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Drought tolerance of bioenergy grass *Saccharum spontaneum* L. enhanced by arbuscular mycorrhizae

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ABSTRACT

This study explores the effects of arbuscular mycorrhizal (AM) represented by *Glomus* spp. colonization on *Saccharum spontaneum* (L), a potential bioenergy grass subjected to drought stress under greenhouse conditions. The different treatments were: (1) watered control plants without AM association, (2) watered control plants with AM association, (3) drought stressed plants without AM association, (4) drought stressed plants. The AM association was established with *Saccharum spontaneum* (L) as evident from the increase in percentage of root infection and distribution frequency of vesicles. AM plants maintained a higher leaf osmotic potential and relative water content as compared to non-AM plants to counter osmotic stress in *S. spontaneum*. Accumulation of sugar (71% and 47% in AM and non-AM plants, respectively), proline (8.5 fold and 4 fold in AM and non-AM plants, respectively) and phenolic content (101% and 68% in AM and non-AM plants, respectively) were higher in AM plants as compared to non-AM plants on 12 d of imparting drought stress. Protein (3.4 and 2.4 fold in AM and non-AM plants, respectively) and amino acid (63% and 46% in AM and non-AM plants, respectively) content were significantly increased in AM plants over non-AM plants on 8 d of drought stress. Similarly, activities of SOD (superoxide dismutase), APX (ascorbate peroxidase) and GPX (guaiacol peroxidase) were higher in AM plants as compared to non-AM plants. Lipid peroxidation (83% and 129% in AM and non-AM plants, respectively) was more pronounced in non-AM plants as compared to AM plants on 12 d of imparting drought stress. Reduction in total chlorophyll content (63% and 85% in AM and non-AM plants, respectively) and activities of photosystem I (33% and 42% in AM and non-AM plants, respectively) and photosystem II (27% and 57% in AM and non-AM plants, respectively) was more evident in non-AM plants as compared to AM plants. The maximum quantum efficiency of PS II primary photochemistry (F_v/F_m), potential photochemical efficiency (F_v/F_o) and the maximal fluorescence (F_m) were higher in AM plants than non-AM plants. Based on these results, it can be deduced that the AM association helps the acclimation of *S. spontaneum* grown in drought stress conditions. AM association aid in enhancing the inherent drought tolerance potential of *S. spontaneum* by maintaining better osmotic status and enhanced antioxidation which have resulted in lesser decline of total chlorophyll and photosynthetic capacity.

1. Introduction

Biofuels are renewable source of energy and can be used as an alternative for fossil fuels and will help to reduce the energy dependency on fossil fuels (Huang et al., 2011a, 2011b). Bioethanol has been projected as a principal candidate for the next generation biofuels. The first generation bioethanol is produced mainly from food crops such as cassava, sweet potato and the second generation bioethanol (cellulosic ethanol) is produced from non-food crops such as energy grasses which include elephant grass, miscanthus, switch grass etc. (Ohimain, 2013). The second generation bioethanol has many advantages, which can overcome many limitations of the first generation bioethanol (Ladislao and Gomez, 2008). But the challenge is to identify the plant species that accumulate high biomass and that can be grown in marginal land, so that biomass production does not interfere with the existing crop production in arable land. Reclamation of marginal land is a challenge for the growth of the plants, as these lands are adversely affected by various stresses including water deficit, salinity etc. (Quinn et al., 2015).

Drought is a major abiotic stress experienced by marginal land, which reduces the ability of plants to take up water from soil and result in the suppression of plant growth. Extreme drought lowers the soil osmotic potential and further cause's ionic stress and nutritional imbalance (Parida and Das, 2005). To encounter drought stress, plants

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undergo various changes at the cellular and metabolic levels, such as an increase in the concentration of a variety of compatible solutes viz. proline, betaines and sugar alcohols (Chen and Murata, 2002). Drought stress increases reactive oxygen species (ROS) production, which causes damage to lipid membranes, proteins and nucleic acids. Plants alleviate this oxidative damage by producing various antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR). Non-enzymatic means of ROS detoxification is by the action of several small molecules that are antioxidants in nature which includes quaternary ammonium compounds, polyamines, polyols, alpha tocopherol, ascorbic acid and carotenoids (Sairam et al., 2005).

The association of soil with microorganisms has proved to augment the existing abiotic stress tolerance mechanism within the plants and thus helps to alleviate the detrimental effects of stresses. Arbuscular mycorrhiza (AM) fungi, a beneficial soil microorganism which gets associated with plant roots can improve plant water relations and also increase tolerance towards drought by producing a very elaborate network of hyphae, connecting the soil with the host plant and thus facilitates water uptake at minimum soil moisture levels (Navarro et al., 2011). AM association also facilitates plant growth by assimilating less mobile elements such as P, Zn, and Cu and improves the structure of soil and its stability by producing a glycoprotein (glomalin) into the soil (Wu et al., 2013). Our earlier studies have showed that *S. spontaneum* is a drought tolerant grass as compared to some other energy grasses studied. But acclimatization in marginal lands is a challenging task considering the poor soil quality and various abiotic stresses prevailing over there. Therefore, in order to carry out successful acclimatization, it is necessary to improve soil quality as well as the ability of the plant species to tolerate various abiotic stresses which are the characteristic features of marginal lands. AM fungi have been considered as a valuable tool in the reclamation of disturbed and marginal lands. AM fungi enhance reclamation of marginal lands by modifying root system for better nutrient acquisition by the host plant as well as impart resistance to biotic and abiotic stresses (Fagbola et al., 2001). *Saccharum spontaneum*, a prominent energy grass was shown to develop AM association in natural habitats (Mirshad and Puthur, 2015). Therefore, enhancing inherent potential of drought tolerance in *S. spontaneum* associated with *Glomus* spp. was studied.

2. Materials and methods

2.1. Plant material and AM inoculum

The AM inoculum was produced from a pot culture of *S. spontaneum* associated with *Glomus* spp. The inoculum was prepared when the root infection rate of AM in *S. spontaneum* was 90%. The inoculum contained a mixture of soil and root fragments (40 g), which approximately consisted of 520 spores.

2.2. Experimental design

Soil was sterilized by the method of solarization for 30 d according to the method of Raj and Sharma (2009). Healthy stem cuttings (20–30 cm long) of *S. spontaneum* (collected from different areas of Kozhikode district, Kerala, India) were planted in polybags (30×28 cm) containing 4 kg of sterilized soil. Plants were maintained in greenhouse at a relative humidity of 60 ± 5%, temperature of 28 ± 2 °C and day light of 800 ± 100 μmol/m^2/s. Two sets of plants were maintained, one without AM (*Glomus* spp.) inoculation and the other with inoculum (40 g of inoculum was provided at the base of each stem cutting placed in polybags with sterilized soil). After 50 d of AM inoculation (as described earlier) and plant growth, one half from each set was irrigated and other half was imposed with drought treatments for 16 d by withholding irrigation and was maintained in the green house. Various analyses were carried out in fresh leaves samples at 4 d intervals up to 16 d (4, 8, 12 and 16 d).

2.3. Soil water content (SWC)

Soil samples were collected on 4, 8, 12 and 16 d after withholding the irrigation. Soil water content (SWC) was calculated according to Robert et al. (1987).

2.4. Assessment of AM root colonization

AM colonization in roots was detected as described by Phillips and Hayman (1970) and assessment of percentage colonization of AM was calculated using the following formula;

$$\text{Root colonization (\%)} = \frac{\text{Number of segments colonized with AM}}{\text{Total no. of segments observed}} \times 100$$

2.5. Determination of leaf ψ_s and relative water content

Leaf ψ_s was measured according to Hura et al. (2007), using a vapour pressure osmometer (Wescor 5520, USA). Calibration of the chamber was done using 100, 290 and 1000 mmol/kg standard solutions (Wescor).

2.6. Estimation of metabolites

Proline content was estimated according to Bates et al. (1973). Total sugar was estimated according to Montgomery (1957). Total polyphenols were extracted in 80% ethanol and estimated according to the procedures described by Folin and Denis (1915). Total protein was estimated using Folin-Ciocalteau reagent according to the method of Lowry et al. (1951). Free amino acids were extracted from leaf samples by 70% ethanol and estimation was carried out following the method of Moore and Stein (1948).

2.7. Estimation of malondialdehyde (MDA) content

Malondialdehyde (MDA) was estimated according to the method of Heath and Packer (1968).

2.8. Enzyme assay

The enzyme extract was prepared by the method of Yin et al. (2009). The activity of superoxide dismutase (SOD) was assayed by the method of Giannopolitis and Ries (1977). Ascorbate peroxidase (APX) activity was assayed as described by Nakano and Asada (1981). The activity of guaiacol peroxidase (GPX) was estimated according to Gaspar et al. (1975).

2.9. Estimation of pigment composition and photosystem activities

Chlorophyll estimation was carried out by the method of Arnon (1949). Thylakoids from leaves were isolated according to Puthur (2000). Photochemical activities of isolated thylakoids were assayed polarographically with a Clark-type oxygen electrode (DW1/AD, Hansatech, Norflok, UK) which was connected to a digital control box (OXYG1, Hansatech). The light dependent O_2 uptake/evolution was measured by irradiating the sample with saturating intensity of white light (1800 μmol m^{-2} s^{-1}), provided by a 100 W halogen lamp (LS2, Hansatech). The activity of Photosystem (PS) I and PS II was expressed in terms of μmol O_2 consumed/evolved min^{-1} mg^{-1} chlorophyll.