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Research Article

Influence of Arbuscular Mycorrhizal Fungi on Proline and Chlorophyll Content in *Zingiber Officinale* Rosc Grown Under Water Stress

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ABSTRACT
The plants of *Zingiber officinale* Rosc, were grown in pots without mycorrhiza and with mycorrhiza i.e. control and experimental. The water stress was given to plants at the interval of 5, 7, 9 and 11 days. The ginger plants were grown under water stress condition for four months. The estimation of chlorophyll a, chlorophyll b and total chlorophyll along with proline contents was carried out from mycorrhizal and non mycorrhizal plants. The amount of chlorophyll content was found to be decreased due to increase in water stress however the chlorophyll contents in mycorrhizal plants recorded more than non mycorrhizal plants. The amount of proline content increased with increase in stress but the non mycorrhizal plants showed slightly higher amount than the mycorrhizal plants.

Key Words: Water stress, AM fungi, *Zingiber officinale* Rosc, rhizome, chlorophyll, Proline

INTRODUCTION
The arbuscular mycorrhizal (AM) fungi enhance tolerance of plant to water deficit through the alteration of plant physiology (Ricardo Aroca *et al.*, 2008). In most of the experiments it has been indicated that Arbuscular Mycorrhizal (AM) fungi are able to alter water relation of its host plants. AM fungi enhance the growth of the plant by improving properties of soil in rhizosphere. They absorb P and other nutritional elements and then improve nutritional status of host plant.

Zingiber officinale Rosc belongs to the family "Zingiberaceae" commonly called as 'adrak' or ginger. It has an underground stem called as rhizome which is covered by a scaly leaves and possess fibrous roots. It is native of south-east Asia; it is cultivated in most of the countries. It grows well at an elevation of 1,500 meters from the mean sea level. Fresh ginger, if dried after application of calcium chloride for three to five days in the sun becomes '*sunth*' after drying. It is valued not only for the aromatic flavour but also acclaimed in ayurvedic preparations, in allopathic preparations, spice and household remedies throughout the world. The ancient Egyptians, Greeks and Romans were well aware of its stomachic benefits. The Chinese herbalists use it in medicines since more than 2,500 years. It is carminative and a good laxative as it expels stagnant food remains from the digestive system. It is an expectorant and warms up the lungs and dries excess moisture. Ginger is propagated by its fresh rhizome by cutting into moderate pieces of about 25 to 30 g. with one or two eye buds on each piece which forms new shoots within 20-25 days after sowing. It is grown either in flatbeds or ridges. Ginger grows in the temperature range around 28-30^{o} C. Extremely high temperatures are desiccating and result in death of the plants. At the beginning, temperature requirement is relatively high but after germination it requires relatively less temperature. Ginger grows well in sandy clay and clayey loam soils with adequate organic matter like farm yard manure for retention of moisture. The range of pH of 5.5 to 6.5 with drainage is found suitable for it. Arbuscular mycorrhizal (AM) fungi are natural plant growth regulators and stimulants (Wood and Cummings 1992). Most of the terrestrial plants form mycorrhizas. Many mycorrhizae have been shown to enhance plant survival and fitness through mechanisms such as increasing water and nutrient uptake (Marschner and Dell 1994; Peterson *et al*. 2004; Pasqualini *et al*. 2007; Plassard and Dell 2010). Mycorrhizal fungi form symbiotic relationship with host plants. Most of the experiments have indicated that arbuscular mycorrhizal fungi are able to alter water relation of their host plants Huixing Song (2005). They grow in close association with the roots and play an important role in the concentration and transfer of soil nutrients to the plant. In exchange, the plant supplies the fungus with sugars. Mycorrhizal fungi have been suggested as having a role in mediating the uptake of water at times of drought stress. Root systems of crop and native plants are commonly colonized by one or more mycorrhizal fungi, naturally occurring soil fungi that increase nutrient absorption and improve soil

structure and fertility. The hyphae of arbuscular mycorrhizal fungi penetrate the roots and grow extensively between and within living cortical cells, forming a very large and dynamic interface between symbionts. The hyphae also extend from root surfaces into the surrounding soil, binding particles and increasing micro- and macro-aggregation (Auge, 2001). Mycorrhizal fungi can increase absorption of phosphorus by symbiosis with plant roots. (Aliabadi Farahani *et al.* 2008)**.** In present investigation, ginger plants were grown under different water stress conditions. They were watered at the interval of 5, 7, 9 and 11 days. During the vegetative growth stages the effects of water stress on chlorophyll contents from leaves and proline contents from leaves and rhizomes were examined.

MATERIALS AND METHODS

The plants of *Zingiber officinale* Rosc. were collected from Rahimatpur village of Satara District from Maharashtra state. The plant were identified and confirmed from the Botanical Survey of India, Western regional office Pune, before starting the experiment. Soil used for the experimentation was prepared by mixing soil, farmyard manure and sand in 3:1:1 ratio. This soil, farmyard manure and sand mixture was autoclaved at 120 lbs pressure for 20 minutes, cooled overnight and then filled in plastic pots of uniform size, which comprised 8 kg soil/pot. Healthy and disease free rhizomes were selected for cultivation. The rhizomes were cut in to small pieces of about 25-30 g. with one or two eye buds on each. These pieces were placed in 0.5% $HgCl_2$ for 30 minutes and then washed with water by placing under tap water for 4 Hrs. The pots were then filled with the mixture of mycorrhiza from the pot culture i.e. mycorrhizal soil 200g/pot. The soil mixture used for pot culture included the species of *Acaulospora appendiculata, A. gerdmanni, Glomus convolutum, G. fasciculatum and Scutellispora calospora*. For each treatment three replicates were maintained. The washed rhizomes were then grown in each pot. The pots were placed under shade net and irrigated with normal water for 1 month at the interval of 5 days. The ginger plant took 20-25 days to germinate. The first water stress treatment was given after one month. Four sets of 5, 7, 9 and 11 days each were prepared for the present study. The water stress of 5 days for 1^{st} set, 7 days for 2^{nd} set, 9 days for 3^{rd} set and 11 days for 4^{th} set were given for four months. The first set received the water for maximum times and the last set received water for minimum times. Every time 500 mL of water was added in each pot. The water used for stress treatment for all plants was of neutral pH. At the very beginning of an experiment plants were not exposed to water stress to avoid desiccation and to ease the germination. In the beginning ginger plant requires optimum amount of water otherwise plants do not grow properly. Plants were harvested after four months stress treatment. The average of three replicates was taken for study of each parameter. The amount of proline was determined by Bates *et al.* (1973) method. The plant material used for proline estimation was rhizome and leaves. The procedure for proline estimation was followed as under. Extracted 0.5 g. of plant material in 10 mL of 3 % aqueous sulphosalicylic acid and homogenized it. The homogenate was then filtered through Whatman No.2 filter paper. Two mL of filtrate was added with 2 mL of glacial acetic acid and 2 mL acid ninhydrin. This mixture was heated in boiling water bath for 1 hr. The reaction was then terminated by placing the tubes in ice bath. After cooling the reaction mixture, added 5 mL toluene and stirred well for 20-30 seconds. Then the toluene layer was separated and placed at room temperature and absorbance was measured at 520 nm wavelength. Standard graph of proline was drawn by using standard proline. The amount of proline was calculated with the help of standard graph. The photosynthetic pigments i.e. chlorophyll a; chlorophyll b and total chlorophyll were determined by Arnon's method (1949). Chlorophyll extract was prepared from fresh leaves (100 mg) by grinding in a mortar and pestle, together with 10 ml of ice cold 80% acetone. The homogenate was centrifuged at 3000 rpm for 2 minutes. The supernatant was saved and pellet was re-extracted twice with 5 ml of 80% acetone. All the supernatants were pooled and saved.

The absorbance of the extract was recorded at 663 nm, 645 nm and the concentration of chlorophyll a, chlorophyll b and total chlorophyll was calculated using Arnon's equations as follows.

Total Chl: = (20.2 × A 645-8.02 × A 663) × 100 /mg leaf weight

Chl-a = (12.7 × A663-2.69 × A645) × 10 /mg leaf weight

Chl-b = (22.9 × A645-4.61 × A663) × 10 /mg leaf weight

RESULTS AND DISCUSSION

The effect of water stress on proline and chlorophyll content in ginger was studied in pot culture experiment. The amount of proline was increased significantly as

there was increase in water stress interval. The amount of proline was recorded more in leaves as compared to the rhizome. The amount of proline was found more in non mycorrhizal plants than mycorrhizal plants (Figure No.1). This was due to AM fungi which helps the host plant during water stress condition. Mycorrhizal plants synthesize less amount of proline than non mycorrhizal plants. Proline is an important amino acid in plant under drought stress that prevents oxidation of cells from inside. It also regularizes osmotic pressure of plant under drought stress for absorbing water.

Therefore proline accumulation rate increased in ginger under drought stress, similar results were recorded by Aliabadi Farahani *et al* (2008) in *Coriandum sativum.* Water deficits induce dramatic increases in the proline concentration of phloem sap in alfalfa Girousse *et al.*, (1996), suggesting that increased deposition of proline at the root apex in water stressed plants Voetberg and Sharp, (1991). The results of the pot culture experiment showed that there was clear influence of water stress on the chlorophyll contents of ginger plants.

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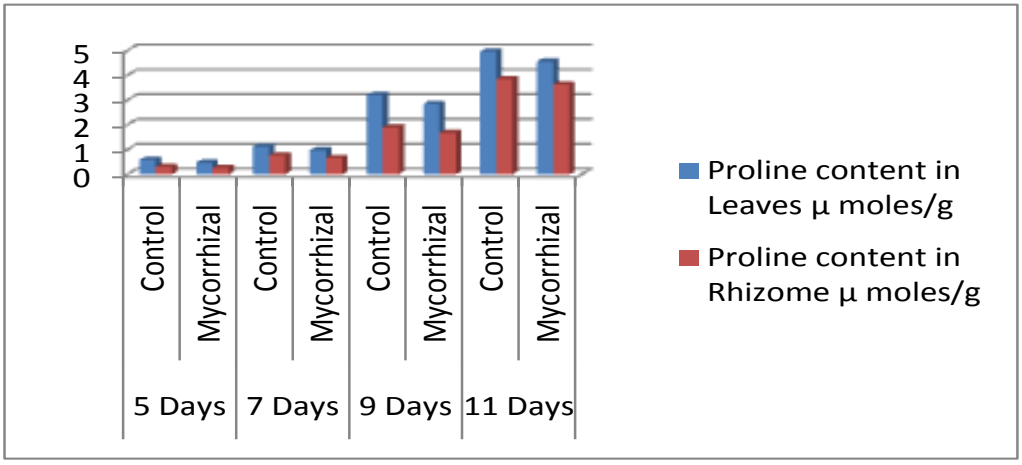

Figure 1: Proline contents in leaves and Rhizome in µ moles/g.

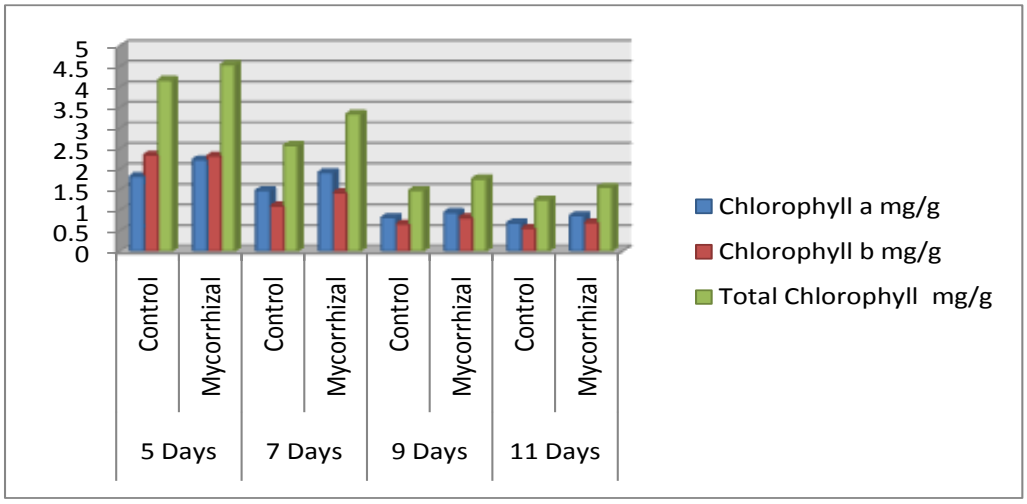

Figure 2: Chlorophyll a, Chlorophyll b and total chlorophyll contents in leaves mg/g.

In all three replicates of different water stress intervals, the amount of chlorophyll recorded was more than control plants. The increase in water stress interval reduced the amount of chlorophyll. The maximum amount of chlorophyll a, chlorophyll b and total chlorophyll recorded at 5 days interval and lowest at 11 days interval (Figure No. 2). The values for graph are taken as average of three replicates as compared with control. It was observed that the more the water stresses least the chlorophyll contents and least the water stress more the chlorophyll contents (Figure No.2). Mycorrhizal plants very often show higher rate of photosynthesis than non mycorrhizal plants Huixing Song (2005). Arbuscular mycorrhizal symbiosis increased the rate of photosynthesis, and so as to increase the rates of photosynthetic storage and export at the same time Auge R.M. (2001). It has been proved that the amount of chlorophyll in mycorrhizal plants was higher than non mycorrhizal plants Gemma *et al* (1997); Davies *et al* (1993); Mathur and Vyas (1995) and higher concentration of chlorophyll is associated with higher photosynthesis rate Davies *et al* (1993). Different VAM fungi have different effects on photosynthesis in the condition of drought stress Dixon *et al*. (1994); Ruiz-Lozano and Azcon found that in comparison with non mycorrhizal plants one species of *Glomus* increased photosynthetic efficiency of its hostThus, it is concluded that the water stress influences the amount of chlorophyll in leaves. The rate of photosynthesis is least in stressed plants resulting yellowing of leaves. The amount of proline was recorded more in non mycorrhizal plants than that of the mycorrhizal plants. This clearly indicated that the mycorrhiza helps the plants during water stressed conditions hence they do not synthesize proline in more concentration, whereas the non mycorrhizal plants need proline as an osmolytes for its survival so that they synthesized more proline than mycorrhizal plants. The amount of proline was recorded more in leaves than that of rhizome because transpiration is taking place through leaves but not through the rhizome so that proline is required as an osmolytes in leaves.

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