

Responses of crops towards water stress mitigation through plant growth regulators and Vasicular Arbuscular Mycorrhizae

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SUMMARY

Plants in nature are continuously exposed to several biotic and abiotic stresses. Among these stresses, drought stress is one of the most adverse factors of plant growth and productivity and considered a severe threat for sustainable crop production in the conditions on changing climate. Drought triggers a wide variety of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Understanding the biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions. This review describes role of plant growth regulator and vasicular arbuscular mycorrhizae on mitigation of drought as well as some aspects of drought induced changes in morphological, physiological and biochemical changes in plants. Drought stress progressively decreases CO₂ assimilation rates due to reduced stomatal conductance. It reduces leaf size, stems extension and root proliferation, disturbs plant water relations and reduces water-use efficiency. It disrupts photosynthetic pigments *i.e* chlorophyll molecule and reduces the gas exchange leading to a reduction in plant growth and productivity. The critical roles of osmolytes accumulation under drought stress conditions have been actively researched to understand the tolerance of plants to dehydration. In addition, drought stress-induced generation of active oxygen species is well recognized at the cellular level and is tightly controlled at both the production and consumption levels, through increased anti-oxidative systems. This review focuses on the ability and strategies of higher plants to respond and adapt to drought stress.

Key Words : Drought stress, PGR, VAM, Photosynthetic pigments, Osmolytes, Growth, Yield

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Moisture stress is one of the major constraints for growth and productivity of crop plants. The adaptation of plants and their ability to tolerate different abiotic stresses is of greater relevance for genetic manipulation to develop improved genotypes for higher productivity under these conditions. Moisture stress affects many metabolic aspects of plant and induce anatomical and morphological changes resulting in reduced growth. The use

of plant growth regulators (PGR) and VAM (Vesicular Arbuscular Mycorrhizae) are an alternative approach to ameliorate the effect of moisture stress on crops. Crop productivity is decreasing due to detrimental effects of various biotic and abiotic stresses; therefore, minimizing these losses is a major area of concern to ensure food security under changing climate. Environmental abiotic stresses, such as drought, extreme temperature, cold, heavy metals, or high salinity, severely impair plant growth and productivity worldwide. Drought, being the most important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factor. Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. Available water resources for successful crop productions have been decreasing in recent years. Furthermore, in view of various climatic change models scientists suggested that in many

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regions of world, crop losses due to increasing water shortage will further aggravate its impacts. Drought impacts include growth, yield, membrane integrity, pigment content, osmotic adjustment water relations, and photosynthetic activity (Praba *et al.*, 2009). Drought stress is affected by climatic, edaphic and agronomic factors. The susceptibility of plants to drought stress varies independence of stress degree, different accompanying stress factors, plant species, and their developmental stages (Demirevska *et al.*, 2009). Acclimation of plants to water deficit is the result of different events, which lead to adaptive changes in plant growth and physiobiochemical processes, such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defences (Duan *et al.*, 2007). It has become imperative to elucidate the responses and adaptation of crops to water deficit, and take actions to improve the drought resistance ability of crop plants and to ensure higher crop yields against unfavourable environmental stresses. This article attempted to provide an overview of morph physiological and biochemical responses of plants to drought stress

The germinating phase is of prime importance in the cycle of plants as it determines the stand establishment and the final yield of crop. During the process of germination first seed has to imbibe water which depends on the composition of external factors as well as internal particularly the water potential. Germination rate decreases with decreasing external water potential and for each species there is critical level of water potential, below which germination will not occur. This particular character determines the plant stand, especially under unfavourable environmental conditions such as water stress. The plant response to water deficit are however, known to be dependent on factors such as the degree of stress, duration of stress and the stage of growth (Levitt, 1980). It has been reported that the early drought reduces the effects of subsequent drought through the process of adaptation to water deficit, which is controlled by modification in several physiological and biochemical mechanisms. The effect of drought on plants is complex and responds with many protective adaptations. During drought, the plant suffers from dehydration of cells and tissues (Henkel, 1962). To overcome the problem of drought there are many avenues *viz.*, development of resistant genotypes, seed hardening, use of antitranspirants and alcohols (RaoGangadhar *et al.*, 1999). In recent years, the technique of seed hardening is gaining importance and has been used by farmers on large scale in many crop species. Under adverse conditions; growth regulators and inoculation with VAM fungus ameliorate the adverse affects of drought. Mycorrhiza is an association or symbiosis between the roots of most land plants and many soil fungi that colonize the cortical tissue of roots during periods of active plant growth, from which both partners benefit; vesicular arbuscularmycorrhiza (VAM) is the most common and universal mycorrhiza. The use of VAM fungi in

cultivated crop is an important factor for increasing productivity under adverse conditions as well as enhancing a more rational use of fertilizers (Menge, 1983). Under low moisture levels, water absorption capacity of roots might be enhanced by mycorrhiza. The improved water status and enhanced drought tolerance caused by VAM infection was due to absorption and translocation of water by external hyphae (Liu, 1989). It has been suggested that VAM plants acclimatize more efficiently to water stress (Sweat and Davies, 1984). Same as various plant growth regulators (PGR) have also been used in pre-treating the seeds to induce drought resistance. Plant growth regulators are substances that influence physiological processes of plants at very low concentrations. When produced endogenously by plants, they are often referred to as phytohormones (plant hormones). Plant hormones have been viewed as chemical messengers regulating the normal progression of developmental changes as well as responses to environmental signals. PGR as either naturally or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, or facilitate harvesting. Both the term PGR and phytohormones has been used interchangeably, particularly when referring to auxins, gibberellins, cytokinin, ethylene and abscisic acid. Cytokinin plays a role in stomatal regulation under water stress. It has been reported that water stress lowered cytokinin activity, and rewatering of drought-stressed plants restored the activity (Pospisilova *et al.*, 2000).

Reponses of physiological parameters :

Root: shoot ratio

Root elongation during drought may help plants get deeper water, thus avoiding water deficits near the soil surface. Elongation also could reduce the water lost by drainage when precipitation allows recovery after the drought (Ludlow and Muchow, 1990). If, however, water is unavailable deeper in the soil profile, longer roots may reduce shoot dry weight and harvest index by allowing the preferential partitioning of photosynthate to roots at the expense of shoots. Kothari *et al.* (1990) reported the effect of VAM and rhizosphere microorganism on root and shoot morphology, growth and water relation in maize. VAM and microorganism showed higher root: shoot dry weight ratio and water uptake. While Pospisilova *et al.* (2000) is focused on the interactions between water stress and cytokinins (CKs). CK treatment resulted in increased shoot/root ratio and/or decreased stomatal regulation of gas exchange and it has the ameliorative effect in water stress. The finding of Abdel *et al.* (2002) indicated that the effect of mycorrhizae on drought stressed plants in broad bean which had significant higher shoot dry weight. Same as result also reported by Khan and Shahabaz (2003) the influence of VAM on *Avena sativa* at two water regimes (100 % field capacity and 50 % field capacity) which resulted

in maximum shoot and root dry weight. During drought, soil begins to dry at the surface and drying extends to the lower soil horizon. Under such conditions the growth and yield of cotton depends on deep and proliferated roots which can effectively make use of stored soil moisture in the sub soil. The shoot length decreased with increase in the intensity of stress. Increased root growth may result in reduced growth of shoot and results in change in the root: shoot ratio. Low resistance to water movement through roots and conducting system to the shoots by an increase in vessels or vessel diameter also aids in maintaining water uptake under the stress (Hale and Oracett, 1987). VAM significantly increased the root length and shoot length and decreased root: shoot ratio at both the stages of the crop. In cycocel treated plants, there was increase in root length and decreased shoot length which resulted in increased root: shoot ratio at both the stage of the crop. Similar results were reported by Turner and Begg, (1978). This might be due to a greater decrease in growth of tops, to minimize water loss and a rapid increase in root growth in search of moisture under moisture stress condition. Subramanian and Charest (2004) reported in maize that plants treated with VAM had higher shoot and root length than non-VAM; this provides the clear evidence that the external mycelium of VAM fungus transports considerable amounts of tracers to the plants under drought conditions. Decreased root to shoot ratio was observed in VAM treatment at both stages of crop growth. The similar result was reported by Busse and Ellis (1985), indicating enhanced drought tolerance by VAM in different field crops.

Photosynthesis :

Environmental stresses have a direct impact on the photosynthetic apparatus, essentially by disrupting all major components of photosynthesis including the thylakoid electron transport, the carbon reduction cycle and the stomatal control of the CO₂ supply, together with an increased accumulation of carbohydrates, peroxidative destruction of lipids and disturbance of water balance. The ability of crop plants to acclimate to different environments is directly or indirectly associated with their ability to acclimate at the level of photosynthesis, which in turn affects biochemical and physiological processes and, consequently, the growth and yield of the whole plant (Chandra, 2003). Drought stress severely hampered the gas exchange parameters of crop plants and this could be due to decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence, oxidation of chloroplast lipids and changes in structure of pigments and proteins (Menconi *et al.*, 1995). Anjum *et al.*, (2011a) indicated that drought stress in maize led to considerable decline in net photosynthesis (33.22%), transpiration rate (37.84%), stomatal conductance (25.54%), water use efficiency (50.87%), intrinsic water use efficiency (11.58%) and intercellular CO₂ (5.86%) as compared to well

water control. Many studies have shown the decreased photosynthetic activity under drought stress due to stomatal or non-stomatal mechanisms (Ahmadi, 1998; Del Blanco *et al.*, 2000; Samarah *et al.*, 2009). Stomata are the entrance of water loss and CO₂ absorbability and stomatal closure is one of the first responses to drought stress which result in declined rate of photosynthesis. Stomatal closure deprives the leaves of CO₂ and photosynthetic carbon assimilation is decreased in favour of photorespiration. Considering the past literature as well as the current information on drought-induced photosynthetic responses, it is evident that stomata close progressively with increased drought stress. It is well known that leaf water status always interacts with stomatal conductance and a good correlation between leaf water potential and stomatal conductance always exists, even under drought stress. It is now clear that there is a drought-induced root-to-leaf signalling, which is promoted by soil drying through the transpiration stream, resulting in stomatal closure. The non-stomatal mechanisms include changes in chlorophyll synthesis, functional and structural changes in chloroplasts, and disturbances in processes of accumulation, transport, and distribution of assimilates.

Germination :

Moisture stress affects various aspects of plant growth right from germination to boll opening and effects vary with different stages of growth. The effects on growth and yield parameters is through effect on various physiological and biochemical processes like cell division, cell elongation etc. Germination itself begins with water uptake by the seeds (imbibitions) and ends with beginning of elongation by the embryonic axis, usually the radical. The water potential that prevents germination is critical for species and study of such stress condition for crop plants furnishes valuable information (Levitt, 1972). The germination phase of seeds is considered as critical for achieving a successful crop, since it indirectly affects yield, especially under arid conditions (Hadas, 1976). Several studies have shown that moisture stress reduces the germination. Germination of rice decreased with increasing water potential of more than -1 atm of polyethylene glycol (PEG) solution (Deka and Baruah, 1998). Similar reduction in germination under stress situation was observed in rice by Deka and Baruha (2000) and Abugrab and Ebrahim, (1998) who reported increase in germination of onion due to growth regulators as compared to control. Sawan *et al.* (2000) reported the effect of kinetin on seed germination, yield and yield components of cotton plants. The results showed that use of kinetin at 5 mg/litre for pre-soaking seeds before planting and spraying cotton plants at 60 and 75 DAS with the same concentration could improve cotton germination, seed cotton and lint yields. Treatments showed variation in the per cent germination. Maximum reduction in germination was observed at higher stress (50% FC) followed by moderate stress (70%

FC). This might be due to decrease in water potential and increase in osmotic potential with the increased level of stress which may have additive effect on imbibitions of water by the seed during germination. The treatment VAM showed highest germination followed by CCC and least was noticed in control under higher moisture stress (50% FC) (40.8%). This higher germination could be due to maintenance of higher water potential under water stress situations in the treatments of VAM and CCC. Similar reduction in germination under stress situation was observed in rice by Deka and Baruha (2000) and Abugrab and Ebrahim, (1998) who reported increase in germination of onion due to growth regulators as compared to control.

Seedling vigour :

Seedling vigour index (SVI) is an important factor in the establishment of a crop and is independent on the root and shoot length that is governed by the factors which control the process of germination. This was also affected due to the moisture stress and was maximum under non stressed conditions. It decreased with an increase in the intensity of stress, the highest SVI was noticed in the treatment VAM which also possessed higher root length, Shoot length and germination percentage. The finding of Sawan *et al.* (2000) indicating the effect of kinetin on seed germination, seedling vigour, seed cotton and lint yields of cotton plants. The results showed that use of kinetin at 5mg/litre for pre-soaking seeds before planting and spraying cotton plants at 60 and 75 DAS with the same concentration improved cotton germination, seed cotton and lint yields. Similarly Garai and Datta (2003) indicated that foliar application of cycocel reduced plant height, increased number of branches per plant and nodules per plant in green gram. Hardening as well as pelleting treatment enhanced seed germination and seedling vigor as compared to control in black gram (Begum and Krishnasamy, 2003). Similarly, Davies *et al.* (1996) reported that mycorrhizae end to alter root morphology and carbon allocation patterns of shoots and roots. Seedling vigour index (SVI) is an important factor in the establishment of a crop and is independent on the root and shoot length that is governed by the factors which control the process of germination. This was also affected due to the moisture stress and was maximum under non stressed conditions. It decreased with an increase in the intensity of stress, the highest SVI was noticed in the treatment VAM which also possessed higher root length, shoot length and germination percentage. The results are in agreement with those of Abdel *et al.* (2002) in the broad bean plants. Begum and Krishnasamy (2003) also indicated the similar results in black gram treated with CCC.

Relative water content :

Relative water content (RWC) is measure of the amount of water present in the leaf tissue in relation to turgid condition

and the treatments having higher RWC under drought condition would be preferable to maintain higher water balance. In present so many studies are indicating that the treatment VAM recorded significantly higher RWC followed by CCC, CK compared to control during both stages. These results are in concurrence with Abdel *et al.* (2002) in broad bean and Farooqi *et al.* (2005) in aromatic grasses. The increase in RWC due to the application of plant growth regulators could be due to their ability in hormone directed translocation of photosynthates leading to better osmoregulation and enhanced RWC and also due to mycorrhizal infection there is an increase in water content of stress plants by mycorrhizal colonization. LRA-5166 cotton variety in present investigation increased of relative water content in VAM was 10.4 per cent and 15.9 per cent over control at both stages of crop growth. Similarly Thadapani and Subharayalu (1980) reported that soaking cotton seeds in 500ppm CCC solution for 12hrs and drying for 5hrs in shade increased seed cotton yield proline accumulation, chlorophyll stability index and RWC at different growth stages as well as dry matter production under drought conditions. Singh *et al.* (1999) reported the effect of benzyl adenine during water stress in Senna (*Cassia anustifolia* Vahl.) and showed decrease in RWC by 85 to 54 per cent. While Prakash and Ramchandran (2000) find the effect of three chemical ameliorants namely CCC, lime wash and potassium chloride on stomata frequency, leaf water potential, RWC, in brinjal plants under moisture stress conditions and among these CCC proved best in the case of relative water content. Abdel *et al.* (2002) reported the influence of VAM on growth and metabolic changes of broad bean plants grown under drought conditions. Arbuscular mycorrhizal inoculation significantly increased the relative water content compared to non mycorrhizae treatment. Similarly Atteya (2003) reported the influence of drought stress on internal water status of three Egyptian corn (*Zea mays* L.) genotypes; GIZA2, TWC310 and TWC320 at different developmental stages. During drought stress there was decrease in relative water content (RWC) of 86.6 to 69.6 per cent in genotype, GIZA2. Farooqi *et al.* (2005) find out the effect of chlormequat chloride treated to aromatic grass (*Cymbopogon martini*, *Cymbopogon winterianus*) under drought stress, result was RWC decreased significantly under drought stress, the greater decrease was with *Cymbopogon winterianus* (23-25% compared to control) than in *Cymbopogon martini* (14-18% over control). Same as Mathur *et al.* (2005) reported that the effect of CCC was observed in stressed plants of different varieties of a Japanese mint and showed increased RWC (59-75%). Increase in yield and yield components Misra and Malik (1971) findings are indicating the decrease in plant height by increase in concentration of cycocel and highest yield of seed cotton was obtained by 40ppm and 160ppm spray of cycocel with variety of J-205 and Lohit, respectively. Similarly Mohamed *et al.* (1971) observed the cotton grown under water

stress condition showed increase in yield by the application of CCC. Appleby *et al.* (1996) revealed the increase in test weight and grain yield of wheat with 5 per cent cycocel seed treatment. According to Nawalagatti *et al.* (1991) that foliar spray of CCC at 1000 ppm increased total dry matter production and pod yield in groundnut. Lovett and Orchard (1977) reported that CCC not only reduces the plant growth but also there was reduction in the accumulation of dry matter in stems, leaves and petioles of sunflower besides reduction in leaf area. Similarly RamaKrishnayya (1978) reported that foliar spray of kinetin reduced the drought injury by increased grain number, higher shoot weight and harvest index in rice. Tholkappian *et al.* (2001) studied that the mycorrhizal soybean plant recorded lower levels of proline accumulation than non-mycorrhizal plants. The nitrogen content and pod number per plant increased in mycorrhizal soybean than non mycorrhizal plants. Dighe *et al.* (1983) opined that among the different methods of cycocel treatments, foliar spray of cycocel in wheat produced higher grain yield than the application of cycocel through soil drenching. Ellis *et al.* (1985) inoculated the drought resistant wheat plants with vesicular arbuscular mycorrhizae and plants were subjected to low level and severe stress and they were kept up to harvest and there was increase in leaf area, root weight, total dry weight and VAM plants which had undergone stress periods had twice the biomass yield and grain yields non-VAM plants subjected to same stress periods. Same as the finding of Singh and Kakralya (1992) indicating that 50mg/l of etherel or GA₃ or benzyladenin may be applied as pre sowing seed soaking treatments for 12hrs, increased the germination per cent, crop stand density, seedling growth and yield of pigeonpea. Sylvia *et al.* (1993) recorded the field response of maize to VAM fungus and water management of plant grown in growth chambers and green house. There was increase in growth and yield in the maize. Bio regulators NAA, kinetin and KNO₃ sprayed at bud initiation and pod formation stage of chick pea increased the plant height, number of flowers, vegetative growth and yield. NAA 20 ppm induced early flowering, whereas, kinetin delayed it slightly (Upadhyay, 1994). Carvalho *et al.* (1994) reported the effects on yield and fibre quality of applying the growth regulator cycocel in cotton. Growth regulator treatments increased boll and seed weights while fibre percentage was decreased. Saha and Gupta (1997) observed the effect of plant growth retardants *viz.*, triazols and CCC when applied as soil drench, improved growth, photosynthetic activity and yield of mungbean under salinity. Satvir Kaur *et al.* (1998) observed that germination and seedling growth of chickpea decreased with increasing concentration of PEG and addition of GA₃ (6µm) increased the germination, cotyledonary and seedling growth under stress. Garai and Datta (1999) reported that sesame treated with CCC (200ppm) showed higher yield of more than 53 per cent in comparison to water soaked seeds. So CCC might be utilized for enhancement of

yield of summer sesame under moisture stress. Najma *et al.* (2000) conducted an experiment to evaluate the effect of VAM on plant growth and yield under controlled and drought conditions. Plant growth regulators GA₃ was used in combination with VAM under drought stress. The treatment with VAM showed better vegetative and reproductive growth than non VAM plants. And the plants which treated with combination with GA₃ gave better shoot and root dry weight and yield than only VAM in *Vigna radiata*. Ghourab *et al.* (2000) reported the effect of mepiquat chloride application on the productivity of cotton plants cv. Giza 80. The results revealed, that the application of mepiquat chloride reduced plant height and length of internodes, tended to increase number of opened bolls per plant significantly and increased the seed cotton yield (in kentars per feddan), while seed index, lint percentage, were not significantly affected. Prasad and Ram, (2000) reported the effect of mepiquat chloride [mepiquat] which suppressed vegetative growth in cotton by reducing plant height. Bolls plant⁻¹, boll weight, seed cotton yield and fibre strength were significantly higher in a mepiquat chloride spray compared to the untreated control. Sawan *et al.* (2000) observed the effect of kinetin on seed germination, yield and yield components of Egyptian cotton cultivar Giza 75 (*Gossypium barbadense*). The results showed that use of kinetin at 5 mg/litre for pre-soaking seeds before planting and spraying cotton plants at 60 and 75 DAS with the same concentration could improve cotton germination, seed cotton and lint yields. The effect of mepiquat chloride (Pix), benzyl adenine (BA), ethephon (Prep) and their combinations on the growth and yield of cotton cv. Giza 83 were determined in a field experiment by Abed (2001). All the treatments increased the number of bolls, average boll weight, yield of seed cotton and lint yield per plant and average weight of 100 seeds, while reducing the number of bad bolls. Garai and Datta (2001) reported that, summer sesame with three levels of moisture regimes and five levels of growth regulators, resulted in higher root: shoot ratio, stomatal frequency, leaf protein content and seed yield when CCC @ 100 and 200mg/l was applied. Ram *et al.* (2001), observed the effect nitrogen, chlormequat chloride and farm yard manure (FYM) on cotton growth, yield and quality. Spraying 50 ppm chlormequat chloride increased bolls/plant, boll weight and seed cotton yield. Stephan and Cothren (2001) documented the effect of mepiquat chloride (MC) and PGR-IV on yield and flowering of cotton. MC yield was 3540.9 kg/ha, all PGR treatments resulted in increased yields and boll numbers. Ghazi *et al.* (2003) conducted the study to determine the effects of arbuscular mycorrhizal fungi on growth and grain yield of two winter wheat (*Triticum aestivum* L.) cultivars grown under field conditions. There was increase in biomass yield and grain yield in mycorrhizal treatment than non-mycorrhizae. Uday Burman and Kathju (2003) observed that kinetin (5ppm) applied as either presowing seed treatment or foliar spray or both significantly improved the growth, dry

matter production and seed yield of cluster bean under moisture deficit condition Huixing (2005) observed the effect of VAM on host plant in the condition of drought stress. VAM enhanced resistance to drought stress in host plant by increase dry matter than non-VAM plants. Muhammad *et al.* (2007) reported higher seed cotton yield can be achieved with use of Pix by managing the excessive plant growth

Reponses of biochemical characters :

Chlorophyll content :

Chlorophyll is known to influence the photosynthetic rate and in turn influence growth and development of cotton (Krasichkova *et al.*, 1989). However, under moisture stress conditions there will be degradation in pigment composition, which induce decrease in chlorophyll content. Chlorophyll 'a' and chlorophyll 'b' and total chlorophyll content of leaf decreased with increase in moisture stress at both stages. Higher persistence of chlorophyll content under stress due to growth regulators and VAM may be attributed to decreased chlorophyll degradation and increased chlorophyll synthesis. These results are in accordance with Jayakumar and Thangaraj (1998) who explained that the application of cycocel to groundnut resulted in higher chlorophyll content. The delay in leaf senescence could also be attributed to higher chlorophyll content. Among the treatments, VAM had significantly higher chlorophyll content at both the stages of crop growth. The increase in total chlorophyll concentration of drought plants in response to mycorrhizal effects was positively correlated with respective levels of mycorrhizal infection in broad bean plants (Abdel *et al.*, 2002). Such increases were related to the degree of mycorrhizal infection and Huixing (2005) also showed the effect of VAM on host plant in drought condition that enhanced resistance to drought stress by increase in chlorophyll content than non VAM plants. Singh *et al.* (2000) reported that cytokinin enhanced the chlorophyll content in senna leaves. Thus, our results of enhanced chlorophyll content due to plant growth regulators application and VAM are in agreement with the above discussion. Mohamed *et al.* (1971) observed that cotton grown under water stress condition showed significant increases in both protein and chlorophyll contents by the application of CCC. Similarly Thandapani and Subharayalu (1980) observed that soaking of cotton seeds in 500ppm CCC solution for 12 hrs and shade dry for 5 hrs increased the seed cotton yield and proline accumulation, chlorophyll stability index and RWC at different growth stages under drought conditions. In groundnut, foliar spray of different concentrations of cycocel at 100, 200, 300, 400, 500 and 1000 ppm applied at 20 days after sowing showed that with an increase in the concentrations of cycocel, there was an increase in the chlorophyll content in leaves (Sorte *et al.*, 1989). Microschnichenko and Manankov (1991) observed that the application of GA₃ reduced the chlorophyll content in onion

genotypes while increased chlorophyll content was observed when CCC (1000 ppm) was applied to wheat genotypes at tillering and anthesis stage (Sairam *et al.*, 1991). Similarly Ganiger (1992) reported increase in photosynthetic pigments with CCC and mepiquatchloride sprays on seed tuber planted potato over control. Saha and Gupta (1997) observed the effect of plant growth retardants *viz.*, triazols and CCC when applied as soil drench, improved growth, photosynthetic activity and yield of mungbean under salinity in addition to increase in chlorophyll content. Fatima *et al.* (1999) observed the effects of gibberellic acid and water stress on growth of fenugreek plant. Photosynthetic pigments (Chlorophyll a and b, and carotenoids) were high in the leaves treated with GA₃. Singh *et al.* (2000) reported that cytokinin enhanced the protein, chlorophyll content in senna leaves. Same as Yadav and Pandey (1997) reported the effect of CCC (100 ppm) on the water potential and chlorophyll content and these showed increase in the plant under water stress in wheat. The effects of mepiquat chloride, benzyl adenine (BA), ethephon (Prep) and their combinations on the growth and yield of cotton cv. Giza 83 were determined in a field experiment conducted in Egypt. All treatments (except for Prep alone) significantly increased the chlorophyll a and b, and carotenoids in the leaves Abed *et al.* (2001) and Vijayakumari and Janardhanan. (2003) while assessing the effect of biofertilizers on seed germination and seedling growth of silk cotton (*Ceiba pentandra*) in pot culture experiment reported that combined inoculation of biofertilizers *viz.*, *Azospirillum* + phosphobacteria + VAM resulted in improvement in biochemical parameters like chlorophylls, total soluble carbohydrates, reducing sugars, total free amino acids, buffer-soluble proteins and phenolics at 60, 90 and 120 days in leaves after sowing. Huixing (2005) observed the effect of VAM on host plant in the condition of drought stress. VAM can enhance resistance of drought stress in host plant by increase in chlorophyll content than non-VAM plants. Tayeb (2006) reported the study on germination of five *Vicia faba* cultivars (cv. Giza40, Giza 667) exposed to PEG induced water stress. cv. Giza 40 showed increase in Chl-a, Chl-b and carotenoid contents and the Chl a/b and carotenoid /Chl a+b ratios was observed under drought stress than Giza 667.

Proline content :

The physiological significance of proline accumulation is poorly understood. Proline has been assigned the role of cytosolute a storage compounds or a protective agent for cytoplasmic enzymes and cellular structure (Demir, 2000; Pandey and Ganapathy, 1985). Hanson and Hitz (1982) suggested that proline accumulation is a consequence of stress induced damaged to cells. In plants, the role of proline may not be restricted to that of a compatible osmolytes. For example, proline synthesized during water deficit and salt stress may serve as an organic nitrogen reserve that can be

utilized during recovery (Trotel *et al.*, 1989). In the present study, the proline content in the leaves of LRA-5166 cotton genotype increased with increase in moisture stress levels and seed priming treatments and VAM treatment and differed significantly in their ability to accumulate proline under moisture stress. VAM and seed priming treatment with CCC had more proline accumulation at both stages especially at higher moisture stress levels compared to all other treatments and control, which accumulated less proline at both the stages. More pronounced effect of moisture stress on proline accumulation was observed at 65 DAS than at 45 DAS particularly at higher moisture stress levels. Similar results were found by Huixing (2005) in host plant where VAM symbiosis enlarges absorption areas and improves the nutritional status of plant. The increase in amino acid levels by the influence of mycorrhizal fungus on metabolic changes in the tropical maize was noticed by Kizhaeral and Christiane (2004). Increased proline in the stressed plants may be an adaptation, the purpose of which is to overcome the stress conditions and the reduced proline oxidase may be the reason for increasing proline accumulation (Sankar *et al.*, 2007). By the application of kinetin there was slight increase in proline content compared to control (Balasimha, 1983). McMichael and Elmora (1977) reported that when predawn leaf water potentials reached -15bars, leaf proline concentration increased sharply from trace to 86m moles and to 160 m moles per g dry wt at -35bars during the stress period. Proline increased slightly from 4.6-6.6 g/100g leaf protein. Owing to the late rise in leaf proline concentration following water stress, it was concluded that proline was not a good indicator after the onset of water stress. Balasimha (1983) observed the effect of kinetin on growth and proline accumulation in cocoa seedlings under water stress. There was no much difference between control (648µg/g fresh wt.) and kinetin treated seedlings (564 µg/g fresh wt.) while Janagoudar *et al.* (1983) reported that free proline accumulation was highest in the stem followed by leaf and root under short-term stress. Genotypic differences were significant and drought tolerant varieties had the highest rate of proline accumulation during stress and maintained the higher level of proline even after the alleviation of stress. Similarly Ramakrishnan *et al.* (1988) reported the influence of VAM fungus on free proline accumulation in water stressed maize. There was increase in proline content in VAM treated plants than non-VAM treated plants of maize. Singh *et al.* (1999) reported the effect of benzyladenin during water stress in senna (*Cassia anustifolia* Vahl.) and showed higher amino acid accumulation upto 36 per cent during water stress. Ronde *et al.* (2000) reported the effect of water stress in six cotton cultivars grown in greenhouse resulted in increased free proline content with increasing water stress. Porcel *et al.* (2004) reported the effect of arbuscular mycorrhizal (AM) on soybean plants under drought stress. Results showed higher accumulation of proline in AM roots than non-AM roots.

Kizhaeral and Christiane (2004) reported the influence of mycorrhizal fungus on metabolic changes in the tropical maize under drought, there was increase in amino acid levels by 10.7 per cent and 19.2 per cent of leaf dry mass in C08 and C08 genotypes, respectively than nonmycorrhizal plants. Huixing (2005) observed the effect of VAM on host plant in the condition of drought stress. VAM can enhance resistance of drought stress in host plant by increase in proline content than non-VAM plants. Fazeli *et al.* (2006) reported the effect of drought on water potential, proline accumulation, sugars, osmotic potential, and relative water content of leaves of sesame cultivars. The mechanism of drought tolerance in the sesame is through production of proline, changes in sugars and ion accumulation for maintenance of suitable pressure potential. Thomas and Pandey (2006) reported that pre-soaking of groundnut in solution of CCC resulted in higher yield and higher RWC and proline accumulation under drought conditions. Tayeb (2006) reported the study on germination of *Vicia faba* cultivars (cv. Giza 40, Giza 667) exposed to PEG induced water stress. Drought induced the accumulation of proline in both cultivars. However, this accumulation was lower in cv. Giza 667 than in the tolerant cv. Giza 40. *Rehmannia glutinosa* seedlings when pre-treated with choline chloride (CC) accelerated accumulation of proline during drought stress and retarded the drop in proline concentration after rewatering (Huijie *et al.*, 2007). Beemaroo *et al.* (2007) observed the effect water deficit on the proline metabolism in bhendi varieties and there was increase in proline content upto 0.5 to 1 mg/g dw.

Peroxidase activity :

Under moisture and salinity stress there will be higher production of cytotoxic activated oxygen species and the balance between production of activated oxygen molecules and quenching activity of antioxidants is upset (Fridovich, 1986 and Daveis, 1987). The enzyme superoxide dismutase is major scavenger of activated oxygen species and its activity results in the formation of H₂O₂ which is also toxic to plants. Catalase and peroxidase enzymes catalyze the breakdown of H₂O₂ (Chang *et al.*, 1984). Plants with high levels of antioxidants have greater resistance to oxidative damage by H₂O₂ (Harper and Harvey, 1978). In general, peroxidase activity increased with an increase in moisture stress and however it varied among the treatments. Among the various treatments, VAM recorded highest peroxidase activity followed by CCC treatment. These results are in concurrence with Huixing (2005), where in protective enzyme activity increased due to VAM symbiosis Li and Pan (1990) reported the effect of 5 ppm PP 333 (paclobutrazol) or 150ppm.CCC [chlormequat] on groundnut cv. Yue You 551-116 under stress. Both PP 333 and CCC treatment increased peroxidase activity compared with untreated seedlings. Barbara *et al.* (1999) analyzed antioxidative defenses in two wheat cultivars, Adamello and

Ofanto, during dehydration and rehydration. During dehydration, the glutathione content decreased in both wheat cultivars, but only cv. ADAMELLO showed a significant increase in glutathione reductase and hydrogen peroxide and glutathione peroxidase activities. Drought did not cause significant effects on lipid peroxidation. Gopalakrishnan *et al.* (2004) observed the response of the cotton genotypes (LRA5166) subjected to abiotic water stress. The PGR's GA₃ and benzyladenin enhanced the activities of peroxidase and nitrate reductase enzymes under drought. Dina and Abdel, (2001) reported that the plants when subjected to drought and treated with the two concentrations gibberillic acid. The application GA₃ increased significantly the lipid peroxidation and peroxidase activity as compared to control. Treatment with gibberillic acid alleviated the adverse effects of drought and the lower concentration of gibberillic acid was more effective. Isabel and Jennifer (2001) observed the relationship between apoplastic peroxidase activity and cessation of growth in maize (*Zea mays* L). In GA₃-treated leaf blades, there was a transient increase in apoplastic peroxidase activity. A peroxidase iso-enzyme with pH 7.0 occurred in the leaf elongation zone during growth deceleration. Harish *et al.* (2003) reported the influence of plant interference and a mild drought in two cotton species (*Gossypium hirsutum* L. cv. DELTA PINE 5415, and *Gossypium barbadense* L. cv. PIMA S-7) and spurred anoda [*Anoda cristata* (L.) Schlecht.] of the Malvaceae. Mild drought increased APX (ascorbate peroxidase) activity more than 40 per cent in cotton and 26 per cent in spurred anoda. Upon drought recovery, drought-induced APX activity was still higher in cotton and GR activity was higher in previously drought-stressed cotton and spurred anoda plants compared with well-watered plants. Ghorbanli *et al.* (2004) reported the effect of NaCl and mycorrhizal fungi on antioxidant enzymes in the shoots and roots of soybean (*Glycine max*). Activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were increased in the shoots of both mycorrhizal (M) and non mycorrhizal (NM) plants grown under NaCl salinity. Mycorrhizal plants had greater SOD, POD and ascorbate peroxidase activity under salinity. Mathur *et al.* (2005) reported that the effect of CCC was observed in stressed plants of different varieties of a Japanese mint which showed decreased peroxidase activity (3.2-0.7OD/min/mg/protein). Farooqi *et al.* (2005) reported the effect of chlormequat chloride treated to aromatic grass (*Cymbopogon martini*) under drought stress in which peroxidase activity increased significantly in *C. martinii* between 40-151 per cent compared to control. Huijie *et al.* (2007) reported that when *Rehmannia glutinosa* seedlings were retreated with choline chloride (CC) in concentrations of 0, 0.7, 2.1 and 3.5 mM, and then subjected to drought and rewatering treatment. The seedlings pre-treated with CC alleviated the inhibition of SOD and peroxidase activity caused by drought stress.

Osmotic potential :

In most of the cases, poor plant growth is attributed to higher osmotic value of soil solution (Bernstein and Haywards, 1958). For this reason, the availability of soil water is decreased and as a result, the uptake of water by plant root is reduced (Cooper and Dumbu off, 1973). The present study indicated that osmotic potential decreased with increase in moisture stress. However, treatments differed in their ability to maintain osmotic potential at higher moisture stress level. The data on mean osmotic potential indicated that it was minimum in the treatment VAM and followed by CCC treatment. These results are in agreement with Atteya (2003) in corn, the lower osmotic potential could occur for several possible reasons: lower water content, which could cause greater solute concentration, greater tissue elasticity, and/or active accumulation of solutes. Soil water extractions of stressed plants were enhanced by mycorrhiza particularly in deficient 'p' soil. This data supports the hypothesis that mycorrhiza significantly enhanced the water relations of plants under the drought stress condition (Levy *et al.*, 1983; Busse and Ellis, 1985; Auge *et al.*, 1986; El-Tohamy *et al.*, 1999; Meddich *et al.*, 2000). In contrast to these results, Graham *et al.* (1987) reported that under drought stress condition, water relations of citrus plants were affected by mycorrhizal colonization. The interaction of mycorrhiza and drought stress showed that mycorrhiza was more beneficial to P uptake of stressed plants compared with non stressed plant and enhanced water relations of mycorrhizal plants resulted in improved P uptake (Nelson and Safir, 1982; Bethlerfalvay *et al.*, 1987) Subramanian and Charest (1994) investigated the influence of an arbuscular mycorrhizal (AM) fungus on drought tolerance in tropical maize. Plants were subjected to drought stress for three weeks. During this period there was increase in leaf water potential and leaf area in mycorrhizal plants. Atteya (2003) reported the influence of drought stress on internal water status of three Egyptian corn (*Zea mays* L.) genotypes; GIZA2, TWC310 and TWC320 at different developmental stages. In all the genotypes, stressed plants maintained osmotic potentials approximately - 0.47 MPa lower than control. Porcel *et al.* (2004) reported the effect of arbuscular mycorrhizal (AM) on soybean plants under drought stress. Results showed that there was higher shoot biomass and leaf water potential in stressed AM plants (-1.9 Mpa) than in non-AM plants (-2.5 Mpa). Neelam *et al.* (2006) reported that there was reversal effect of salinity stress on mothbean seedlings by gibberillic acid and IAA. There was increase in osmotic potential, leaf water potential and turgor potential in the genotype FMM-96 was more than in genotype IPCMO-912. Huijie *et al.* (2007) reported that when *Rehmannia glutinosa* seedlings were retreated with choline chloride (CC) and then subjected to drought and rewatering treatment. CC pre-treatment maintained higher ψ_w , deferred leaf water loss during drought stress.

Yield and yield components :

Improvement in yield according to Humphries (1979) could happen in two ways *i.e.*, by adopting the existing varieties to grow better in their environment or by altering the relative proportion of different plant parts so as to increase the yield of economically important parts. The influence of plant growth regulators and VAM significantly increased the seed cotton yield. The increased seed cotton yield could be attributed to maintenance of water status of plant, higher accumulation of proline, reducing sugar, enhanced chlorophyll content and peroxidase activity. In the present investigation, it is observed that the number of bolls and seed cotton yield per plant increased due to the soil treatment of VAM and seed priming with growth regulators, CCC, CK and GA₃. The increase in seed cotton yield could be attributed to significant enhancement in the biochemical characters and manipulation in the physiological processes. The present study revealed that increase in seed cotton yield was significantly higher in VAM followed by CCC. This could be probably be due to the beneficial effects of plant growth regulator treatments which are involved in enhancement of photosynthesis and nitrogen metabolism which are the major physiological process influencing plant growth and development. Similar increase in yield of soybean in the treatment VAM is reported by Tholkappian *et al.* (2001) in soybean. Better growth and yield of VAM mycorrhizal plants in moisture stress condition may be due to increased uptake of water as well as efficient P uptake (Nimje and Seth, 1990) and Ayub *et al.* (2000) in *Vigna radiata*.

Conclusion :

Abiotic stress signalling is an important area with respect to increase in plant productivity. Drought is a worldwide problem, constraining global crop production and quality seriously, and recent global climate change has made this situation more serious. Drought stress affects the growth, dry matter and harvestable yield in plants. Timing, duration, severity and speed of development undoubtedly have pivotal roles in determining how a plant responds to water deficit. Following drought, stomata close progressively with a parallel decline in net photosynthesis and water-use efficiency. In addition to other factors, changes in photosynthetic pigments are of paramount importance to drought tolerance. Protective responses at the leaf level must then be triggered quickly in response to the stress effectors to prevent the photosynthetic machinery being irreversibly damaged. Scavenging of reactive oxygen species by enzymatic and non-enzymatic systems, cell membrane stability, expression of aquaporins and stress proteins are vital mechanisms of drought tolerance.

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