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Research Article

Efficacy of AM fungi against drought stress on sweet corn cultivars with special reference to biochemical contents

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Abstract: This study was carried out to investigate the effect of drought stress on biochemical contents of sweet corn cultivars. The plants of sweet corn were grown in pots without mycorrhiza and with mycorrhiza i.e. control and experimental. The pots were placed under shade net and watered with normal water for one month at an interval of 4 days. The water stress treatment was started after one month at an interval of 4, 8 and 12 days. The biochemical analysis was carried out from leaves and kernels of sweet corn plants. The amount of chlorophyll, protein and starch has been decreased significantly due to increase in drought stress. The amount of proline and carbohydrates has been increased significantly with the increase in drought stress. However, these contents were more in mycorrhizal plants as compared to control plants. The results indicated that AM symbiosis alleviates the toxic effect of drought stress via improving water status of plants.

Key words: Water stress; AM fungi; Sweet corn; Chlorophyll; Protein; Proline; Starch and total Carbohydrates

Introduction

Sweet corn (*Zea mays* L. saccharata) also known as sugar corn is a hybridized variety of maize (*Zea mays* L.) specifically bred to increase the sugar content. Sweet corn is introduced to India from USA. The fruit of the sweet corn plant is the corn kernel. It has a sugary rather than a starchy endosperm and a creamy texture. The modern sweet corn varieties are classified as "normal sugary" (Su); "sugary enhanced" (Se) and "shrunken" (Sh2) also called as "super sweet". These differ in sweetness and ratio of conversion of sugar to starch. All these varieties are most popular but "super sweet" are commercially used because it is very sweet and has very low conversion of sugar to starch. When the moisture content is higher than 74 per cent the cobs are immature and below 70 per cent they lose the sweetness, and develop an unpleasant taste and texture. It has a thinner pericarp than the normal corn making it tender (Pradeep *et al.*, 2005).

Drought is undoubtedly one of the most important environmental stresses limiting the productivity of crop plants around the world (Bohnert *et al.,* 1995). Drought stress decreases the rate of photosynthesis (Kawamitsu *et al.,* 2000). Plants grown under drought condition have a lower stomatal conductance in order to conserve water. Consequently, CO_2 fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants. Diffusive resistance of the stomata to CO_2 entry probably is the main factor limiting photosynthesis under drought (Boyer, 1970). Occurrence of drought stress during maize growth period may hamper the nitrogen and water use efficiencies leading to significant yield losses (Saini and Westgate, 2000; Ashraf *et al.,* 2016).

The AM fungi can enhance resistance to drought stress in host plant may include improving the properties of soil in rhizosphere, enlarges root areas of host plants, and improves its efficiency of water absorption, enhances the absorption of phosphorus and other nutritional elements and then improves nutritional status of host plant, activates defence system of host plant quickly, protects against oxidative damage generated by drought. Ommen *et al.*, (1999) reported that leaf chlorophyll content decreases as a result of drought stress. Drought stress caused a large decline in the chlorophyll *a* content, the chlorophyll *b* content, and the total chlorophylls content in all sunflower varieties investigated (Manivannan *et al.*, 2007). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). Abbaspour *et al.*, (2012) found that AM colonization improved the drought tolerance of *Pistacia vera* seedlings by increasing the accumulation of osmotic adjustment compounds, nutritional and antioxidant enzyme

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activity. Plants can partly protect themselves against mild drought stress by accumulating osmolytes. Proline is one of the most common compatible osmolytes in drought stressed plants. Proline content has increased under drought stress in sweet corn (Sanchez *et al.,* 1998; Alexieva *et al*., 2001). Proline accumulation can also be observed with other stresses such as high temperature and under starvation (Sairam *et al*., 2002). Proline metabolism in plants, however, has mainly been studied in response to osmotic stress (Verbruggen and Hermans 2008). Proline does not interfere with normal biochemical reactions but allows the plants to survive under stress (Stewart, 1981). Regulations of physio-biochemical responses of plants under drought stress can be used as markers for drought stress tolerance in selection and breeding (Shakeel *et al.,* 2017). The accumulation of proline in plant tissues is also a clear marker for environmental stress, particularly in plants under drought stress (Routley, 1966). Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio *et al*., 2002). Adebayo and Menkir (2015) stated that sustained yields in maize under drought stress were directly related to better antioxidant activities.

Drought increased the peroxidase and superoxide dismutase activities in both shoots of *Juniperus oxycedrus* seedlings inoculated with exotic AM fungi and grown with composted sewage sludge, but the increase was less than in the plants neither inoculated nor treated with sewage sludge. Both the plants inoculated with exotic AM fungi and the plants grown with composted sewage sludge developed additional mechanisms to avoid oxidative damage produced under water-shortage conditions (Alguacil, 2006).

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).

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, sweet corn plants were grown under different drought stress conditions. They were watered at the interval of 4, 8 and 12 days. During the vegetative growth stages, the effects of drought stress on biochemical contents were examined. The purpose of this study was to contribute a better understanding of the physiological responses of sweet corn plants to toxic effect of drought stress. We have investigated the influence of AM fungi and drought stress on the total chlorophyll content, Protein, Proline, starch and total carbohydrate content in sweet corn.

Materials and Methods

Experimental Set Up: A study was conducted to determine the effect of arbuscular mycorrhizal (AM) fungi inoculation on the biochemical contents of sweet corn grown under water stressed pot culture conditions. Water stress treatment was given at the Fergusson College botanical garden. In this experiment, seeds of Sweet corn were sown in the pots with and without mycorrhiza. Fifteen replicates of both control and mycorrhizal plants were maintained during present investigation. These plants were watered with normal water for one month at an interval of 4 days. The mixture of AM fungi used for current experiment included the species of *Acaulospora, Glomus* and *Scutellospora*. 25 gram of mycorrhizal soil was added in the pots at the time of sowing of seeds in mycorrhizal set. The AM fungi have been shown to help in retaining moisture of soil and also help in uptake of important nutrients during stress conditions (Heikham *et al.,* 2009). The water stress treatment was started after one month old sweet corn seedlings at an interval of 4, 8 and 12 days for next two months. Every time biochemical analysis was done at an interval of 15 days. The different parameters studied in mycorrhizal and non-mycorrhizal plants include biochemical analysis of chlorophyll, proteins, proline, starch and total carbohydrates.

Quantitative estimation of Chlorophyll

Chlorophyll was extracted from the leaves of mycorrhizal and non-mycorrhizal plants by Arnon's method (1949). Fresh leaves of mycorrhizal plants and non-mycorrhizal plants were plucked and one gram sample was weighed. The leaves were crushed in 20 ml acetone using chilled mortar and pestle. The slurry was centrifuged at 5000 rpm for five minutes. The supernatant was collected and the residue was again homogenized with 80% acetone and centrifuged. This was continued till the residue lost all its green pigment and turned white. The supernatant was collected and the final volume was made up to 100 ml by using 80% acetone. The solvent (80 % acetone) was used as blank and the

absorbance of the samples was read at 645 and 663 nm using UV visible spectrophotometer

Quantitative estimation of Protein

The Proteins content of leaves and seeds was estimated by Lowry *et al.,* (1951) method. The mycorrhizal and non- mycorrhizal samples were washed 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.

Quantitative estimation of Proline

The amount of proline was determined by Bates *et al.,* (1973) method. The plant material used for estimation of proline was seeds and leaves. The procedure for estimation of proline was as follows. Extracted 0.5 g. of plant material in 10 ml of 3% aqueous sulphosalicyclic acid and homogenized it. The homogenate was then filtered through Whatman no.1 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 toluene layer was separated and placed at room temperature. The absorbance of fraction with toluene aspired from liquid phase was read at a wave length of 520 nm. Standard graph of proline was drawn by using standard proline. The amount of proline was calculated with the help of standard graph.

Quantitative estimation of Starch

Starch is an important polysaccharide. The amount of starch was quantified using the method suggested by Hedge and Holfreiter (1962). This method is based on the principle that In hot acidic medium starch is hydrolysed to glucose and dehydrated to hydroxymethyl ferfural. This compound forms a green coloured product with anthrone reagent. Standard glucose stock is prepared by using 100 mg in 100 ml water and working standard is prepared by 10 ml of stock diluted to 100 ml with water. For the quantitative estimation of starch 0.1 gm of leaf and kernels samples grown in control and mycorrhizal conditions weighed and homogenized in 80% hot ethanol to remove sugars. The homogenate is centrifuged at 3000 rpm for 10 min and the residue is retained for further estimation. The residue is washed repeatedly with hot 80% ethanol until the washing gives no colour with anthrone reagent. The residue left after washing is dried well over water bath. To this residue add 5 ml of water and 6.5 ml of 52% perchloric acid and extract to this at 0 degree for 20 minutes. The above extract is centrifuged and supernatant is preserved for further analysis. The process of extraction is repeated using fresh perchloric acid and the total volume of supernatant is raised to 100 ml using distilled water. From the above supernatant 0.1 and 0.2 ml of supernatant is pipette out and the volume is raised to 1ml with water. Standard series of solution was prepared by taking working standards and the total volume in each tube is raised to 1 ml water. To each of these tubes containing standards or the sample of unknown concentration add 4 ml of anthrone reagent and heated in water bath for 8 minutes after that all the tubes are rapidly cooled and the intensity of green to dark green colour was recorded at 630 nm. The glucose content in the sample was obtained using the standard graph and to arrive at the starch content the value was multiplied by factor 0.9.

Quantitative estimation of total carbohydrates

Total carbohydrates in the leaves and kernels of sweet corn plants were determined by phenol sulphuric acid method proposed by (Krishnaveni *et al.,* 1984). For the estimation, 100mg of mycorrhizal and non- mycorrhizal tissue was weighed. The tissue was hydrolyzed by adding 5 ml 2.5N HCl and boiling in hot water bath for three hours. After cooling, it was neutralized using solid sodium carbonate until effervescence ceases. The final volume was made to 100 ml and centrifuged. From this sample, 0.1 and 0.2 ml was pipette in two separate test tubes. The volume of the test tube was made to 1 ml by using water. To the test tubes, 1ml phenol and 5 ml 96% H_2SO_4 was added. The tubes were kept for 10 minutes and shaken well. These test tubes were kept in hot water bath at 20-30^{0}C for 20 minutes. The absorbance was taken at 490nm after cooling by using the mixture of 1 ml water, 1 ml phenol and 96% H_2SO_4. For the standard readings, glucose solution was used. A standard glucose solution was prepared by dissolving 100mg in 100ml water. Working standard was prepared by diluting 10ml of standard glucose by 90ml water. In hot acidic medium, glucose gets dehydrated to hydrozymethyl furfural which forms green colour with phenol. The amount to total carbohydrates is calculated by using the standard graph drawn with

the help of readings obtained using working standard.

Results and Discussion

The effect of AM fungi on the content of chlorophyll in the leaves of sweet corn plant was examined under water stress conditions. In all the five replicates of different water stress intervals it was observed that the chlorophyll content was recorded more in mycorrhizal plants rather than control plants. The increase in water stress interval reduced the amount of chlorophyll. The water stress treatment was given to one month old seedlings at an interval of 4, 8 and 12 days. The maximum amount of chlorophyll a, chlorophyll b and total chlorophyll was recorded at 4 days' interval and lowest was at 12 days interval (Chart No.1). It was found that more the water stress interval least the chlorophyll contents and least the water stress interval more the chlorophyll contents (Chart No.1) It has been proved that the amount of chlorophyll content in mycorrhizal plants was higher as compared to 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; Shinde and Khanna, 2014) recorded higher amount of chlorophyll pigments in mycorrhizal plants of potato as compared to non- mycorrhizal plants. (Bhosale and Shinde, 2011) reported similar results in *Zingiber officinale* under water stress condition

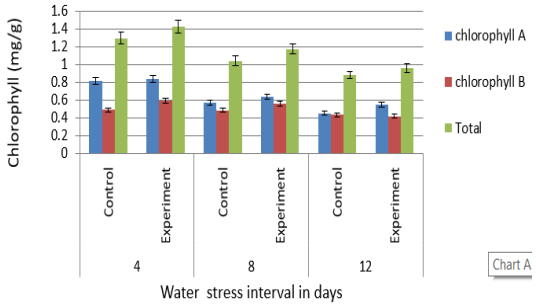

Chart 1: Effect of AM Fungi on chlorophyll content in sweet corn plant at the interval of 4, 8 and 12 days

The amount of protein was more in experimental plant than control plants (Chart No. 2) and (Chart No.3) that means mycorrhiza helped the plants during the drought stress. The increase in the drought stress interval decreases in the amount of protein in the both leaves and kernels. The maximum amount of proteins was present in the plants with 4 days drought stress interval and it was decreased with the increase in drought stress interval. The least amount of protein was present in the plants with the drought stress interval of 12 days. The results of the present studies showed that AM fungi have great influence on protein contents in sweet corn. In the present study drought stress resulted in reduction of protein content both in leaves and kernels. Similar findings were observed by (Mishra and Gupta, 2006; Osmen *et al.,* 2007; Bhosale and Shinde, 2011b; Shinde and Jaya, 2015). Results of our study indicated that there was a decreasing trend in kernels protein of sweet corn plant under water deficit which is in agreement with the findings of (Shinde and Deokule, 2013) who reported that in wheat (*Triticum aestivum*) protein content decreased under water stress condition. Similar results were observed by Gong *et al.,* (2005) in wheat and Mafakheri *et al.,* (2011) in chickpea under drought stress condition.

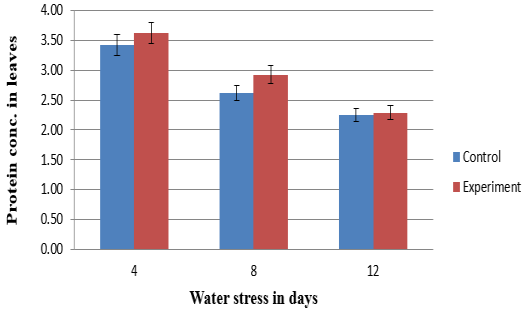

Chart 2: Impact of water stress and AM fungi on the protein content in leaves of sweet corn plant

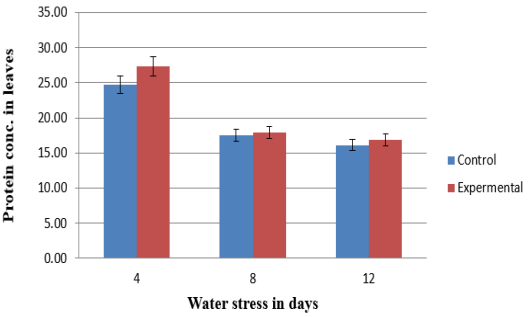

Chart 3: Impact of water stress and AM fungi on the protein content in kernels of sweet corn

The amount of proline was found to be increased significantly as there was increase in level of water stress interval. The amount of proline was found more in leaves in comparison of seeds (Chart No.4) and (Chart No.5) The amount of proline was found to be more in mycorrhizal plants rather than non- mycorrhizal plants (Chart No.4) and (Chart No.5). This was due to AM fungi which helps the host plant during water stress condition. During 4 days water interval, the amount of proline was less in leaves and kernels. At the interval of 12 days, the amount of proline was high in both leaves and kernels. The plant treated with 8 days interval showed intermediate results. (Chart No.4) and (Chart No.5). These findings are in accordance with the findings of (Aranjuelo, 2011; Bhosale and Shinde, 2011b; Shinde and Jaya 2015) who found that water stressed plants could invest a large quantity 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. Plants can partly protect themselves against mild drought stress by accumulating osmolytes.

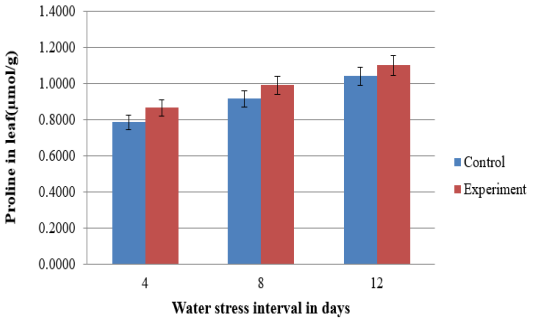

Chart 4: Impact of water stress and AM fungi on the proline content in leaves of sweet corn

Proline accumulation can also be observed with other stresses such as high temperature and under starvation (Sairam *et al.,* 2002). Proline metabolism in plants, however, has mainly been studied in response to osmotic stress (Verbruggen and Hermans, 2008). Proline does not interfere with normal biochemical reactions but allows the plants to survive under stress (Stewart, 1981). The accumulation of some compatible solutes, i.e., proline and other free amino acids increased significantly in *Salicornia brachiata* under PEG-induced water stress that played dynamic roles in osmotic regulation, pH maintenance, protection of cellular macromolecules, and scavenging of free radicals to negate water stress (Parida and Jha, 2013). Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio *et al*., 2002). The above findings are also in accordance with the findings of Shinde and Deokule (2013).

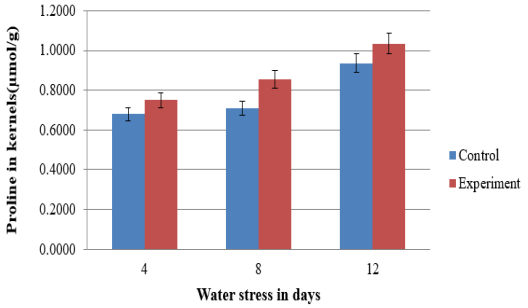

Chart 5: Impact of water stress and AM fungi on the proline content in kernels of sweet corn

The increase in the drought stress causes a decrease in the amount of total starch content in the sweet corn kernels. The more amount of total starch content was present in the kernels of sweet corn plant during 4 days water stress interval and with the increase in drought stress it has been decreased significantly in kernels. The least amount of total starch content was present in the kernels of sweet corn during water stress interval of 12 days. The plant treated with the 8 days water stress interval showed intermediate results (Chart No.6). In the present study drought stress treatment resulted in the reduction of total starch content Similar findings were observed by (Houman and Victoria, 2014; Mir Aafaq *et al.,* 2013). Our results indicated that there was decreasing trend in total starch content of kernels of sweet corn under water deficit which is in accordance with the findings of (Helal *et al.,* 2013) who reported that in cassava starch content decreased under water deficit conditions. Starch depletion in grapevine leaves was noted by Patakas and Noitsakis (2001) in response to drought stress.

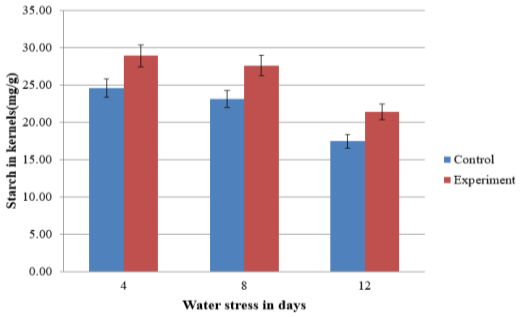

Chart 6: Impact of water stress and AM fungi on the starch content in kernels of sweet corn

A significant increase in the carbohydrates values was clearly observed under drought stress conditions in sweet corn plants. At the interval of 4 days, carbohydrate content was less in leaves and kernels in both control and mycorrhizal plants. At the interval of 12 days the amount of carbohydrate was quite high in leaves and kernels in both control and mycorrhizal plants. The plant treated with 8 days interval showed intermediate results (Chart No. 7) and (Chart No.8). Accumulation of soluble carbohydrate increases the resistance to drought in plant. Soluble carbohydrates have role in osmotic regulation and conservation mechanism (Martin *et al*., 1993). Osmotic stress in plant cells leads to a reduction in carbon assimilation, which is linked to a physiological closure of leaf stomata and to biochemically determined lower photosynthetic activity, which affects carbohydrate economy (Chaves *et al*., 2002). Soluble sugars are acting as osmolytes maintaining cell turgor of leaves, protecting the integrity of the membrane, and preventing the denaturation of proteins (Mohammad Khani and Heidari, 2008). Our results corroborate with those of (Hu et al., 2015) who demonstrated that a significant increase in carbohydrate metabolites especially sugars indicated a diurnal turnover under limited water supply in *Phoebe zhennan* plants, suggesting their availability to be metabolized in source organs or their translocation toward roots.

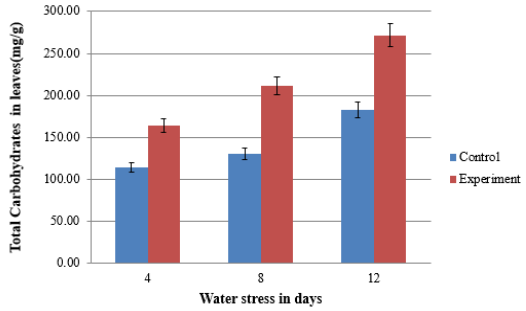

Chart 7: Impact of water stress and AM fungi on the carbohydrate content in leaves of sweet corn plant

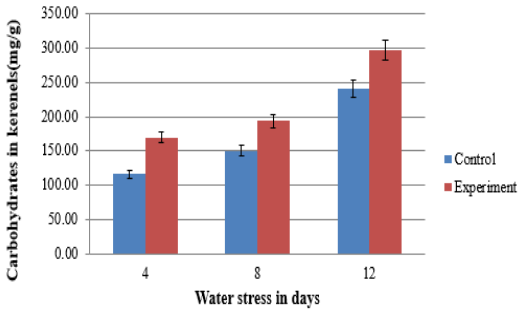

Chart 8: Impact of water stress and AM fungi on the carbohydrate content in kernels of sweet corn

Conclusions

Present work is initiative for studying the different mechanisms under drought stress conditions. On the basis of above findings, it can be concluded that out of five biochemical studied proline and total carbohydrates were significantly increased and chlorophyll, protein and total starch content were decreased under water stress in sweet corn. The AM fungi helped sweet corn plants during drought stress which resulted in increase in biochemical contents in mycorrhizal plants than control plants.

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