Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2011 Vol. 1 (4) October- December, pp.126-130/Bhosale and Shinde
Research Article

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON NUCLEIC ACIDS AND PROTEIN CONTENTS IN GINGER UNDER WATER STRESS CONDITION

***KS Bhosale [1] and BP Shinde [2]**
Department of Botany Nowrosjee Wadia College Pune 411 001 India
Department of Botany Fergusson College Pune 411 004 India
**Author for Correspondence*

ABSTRACT
The rhizomes of ginger, *Zingiber officinale* Rosc. weighing 25-30 g were grown in the pots with and without mycorrhiza. The pots were placed under shade net and watered with normal water for 1 month at the interval of 5 days. The water stress treatment was started after one month at the interval of 5, 7, 9 and 11 days for four months. The estimation of DNA, RNA and proteins from control and experimental plants was carried out after four months. The amount of DNA, RNA and protein contents increased with the increase in water stress. However, these contents were more in control plants as compared to experimental plants. It was evident that mycorrhiza helped the plants during water stress conditions. The mixture of AM fungi used for current experimentation included the species of *Acaulospora appendiculata, A. gerdmanni, Glomus convolutum, G. fasciculatum and Scutellospora calospora*.

Key Words: *Water stress, AM fungi, Ginger, Rhizome, DNA, RNA, Protein*

INTRODUCTION
The Arbuscular Mycorrhizal (AM) fungi improve the assimilation of water and nutrition for plants, and enhance resistant ability to drought (Ahonen-Jonnarth, 2000). It promotes plant growth, increases crop yield and improves the tolerance to drought, saline and alkaline stress by strengthening nutrient absorption (Smith and Gianinazzi, 1988). Symbiosis of plants with mycorrhizal fungi, improves plants survival under drought stress. Moreover, many mycorrhizal fungi can stand up to drought stress and high temperature (Han *et al*., 2006).
Ginger (*Zingiber officinale*) is a plant that grows in India, China, Mexico, and several other countries. The underground rhizome is the active part used. This herb has been used in Ayurvedic medicine for the treatment of inflammation and rheumatism and is used in China and some Western countries as a treatment for nausea. In an ayurvedic system of medicine, effects of ginger have been reported in the treatment of rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes.
Ginger is propagated by its fresh rhizomes by cutting into the pieces of about 25 to 30 g. with one or two eye buds on each piece which then forms new shoots within 20-25 days after growing. 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). 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 (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 DNA, RNA and Protein contents from rhizomes were examined.

MATERIALS AND METHODS

The plants of ginger, *Zingiber officinale* Rosc. were collected from Rahimatpur village of Satara District from Maharashtra state. The plant was identified and confirmed from the Botanical Survey of India, Western regional office Pune region (Ref No. BSI/WRC/Tech/2010/), before starting the experiment. The soil used for the experiment 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 60 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 piece. These pieces were placed in 0.5% $HgCl_2$ for 30 minutes and then washed with water by washing under tap water for 4 Hrs. The pots were then filled with the mixture of mycorrhiza from the pot culture i.e. mycorrhizal soil 200 g / pot. The mixture of soil used for pot culture included the species of *Acaulospora appendiculata, A. gerdmanni, Glomus convolutum, G. fasciculatum and Scutellospora calospora*. For each treatment three replicates were maintained. The rhizomes after washing were 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. 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. The ginger rhizome took 20-25 days to germinate. The water stress treatment was started after one month at the interval of 5, 7, 9 and 11 days for four months. The first set received the water for maximum times (5 days interval) and the last set received (11 days) water for minimum times. Every time 500 mL of water was added in each pot. The water used for stress treatment for all plants had neutral pH. Plants were harvested after four months stress treatment. The average of three replicates was taken for study of each parameter.

The isolation of DNA was carried out from the rhizomes by the method of Dellaporta *et al.,* (1983) and estimated by Burton's method (1956). Initially prepared three separate tubes containing 1 mL, 2 mL and 3 mL aliquots of the isolated DNA dissolved in standard saline citrate and similar aliquots of a 0.5 mg of standard DNA. The final volume of all the tubes rose along with a separate blank up to 3 mL with H_2O. Then added 6 mL of diphenylamine reagent to each tube, and after mixing, heated the tubes in a boiling water-bath for 10 minutes and cooled the tubes. The absorbance of blue solution was read at 600 nm against the blank. The standard graph was constructed with the standard DNA and the amount of DNA was calculated with the help of it.

Isolation of RNA was carried out by the Brawerman's method (1974). The estimation of RNA was done by Bial's method (1902). The standard RNA was prepared by taking 50 µg RNA/mL solution in ice-chilled 10 mM Tris-acetate, 1mM EDTA buffer (pH 7.2) by dissolving RNA completely. The isolated RNA dissolved in the EDTA buffer (pH 7.2) solution to an approximate concentration 50 µg/mL. Then prepared the series of the tubes containing 0.5 mL, 1 mL, 1.5 mL and 3 mL of isolated RNA. The similar series also was prepared using 0.5 mL, 1 mL, 1.5 mL and 3 ml, of 50 µg standard RNA. The final volume in all tubes was made up to 3 mL with distilled water. In addition the blank was set containing 3

mL distilled water. Then added 6 ml of orcinol acid reagent to each tube. This was followed by addition of 0.4 mL of 6.0% alcoholic orcinol to each tube. The tubes were then shaken well to mix the content and heated all tubes in a boiling water-bath for 20 min. The tubes were then cooled and absorbance was read at 660 nm against the blank. The standard graph was drawn using standard RNA. The amount of isolated RNA was calculated using the standard graph.

Proteins were estimated by Lowry *et al.,* (1951) method. The rhizomes of ginger from control and experimental plants were cut into small pieces separately and 0.5 g plant material was extracted with 5 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved in 2 ml of 1.0 N NaOH solution. This was used as a sample and 0.2 ml was taken for the estimation of proteins. The working standard of BSA and plant extract was taken in a series of test tubes and final volume was adjusted to 1 mL in each tube. Then 5 mL of reagent C was added in all the tubes and incubated the mixture for 10 min. This was followed by addition of 0.5 mL of folin ciocalteau and incubated at dark for 30 min. The blue colour developed in the reaction mixture was read at 660 nm on UV-visible spectrophotometer. Bovine serum albumin fraction V (BSA) was used at the concentration of 50 mg and dissolved in distilled water and used as a standard protein to prepare the standard graph. The amount of protein was calculated with the help of standard graph.

RESULTS AND DISCUSSION

Water stress showed an influence on DNA, RNA as well as Protein contents in ginger plants grown under water stress conditions. The amount of DNA was more in the control plants than that of experimental plants, (Fig.1). The DNA contents in any cell of the same plant remains same in all conditions. The water stress is considered one of the most important stressful environmental conditions to plant survival in arid and semi-arid regions Chaves *et al*., (2003). Thus, the water stress results in the shrinkage of cell. The shrinkage results in the increase of cell number per gram of tissue and that's why the amount of DNA increases per gram of plant tissue. The DNA content in control plant was more than experimental plant because of the shrinkage there was increase in cell number and DNA content. On the other hand in experimental plant mycorrhiza helped the ginger plant during water stress and there was less shrinkage of cells so that number of cells remains as it was along with DNA content. The maximum amount of DNA was present in the plant with the water stress interval of 11 days and least amount of DNA was observed in the plant with the water stress interval of 5 days.

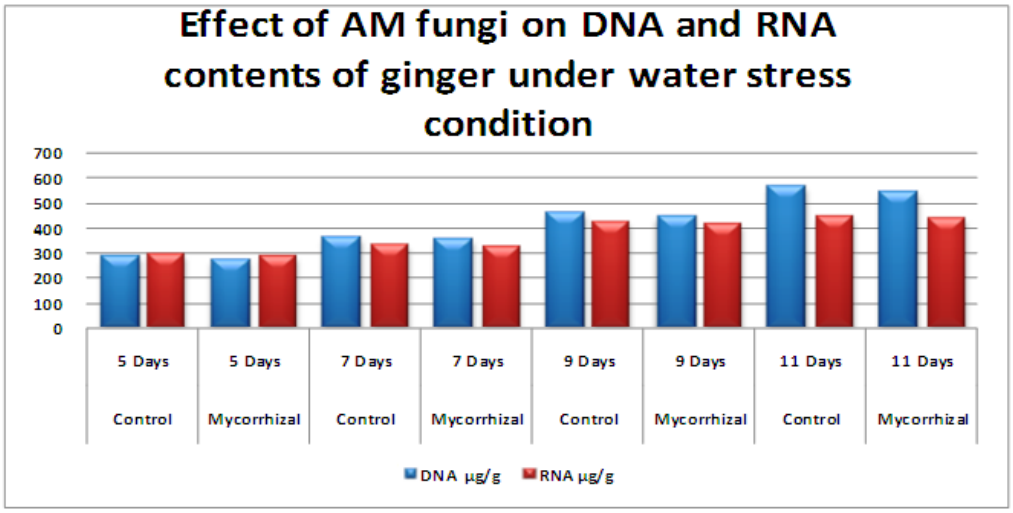

Figure 1. Effect of AM fungi on DNA and RNA contents of ginger under water stress condition.

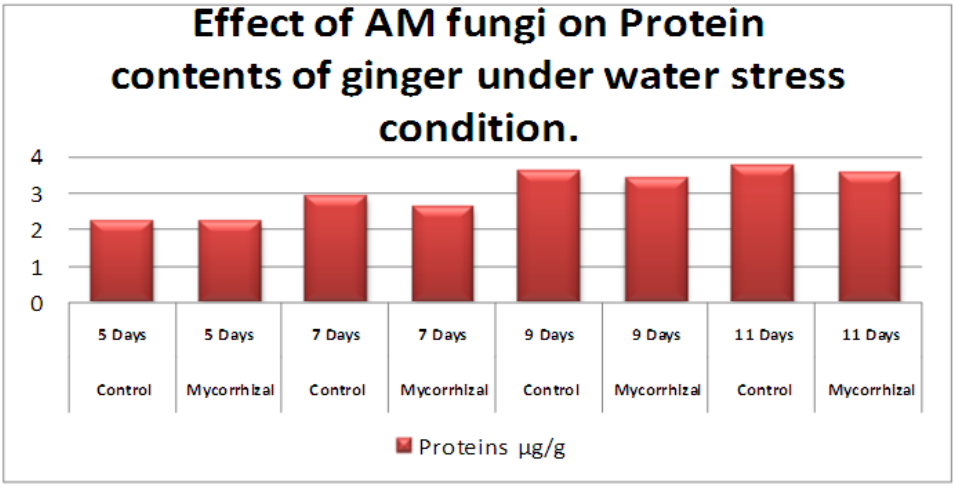

Figure 2. Effect of AM fungi on Protein content of ginger under water stress condition.

Hence it was evident that AM fungi helped the ginger plant during water stress. The DNA content had influence on the RNA contents in plants and hence RNA content was more in control plant than experimental plants (Fig. 1). The amount of RNA similarly was more in the plants with the water stress interval of 11 days and least in the plants with the interval of 5 days.

The DNA and RNA have direct relationship with the protein content in plants. The increased amount of DNA and RNA resulted on protein content. The amount of protein was more in control plant than experimental plant (Fig. 2), that means mycorrhiza helped the plants during the water stress.

The protein content in control plant was more than that of mycorrhizal plant. This clearly indicates that in absence of AM fungi plant need more proteins and thus plant store more proteins. The experimental plants got more nutrients because of mycorrhiza and hence it showed less protein. The increase in the water stress increases in the amount of protein in the ginger rhizomes. The least proteins were present in the plants with 5 days water stress interval and it was increased with the stress interval. The maximum amount of protein was present in the plants with the stress interval of 11 days. The results of the present studies showed that AM fungi have great influence on nucleic acids and protein contents in ginger rhizome. Thus the AM fungi are helpful for the growth of ginger under water stress conditions.

REFERENCES

Ahonen-Jonnarth U (2000). Growth, nutrient uptake and ectomycorrhizal function in *Pinus sylvestris* plants exposed to aluminium and heavy metals. Doctoral Thesis. Swedish University of Agricultural Sciences. Uppsala.

Auge RM (2001). Water relation, drought and VA mycorrhizal simbiosis. *Mycorrhiza*. **11** 3-42.

Bial M (1902). Estimation of RNA nucleic acid *Den Med Woch.* **28** 253.

Brawerman G (1974). In: *Methods in Enzymol.* 30 *(Eds.* Moldave and Grossman, L.) Academic Press. New York 605.

Burton K (1956). A study of the Conditions and mechanism of the diphenylamine reaction of the colorimetric estimation of Deoxyribose nucleic acid *Biochemical Journal,* **62** 315.

Chaves MM, Maroco J P and Pereira J S (2003). Understanding plant responses to drought-from genes to the whole plant. *Functional Plant Biol*ogy **30** 239-264.

Dellaporta SL, Wood J and JB Hicks (1983). A plant DNA mini preparation: version II. *Plant Molecular Biology Reporter 1(4)* 19-21.

Farahani A, Lebaschi H, Hussein M, Shiranirad A., Valadabadi AR and Daneshian J (2008). Effects of arbuscular mycorrhizal fungi, different levels of phosphorus and drought stress on water use efficiency,

relative water content and proline accumulation rate of Coriander (*Coriandrum sativum* L.). *Journal of Medicinal Plants Research*. **2**(6) 125-131

Han XL, Jia GX and Niu Y (2006). Research of the mechanism of ectomycorrhizae to drought resistance. *Research of Soil and Water Conserv*ation **5** 42-44.

Huixing S (2005). Effects of VAM on host plant in the condition of drought stress and its mechanisms *Electronic Journal of Biology,* 2005 **1**(3) 44-48

Lowry OH, Rosebrough NJ, Farr AL and RJ Randall (1951). Protein measurement with the Folin-Phenol reagents. *Journal of Biological Chemistry*. 193 265.

Marschner H and Dell B (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* **159**, 89–102.

Pasqualini D, Uhlmann A and Sturmer LS (2007). Arbuscular mycorrhizal fungal communities influence growth and phosphorus concentration of woody plants species from the Atlantic rain forest in South Brazil. *Forest Ecology and Management* **245** 148–155.

Peterson RL, Massicotte HB, Melville LH (2004). Mycorrhizas: anatomy and cell biology. *National Research Council of Canada, Ottawa, Ontario, Canada*.

Plassard C and Dell B (2010). Phosphorus nutrition of mycorrhizal trees. *Tree Physiology* **30** 1129–1139.

Smith SE, Gianinazzi-Pearson V (1988). Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Annual Review of Plant Physiology and Plant Molecular Biology **39** 221-244

Wood T and Cummings B (1992). Biotechnology and the future of VAM commercialization. In: Allen MF (ed) Mycorrhizal functioning. Chapman and Hall, London 468-487