue water potential) (Bohnert *et al.* 1994) appear to be affected by AMF colonization

Primary metabolites are of high importance and essentially required for growth of plants, these include sugars, proteins and lipids. These are integral part of protoplasm and the plants are dependent on these compounds for their growth and differentiation.

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Plectranthus barbatus (= Coleus forskholii forskholii) is a perennial herb belonging to the family Lamiaceae, with medicinal importance because of forskolin content. It is used to treat heart disease, convulsions, spasmodic pain (Dubey *et al.* 1981).

Mentha piperita piperita also belongs to Lamiaceae family and is a herbaceous rhizomatous perennial herb. It has a high menthol content. It acts as a carminative, *cholagogue* and antibacterial agent with cooling action (Eccles, 1994).

This study is to evaluate the influence of AM fungi on water potential, RWC and free proline content in *Coleus forskholii* and *Mentha piperita* under well watered and water stress conditions.

MATERIALS AND METHODS

The plant saplings *Coleus forskholii forskholii* and *Mentha piperita piperita* were procured from University of Agricultural Science, Bengaluru. The plants were further maintained in the pots in botanical garden, Mount Carmel College. The inoculums of *Glomus mosseae* were obtained from the Department of Microbiology, University of Agricultural Science, Bengaluru, which consists of sand and soil mixture with spores, extra radical mycelia and mycorrhizal root segments of Rhodes grass. The plants were fed with 10% PEG – 6000 on alternate days for 45 days. Experiment was designed in randomized block design with 12 replicates of each treatment (one plant/one pot). The following treatments were given

1.Plants fed with water – control C
2.Plants watered with 10% PEG solution — S
3.Plants inoculated with *G. mosseae* — GM
4.Plants inoculated with *G. mosseae* and 10% PEG — GMS

Physiological parameter

Determination of RWC (%): The RWC of *Coleus forskholii* forskholii and *Mentha piperita piperita* leaves were measured following the method used by Barrs and Weatherley (1962) and calculated as,

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100$$

where FW is fresh weight, TW is turgid weight (weight after leaf samples were soaked in distilled water for 24 hrs), and DW is dry weight.

Determination of water potential : Leaf water potential was determined by Handa *et al.* (1986) method. The excised leaf samples were kept in graded sucrose solutions of 0.1 – 1molal solutions and incipient plasmolysis was observed. The water potential was calculated by the formula

$$\psi = - CST$$

Estimation of proline : Using Bates *et al.* (1973) method.Leaf samples were taken and homogenized in 3% sulphosalicylic acid. The filtrate was taken and glacial acetic acid and acid ninhydrin were added. It was boiled and then cooled and added toluene and intensity was read at 520nm. Pro content was calculated using the formula

$$\mu \text{ moles/g tissue} = \frac{\mu g \text{ proline } X \text{ ml toluene}}{115.5} X \frac{5}{g \text{ sample}}$$

***Data analysis*:** Twelve replicates per treatment were established (one plant / one pot). The data thus obtained from the experiment was subjected to ANOVA. Significant ratios between the group means were further subjected to DMRT using SPSS version 1.5. Probability values <0.05 were considered as significant (Snedecor and Cochron, 1994).

RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi are important in sustainable agriculture because they improve plant water relations and thus increase the drought resistance of host plants (Smitha and Read, 2008). Although the relationship between AM fungi and their host plants is usually considered non specific, this relationship is tightly regulated at both the structural and physiological levels (Ruiz-Lozano *et al.* 1995).

When plants suffer from water stress, osmotic adjustment occurs in order to decrease their water potential to maintain a beneficial gradient for water flow from soil into plant roots. AM plants have a greater osmotic adjustment than non AM plants (Porcell and Ruiz-Lozano, 2004).

RWC is a physiological parameter to assess the drought tolerant capacity and photosynthetic efficiency of plants. It is an appropriate estimation of

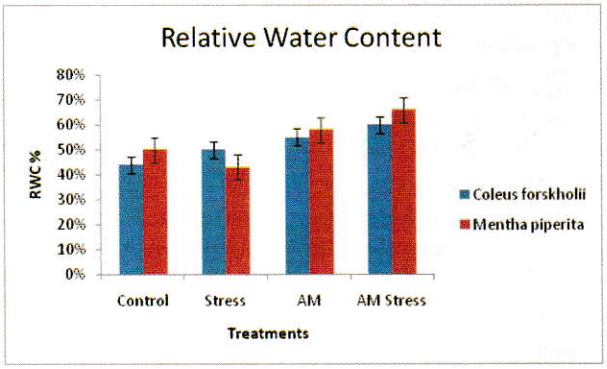

Fig. 1: Effect of AM fungi on RWC in *Coleus forskholii forskholii* and *Mentha piperita piperita* under water stress

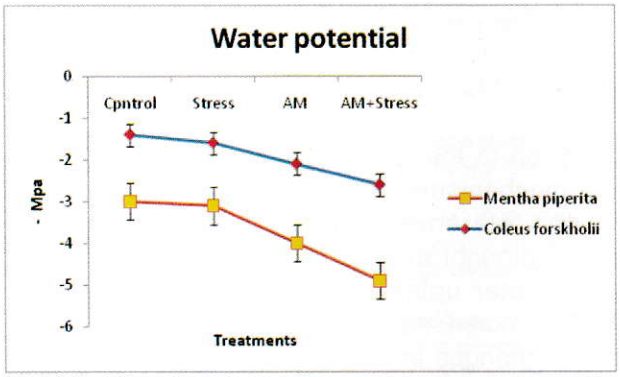

Fig. 2: Effect of AM fungi on leaf water potential in *Coleus forskholii forskholii* and *Mentha piperita piperita* under water stress

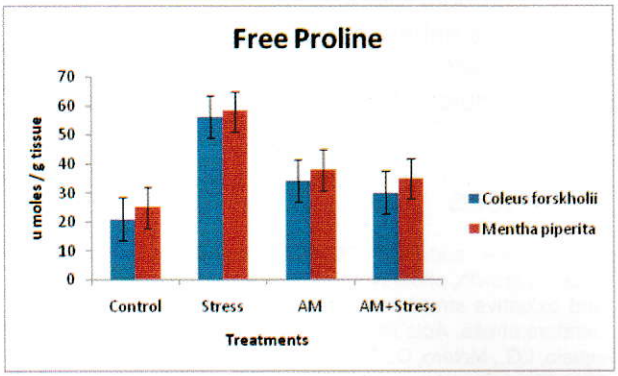

Fig. 3: Effect of AM fungi on free proline content in *Coleus forskholii forskholii* and *Mentha piperita piperita* under water stress

plant water status in terms of cellular hydration under the possible effect of both leaf water/osmotic potential and osmotic adjustment (Barrs and Weatherley, 1962). The RWC was markedly affected by the AM fungal treatment and drought stress and also by the combination of AM fungal treatment and water stress. The RWC of mycorrhizal plants were 55% and 58% in *Coleus forskholii* and *Mentha piperita* respectively, as compared to non mycorrhizal plants which showed 44% in *Coleus forskholii* and 50% in *Mentha piperita* (Fig. 1).The RWC were significantly increased by AM colonization under control and water stress conditions, in both the plants than non mycorrhizal plants. Water deficit reduces the absorption power of roots, but the colonization with AMF seems to compensate for this (Barea and Jeffries, 1995). AM fungi can be beneficial for water transportation through plants and can help plants to keep stomata open (Zhu *et al.* 2012). In this study, the RWC in the mycorrhizal and non mycorrhizal *Coleus* and *Mentha* plants showed significant difference when grown under normal and stress conditions. The mycorrhizal plants showed increased RWC under water stress conditions. These results are in correspondence with Liu *et al.* (2015), who also observed that mycorrhizal seedlings improved their water uptake compared to non mycorrhizal seedlings under drought stress conditions in poplar seedlings. Similar results by Gong *et al.* (2013), were in agreement with the present findings showing mycorrhizal plants improved their water uptake compared to non mycorrhizal plants under drought stress condition.It is likely that the hyphae of the AM fungus expanded the absorption region of the host plant roots (Muthukumar and Udaiyan, 2010) and enhanced absorption of water by the roots of the host plant (Huang *et al.* 2011).

The leaf y determined at the end of the drought period that is after 45 days was similar in AM and non AM plants (-1.4MPa and -1.6MPa and -1.6MPa and -1.5MPa) grown under normally watered conditions in *Coleus forskholii* and *Mentha piperita* respectively (Fig. 2). Water stress decreased ø , but the decrease was more in non mycorrhizal plants (-2.1MPa in Coleus and -1.9MPa in *Mentha*) than in mycorrhizal plants (-2.6MPa in Coleus and -2.3MPa in *Mentha*). Drought tolerance is the ability to withstand water deficit with low tissue water potential (Mitra, 2001). The decline in leaf water potential in response to water stress does not directly affect the metabolism of plants, but it is a good indicator of a plant water status.The adjustment of leaf osmotic potentials requires intracellular osmotic balance. A greater osmotic adjustment has been reported in leaves of mycorrhizal basil plants than in non mycorrhizal ones during a lethal drought period. In the same way, AM plants had postponed declines in leaf water potential in maize plants during drought stress (Porcel and Ruiz-

Lozano, 2004).

Accumulation of osmolytes could have provided the root with osmotic mechanism to maintain a favourable water potential gradient for water entrance into the roots (Irigoyen *et al.* 1992) leading, therefore to a lower stress injury in the plant.

Proline is one of the most common compatible osmolytes in drought stressed plants. It is accumulated in the leaves and enhances osmotic adjustment (Ruiz-Lozano *et al.* 1995; Routley, 1966).Proline does not interfere with normal biochemical reactions but allows the plants to survive under stress (Stewart, 1981).

The proline content of leaves of inoculated *Coleus forskholii* and *Mentha piperita* plants appeared to be a good parameter to measure water stress. The amount of proline has been increased with the increase in water stress concentration.

Osmotic adjustment is considered to be an important component of drought tolerance mechanisms for high plants.Drought stressed plants have been shown to accumulate organic osmolytes such as sugars and amino acids (proline) that are known to contribute to the host plant tolerance under water deficit conditions (Porcel and Ruiz-Lozano, 2004).Under water stress conditions, high plants accumulate some small molecules including organic solutes and inorganic ions to make higher osmotic adjustment (Wu and Xia, 2006; Martinez – Ballesta *et al.* 2004).

Accumulation of proline increased considerably in roots as a consequence of drought stress and AM plants accumulated more than non AM plants (Fig.3). The amount of proline was 30µ moles/g tissue in *Coleus forskholii* and 35µ moles/g tissue in *Mentha piperita* in AM plants under stress and comparatively lower in non AM stressed plants. These findings are in accordance with Shindhe and Thakur (2015) and Aranjeulo *et al* (2011), who found that water stressed plants could invest a large quality of carbon and nitrogen resources into the synthesis of osmoregulants in the leaves such as proline for maintaining cell turgor. However, the control plants showed comparatively less amount of proline as compared to mycorrhizal plants. Proline accumulation may also be part of the stress signal influencing adaptive responses.

Proline an osmoregulator accumulated less in the plants which were inoculated with AM fungi than non AM plants. The higher leaf water potential in stressed AM plants than in non AM plants also suggests that AM fungi had better adaptation towards stress. Proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger, as a solute that protects macromolecules against denaturation and as a means of reducing acidity in the cell (Porcel and Ruiz-Lozano, 2004). This could be an important adaptation for AM plants since drought also induces an oxidative stress, which it has been responsible for many of the degenerative reactions caused by drought.The oxidation of membrane lipids is a reliable indication of uncontrolled free radical production and hence of oxidative stress.

In addition to the above discussed drought tolerance mechanisms, the AM contribution to plant drought tolerance might also have occurred through drought avoidance mechanisms such as hyphal water uptake (Porcel and Ruiz-Lozano, 2004) or increased water uptake related to mycorrhizal changes in root morphology or soil structure (Auge, 2001).

The overall data show that the plants are influenced by AM symbiosis by means of drought avoidance and drought tolerance mechanisms. It seems AM symbiosis enhances osmotic adjustment which could contribute to maintain a water potential gradient favourable to the water passing from soil into the roots.

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