MITIGATION OF DROUGHT STRESS THROUGH PLANT GRWOTH REGULATORS AND VESICULAR ARBUSCULAR MYCORRHIZAE (VAM) IN COTTON

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1. INTRODUCTION

Cotton has been in cultivation in India for thousands of years and the fabrics produced from cotton yesteryears were fascinating as they are today. Cotton remains the most miraculous fiber comes close to duplicating all of the desirable characters combined in cotton.

In India, cotton constituting eighty per cent of the raw materials of textile industry keeps its eminent position with millions of people engaged in its cultivation, processing, marketing and textiles of cotton and plays an important role in the national economy. It is therefore natural that cotton research and development has been getting the attention it deserves.

In India, cotton is primarily grown in dry tropical and sub tropical climates at mean temperatures between 11°C and 25ºC with a rainfall of 250-1500 mm. It is cultivated in India from sub Himalayan region of Punjab in the North to TamilNadu in South and dry regions in the East. It is cultivated on large scale in Maharashtra, Gujarat, Andhra Pradesh, Karnataka, Madhya Pradesh, Punjab, Rajasthan, Haryana, TamilNadu, and Uttar Pradesh.

Cotton (*Gossypium* sp.) is an important fiber crop of India covering an area of 88.20 lakh hectares with production of 242.5 lakh bales and productivity of 467 kg lint per hectare. In Karnataka, it is grown on an area 3.8 lakh hectares producing 7.0 lakh bales with productivity of 312 kg lint per hectare (Anon., 2006a).

The most commonly cultivated species of cotton in the world include *Gossypium hirsutum.* Two additional cultivated species are *Gossypium arboreum* (which originated in the Indo-Pakistan sub continent) and *Gossypium herbaceum* (from South Africa), which are called old world or Asiatic cottons. These two species of cotton with short staple fiber length are known for stress tolerance.

It is generally known that soil productivity depends on its physical and chemical properties. Water is essential for plant growth and development and much concern has been expressed in the recent year towards increase in the area of arid regions of the world. Crop productivity in arid region depends on the length, magnitude and stage of the plant that is affected by moisture stress.

Drought is a serious problem in the state, with about two-thirds having 750 mm or less annual rainfall. The severity and extent of drought not only depends on low rainfall but also on other hydro-meteorological factors like soil moisture, infiltration and moisture-retention capacity of the soil. Aridity of an area depends on rainfall in relation to potential or actual evapo-transpiration and the moisture holding capacity of the soil. Potential evaporation is a measure of the maximum possible evaporation from the soil and the transpiration from vegetation, if the soil is fully saturated. It is a measure of the consumptive use of water by crops. All arid and semi-arid areas of the state have been determined on the basis of ‘moisture index', calculated according to Thronthwaite's method of climatic classification. An area with moisture index between –33 to –100 per cent indicates arid climate while a moisture index between –33 to –60 indicates a semi-arid climate. Considering moderate and severe droughts, the taluks of Karnataka, which have had droughts in 25 per cent or more years, are Chitradurga, Hosadurga, Sira, Madhugiri, Shorapur, Athani and Bagepalli. The taluks with the greatest percentage of drought years are Sira, Madhugiri, Korategere, Kadur, Kushtagi, Shorapur, Shahapur, Yadgir, Bangarpet, Mulbagal, Srinivasapur, Gudibanda, Bagepalli, Athani, Raibag, Saundatti and Gokak. The taluks in the northern drought-prone districts have in general, more years of moderate and severe drought than the taluks in the southern districts. There are some taluks in which drought occurred in three or more consecutive years. The largest continuous period of drought was eight years at Gubbi (Tumkur district) from 1920-1927, at Athani (Belgaum district) from 1965-1972, at Chincholi (Gulbarga district) from 1965-1972 and at Nargund (Dharwad district) from 1920-1927 (www.wao.org).

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 unfavorable 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 (Henkal, 1962). To overcome the problem of drought there are many avenues *viz.* development of resistant genotypes, seed hardening, use of antitranspirants and alcohols (Rao Gangadhar *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 arbuscular mycorrhiza (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).

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, cytokinins, ethylene and abcissic 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). A hypothesis has been proposed that ABA and cytokinin have opposite roles in drought stress. The increase of ABA and decrease of cytokinin under water stress favour stomatal closure and reduce water loss through transpiration. Although stomatal closure was viewed as an adaptive strategy of plants to water stress, it may interfere with gas exchange and result in oxidative stress in plant cells. Pic (Mepiquat Chloride) is the first plant growth regulator in cotton production to make a significant impact on cotton growth and yield. The gibberellins are associated with stem elongation and leaf enlargement, but have been shown to increase fruit retention in cotton. Therefore, in order to overcome/mitigate the effect of drought on cotton crop, an experiment was planned through seed priming with plant growth regulators and VAM (vesicular arbuscular mycorrhiza) treatment to soil in pots with three moisture regimes with the following objectives:

1. To study the biochemical changes due to plant growth regulators and VAM treatment at different levels of drought stress in cotton.
2. To study the effects of plant growth regulators and VAM on yield and yield attributes in cotton.

2. REVIEW OF LITERATURE

The objectives of the present investigation were to assess the response of plant growth regulators (GA3, kinetin and cycocel) and VAM to mitigate drought stress in cotton. Reports on the effect of moisture stress on germination, seedling vigour as well as biochemical changes in cotton/crop plants under laboratory and field conditions are reviewed here under.

The effects of plant growth regulators and VAM on biochemical, yield and yield components of cotton genotype under drought conditions are also reviewed in this chapter:

2.1 Physiological parameters

2.1.1 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 (imbibition) 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,).

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.

2.1.2 Root: shoot ratio

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.

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.

Abdel *et al.* (2002) reported that the effect of mycorrhizae on drought stressed plants in broad bean which had significant higher shoot dry weight.

Khan and Shahabaz (2003) reported 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.

2.1.3 Seedling vigor

Sawan *et al*. (2000) reported 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 5 mg/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.

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).

2.2 Biochemical characters

2.2.1 Chlorophyll content

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.

Thandapani and Subharayalu (1980) observed that soaking of cotton seeds in 500 ppm 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 GA_3 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).

Ganiger (1992) reported increase in photosynthetic pigments with CCC and mepiquat chloride 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 mung bean 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 GA_3.

Singh *et al*. (2000) reported that cytokinetin enhanced the protein, chlorophyll content in senna leaves. 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).

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 *Giza* 40, *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.

2.2.2 Proline content

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 (648 µg/g frsh wt) and kinetin treated seedlings (564 µg/g frsh wt).

Janagoudar *et al*. (1983) reported that free proline accumulation was highest in the stem followed by leaf and root under shot-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.

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 (*Cassis anustifolia* vahl.) and showed higher amminoacid accumulation upto 36% 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 soyabean 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 aminoacid levels by 10.7% and 19.2% of leaf dry mass in C0 and C8 genotypes, respectively than non-mycorrhizal 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 presoaking 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 pretreated with choline chloride (CC) accelerated accumulation of proline during drought stress and retarded the drop in proline concentration after rewatering (Huijie Zhao *et al*., 2007).

Beemarao *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 to1 mg/g dw.

2.2.3 Reducing sugar

Vora *et al*. (1975) observed that when Bajra seeds were pretreated with distilled water and 100 mg/l GA_3. GA_3 treatment enhanced the reducing sugar and length of seedlings

Fatima *et al.* (1999) observed the effects of gibberellic acid and water stress in Fenugreek plant. There was more accumulation of monosaccharides by the application of gibberellic acid under water stress.

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 total sugars compared to non mycorrhizae

Prabhjot *et al.* (2002) reported the effect of osmotic stress on soluble sugar content in *Sorghum bicolor* (CSH 9) seeds and seedling components (endosperm and embryos) under stress and it was increased by 8.9 to 15.3 mg/g dry wt in germinating embryos and endosperm in sorghum.

Combined inoculation of biofertilizers viz., Azospirillum + phosphobacteria + VAM improved reducing sugars and total free amino acids in *Ceiba pentandra* (Vijayakumari and Janardhanan*,* 2003).

Kizhaeral and Christiane, (2004) reported the influence of mycorrhizal fungus on metabolic changes in the tropical maize under drought. Mycorrhizal plants had more total and reducing sugars than non-mycorrhizal 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 soluble sugars than non-VAM plants.

Tayeb (2006) reported the study on germination of five *vicia faba* cultivars (CV *Giza* 40, *Giza* 667) exposed to PEG induced water stress and indicated that cv *Giza* 40 showed higher germination than Giza 667. Drought induced the accumulation of soluble sugars in both cultivars. However, the accumulation was lower in cv *Giza* 667 than in tolerant cv *Giza* 40. These results indicate that cv Giza 40 showed better protection against drought-induced oxidative stress than cv *Giza* 667.

2.2.4 Peroxidase activity

Li and Pan, (1990) reported the effect of 5 ppm PP 333 (paclobutrazol) or 150 ppm. 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 GA_3 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 gibberllic acid .the application GA_3 increased significantly the lipid peroxidation and peroxidase activity as compared to control. Treatment with gibberllic acid alleviated the adverse effects of drought and the lower concentration of gibberllic 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 GA_3-treated leaf blades, there was a transient increase in apoplastic peroxidase activity. A peroxidase isoenzyme 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% in cotton and 26% 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 (3.2-0.7 OD/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% compared to control.

Huijie *et al*. (2007) reported that when *Rehmannia glutinosa* seedlings were pretreated 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 pretreated with CC alleviated the inhibition of SOD and peroxidase activity caused by drought stress.

2.2.5 Osmotic potential

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 mychorrhizal (AM) on soyabean 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 moth bean seedlings by gibberllic 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 pretreated with choline chloride (CC) and then subjected to drought and rewatering treatment. CC pretreatment maintained higher Ψw, deferred leaf water loss during drought stress.

2.2.6 Relative water content

Thandapani and Subharayalu, .(1980) observed that soaking cotton seeds in 500 ppm 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 (*Cassis anustifolia* vahl.) and showed decrease in RWC by 85 to 54 %.

Prakash and Ramchandran (2000) observed 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.

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 % in genotype, GIZA2.

Farooqi *et al.* (2005) reported 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).

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 %).

2.3 Yield and yield components

Misra and Malik, (1971) observed 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.

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. Nawalagatti *et al* (1991) reported 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.

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) reported 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 mychorrhizal 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 upto 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 yield as non-VAM plants subjected to same stress periods.

Singh and Kakralya (1992) reported that 50mg/l of etherel or GA_3 or benzyladenin may be applied as pre sowing seed soaking treatments for 12hrs, increased the germination percent, crop stand density, seedling growth and yield of pigeon pea

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.

Bioregulators NAA, kinetin and KNO_3 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, where as, 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 mung bean under salinity.

SatvirKaur *et al.* (1998) observed that germination and seedling growth of chickpea decreased with increasing concentration of PEG and addition of GA_3 (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 % 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 GA_3 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 GA_3 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 farmyard 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.

3. MATERIAL AND METHODS

An investigation was made with an objective to mitigate moisture stress by soaking cotton seeds of variety LRA-5166 with growth regulators (GA_3, CK and CCC) and treating soil with VAM before sowing and tested under different levels of moisture stress in a pot experiment.

3.1 EXPERIMENTAL DETAILS

Cotton seeds were soaked with growth regulators for six hours and shade dried to gain its original moisture content and VAM was applied to soil and these were tested under different levels of moisture stress *viz.,* 100 % field capacity, 70 % field capacity and 50 % field capacity in the pot. At early stage smaller pots size (7.2 x 8.3 cm) were used with 4 kg of soil in each pot and at later stage of screening big pots (8.5 x 10.7 cm) with 7 kg of soil were used. These pots were filled with black soil.

Experimental Site

The pot culture experiment was conducted in a green house of the Department of Crop Physiology, College of Agriculture, Dharwad.

3.1.1 Genotype

The cotton variety LRA-5166 was used for the study, LRA- 5166 (*Gossypium hirsutum)* species is a medium staple (25 mm), drought tolerant, high yielding (1500 kg/ha) variety released for south zone under rainfed condition during 1982 from CICR, Nagpur. It is a triple cross derivative from the cross Laxmi x (Reba B.5 x AC -738), having higher number of bolls per plant with high ginning outturn (35.6 %), medium duration (160 days) and resistant to pest and diseases.

3.1.3 Experimental design

The experiment was laid out in the Completely Randomized Design with three replications (Plate 1).

3.1.4 Treatments

The pot experiment consisted of three stress treatments *viz.,* control (100 % field capacity), moderate stress (70 % field capacity) and severe stress (50 % field capacity). The pots were filled with 7 kg of black soil. To achieve different stress levels, different quantity of water was added pots, the details of which are given below:

Moisture Stress levels	FC (%)	Water added (ml)
I1 - Control	100	2240
I2 - Moderate	70	1568
I3 - Severe	50	1120

The following growth regulators were used for soaking for 6 hours and shade dried till the seeds attain original moisture and VAM was treated to the soil.

Treatments	Concentration
T1 - GA_3	100 ppm
T2 - Kinetin	10 ppm
T3 - CCC	200 ppm
T4 - VAM	5 g/kg of soil
T5 - Control	---

Plate 1. General view of the experiment in green house, Department of crop physiology, AC, Dharwad

Table 1. Monthly meteorological data for the experimental year (2006-07) of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

Months	Rainfall (mm)	No. of rainy days	Temperature (ºC)		Relative humidity (%)
			Mean max.	Mean min.	
August 2006	115.2	17	26.3	19.6	85
September	91.4	10	29.2	19.2	77
October	38.6	3	30.0	19.1	67
November	55.4	3	29.2	18.1	70
December	-	-	29.1	12.8	61
January 2007	-	-	30.4	14.0	52
February	-	-	31.9	15.7	62
March	12.8	-	35.3	19.7	45
April	1.5	-	37.1	20.3	49
May	166.8	4	35.1	20.9	61
Total	**481.7**	**37**	**313.6**	**179.4**	**629**

3.1.5 Methodology

The amount of water required to maintain to field capacity was measured gravimetrically as mentioned in the above table. The pots containing soil were weighed daily during the experimentation and based on the loss of weight of the pots water was added regularly to maintain the required moisture level in different stress treatments. After imposing the stress treatments, three seeds in each pot were sown.

3.1.6 Treatment combinations

1. I_1 T_1 (100% FC + GA3 @ 100 ppm)
2. I_1 T_2 (100% FC + Kinetin @ 10 ppm)
3. I_1 T_3 (100% FC + CCC @ 200 ppm)
4. I_1 T_4 (100% FC + VAM)
5. I_1 T_5 (100% FC + Control)
6. I_2 T_1 (70% FC + GA3@ 100 ppm)
7. I_2 T_2 (70% FC + Kinetin @ 10 ppm)
8. I_2 T_3 (70% FC + CCC @ 200 ppm)
9. I_2 T_4 (70% FC + VAM)
10. I_2 T_5 (70% FC + Control)
11. I_3 T_1 (50% FC + GA3 @ 100 ppm)
12. I_3 T_2 (50 % FC + Kinetin @ 10 ppm)
13. I_3 T_3 (50 % FC + CCC @ 200 ppm)
14. I_3 T_4 (50 % FC + VAM)
15. I_3 T_5 (50 % FC + Control)

3.1.7 Fertilizer application and after care

Fertilizer dose of 80:40:40 NPK kg/ha was applied and after care operations were carried out as per the package of practices of UAS, Dharwad, for the pots based on weight of the soil (1ha= $2x10^6$kg).

3.1.8 Sowing

Seeds were sown in each pot on 28th Aug, 2007. Ten pots for each treatment were initially maintained. Seven pots were used for the germination count (12th day), root length, Shoot length, seedling vigour and biochemical parameters at 45 and 65 DAS and three pots were maintained till harvest in each treatment for yield and yield components.

3.2 OBSERVATIONS RECORDED

3.2.1 Physiological characteristics

3.2.1.1 Germination (%)

The seeds which emerged from soil were considered as germinated and observation was recorded on 12th day after seeding and expressed as percentage.

3.2.1.2 Root characteristics

3.2.1.3 Root length (cm)

One randomly selected seedling was scooped out without damaging the seedling roots in each replication and measured from collar region to the tip of the longest root on 45th and 60th day and was expressed in cm.

3.2.1.4 Shoot length (cm)

The shoot length of above selected seedling was measured from collar region to tip of the shoot on 45th and 60th days and was expressed in cm.

3.2.1.5 Root: shoot ratio

The ratio of root length and the shoot length of each seedling selected above was calculated.

3.2.1.6 Seedling vigour

Shoot and root vigour indices were calculated at 45 and 65 DAS as described by Abdul-Baki and Anderson (1973).

Shoot vigour index = Shoot length x germination %

Root length index = Root length x germination %

Seedling vigour index = (Root length + shoot length) x germination%

3.3 BIOCHEMICAL PARAMETERS

3.3.1 Estimation of chlorophyll (mg/g fresh weight)

Chlorophyll content in the leaves of the LRA5166 subjected to different drought levels was determined colorimetrically as per the DMSO (Dimethyl sulphoxide) method of Shoaf and Lium (1976).

Procedure

Fresh leaf tissue of 100 mg was cut into small pieces and incubated in 7 ml DMSO at 65ºC for 30 minutes at the end of the incubation period supernatant was decanted and leaf tissue was discarded and volume was made upto 10 ml with DMSO. Absorbance of the extract was read at 645 nm, 652 nm and 663 nm using DMSO as blank. The chlorophyll 'a' and chlorophyll 'b' and total chlorophyll contents were calculated using the formulae.

$$\text{Chlorophyll 'a' (mg/g fr. wt)} = 12.7 (A_{663}) - 2.69 (A_{645}) \times \frac{V}{1000 \times W \times a}$$

$$\text{Chlorophyll 'b' (mg/g fr. wt)} = 22.9 (A_{645}) - 4.68 (A_{663}) \times \frac{V}{1000 \times W \times a}$$

$$\text{Total chlorophyll (mg/g fr. wt)} = 27.8 (A_{652}) \times \frac{V}{1000 \times W \times a}$$

where,

A = Absorbance at specific wave length (645, 663 and 652 nm)

V = Final volume of the chlorophyll extract (ml)

W = Fresh weight of the sample (g)

a = Path length of light (1cm)

3.3.2 Determination of proline content (µg/gm frsh wt.)

Free proline content in the leaves of LRA5166 cotton genotype subjected to different drought levels were determined colorimetrically as per the method of Bates *et al.*(1973).

Procedure

A known weight (0.5 gm) of fresh leaf sample was ground well in a mortar using fine sand and extracted with 10 ml of 3% sulphosalicylic acid. The extract was filtered and 2 ml of the filtrate was used for proline estimation. To this 2 ml filtrate, 2 ml of acid ninhydrin reagent (2.5 gm of ninhydrin was dissolved in 40 ml of 6 m orthophosphoric acid and 60 ml of glacial acetic acid) and 2 ml of glacial acetic acid were added and placed in a boiling water bath for one hour. Following this, the test tubes containing the samples was transferred to ice water bath for cooling. The content of each test tube was transferred to a separating funnel and 6 ml of toluene was added, shaken thoroughly and allowed for few minutes for separation of two layers. The lower layers were discarded and the upper toluene layer containing the color was taken into a test tube. The optical density of the color complex was calculated using the formula,

$$\text{Proline content } (\mu g/g \text{ frsh wt}) = \frac{36.2311 \times OD \times V \times d}{2 \times f}$$

where,

OD = Optical density at 520 nm

V = Total volume of the extract in ml

d = Fresh weight/dry weight ratio

f = Milligrams of fresh sample taken for proline estimation

2 = Volume of the extract taken for proline estimation.

3.3.3 Estimation of reducing sugar (µ/g g frsh wt.)

The reducing sugar was estimated using the leaf as described by Nelson-Somogyi's method (Nelson, 1944, Somogyis, 1952)

Reagents

a. Alkaline copper reagent

Solution A

Dissolved 25 g of anhydrous sodium carbonate, 25 g sodium potassium tartarate, 20 g of sodium bicarbonate and 20 g of sodium sulphate in about 800 ml of distilled water and diluted to 1 litre.

Solution B

Dissolved 15 g of copper sulphate in distilled water to this, one or two drops of concentrated sulfuric acid was added and made upto 100 ml with distilled water. Solutions A&B were mixed in 24:1(v/v) proportion just before use.

b. Arsenomolybdate reagent

Dissolved 25 g of ammonium molybdate in 450ml of distilled water, to this 21 ml of concentrated sulphuric acid was added and mixed well. In another container dissolved 3 g of sodium orthoarsenate in 25 ml water. Both the solutions were mixed with stirring and placed in an incubator at 37ºC for 24-48 hrs. The reagent should be stored in brown bottle which remains stable for a few months.

c. Stock standard solution

Dissolved 100 mg of D-glucose in distilled water in a volumetric flask. This solution contains 1mg of glucose per ml. and this should be prepared fresh on the day of work.

d. Working standard solution

10 ml of stock standard solution was diluted to 100 ml with distilled water in volumetric flask. This solution contains 100 µg of glucose per ml.

Procedure

1. Aliquot of working standard (10-100 µg) was pipetted out in a series of labeled test tubes suitable and made up the volume to 1ml with distilled along with reagent blank with 1 ml distilled water.

2. Added 1 ml of freshly prepared alkaline copper reagent to all the tubes including reagent blank and placed them in boiling water bath for exactly 20 min. kept for cooling under the tap without shaking and added 1 ml of arsenomolybdate reagent with immediate mixing till effervescence die.

3. Made up the volume to 20 ml with distilled water and read the %T of standard and the sample against reagent blank which was set to 100 %T at 510 nm.

4. Reducing sugar content was calculated by using glucose standard curve and expressed in µg/g frsh wt.

3.3.4 Determination of peroxidase activity (∆OD mg/sec protein)

The peroxidase activity was determined as described by Machly and Chance (1954).

A 0.5 gm of fresh leaf sample was weighed and ground well in a mortar with little quantity of chilled phosphate buffer at pH 6.6 and filtered through a double layered muslin cloth to remove the pulp, made upto 25 ml and centrifuged for 30 minutes at 2000 rpm at 4ºC. The clear extract was used as enzyme source. The same enzyme was used for the estimation of soluble protein.

3 ml of 0.05 M guaiacol solution was pipetted out into a test tube to which 0.1 ml of enzyme extract was added. Then 0.5 ml of 1 per cent hydrogen peroxide was added, mixed the contents rapidly and the absorbance was measured in calorimeter at 470 nm at an interval of 20 seconds.

Enzyme activity was calculated by taking the average difference of O.D between two consecutive time intervals and enzyme activity was expressed in terms of ∆OD $sec^{-1}mg^{-1}$ protien (i.e. specific activity).

3.3.5 Estimation of osmotic potential by EC method

The osmotic potential of the leaf sap was determined by electrical conductivity method as proposed by Janardhan *et al.* (1975).

The fully expanded fresh leaf samples were collected, weighed (1 g) and ground thoroughly in a mortar using small quantity of distilled water. The macerate was filtered through a double layered muslin cloth and the extract was made upto 25 ml with distilled water. Electrical conductivity of the extract was measured in a conductivity bridge. A parallel leaf sample with known fresh weight (1 g) was kept in oven to determine the moisture content of the leaf. The osmotic potential of the cell sap was calculated by the following formula:

$$\text{Osmotic potential} = \frac{EC \times 0.36 \times d\,f}{0.987}$$

where,

EC = Electrical conductivity of the fresh leaf extract in dSm^{-1} at 25 ºC

df = The moisture content of the leaf.

0.987 = Factor for converting atmospheric pressure to bars.

3.3.6 Determination of relative water content in leaf tissue

Relative water content was determined by the method of Barrs and Weatherly (1962). Ten leaf discs were collected randomly in each treatment and weighed accurately upto third decimal on a single pan analytical balance. This was considered as fresh weight. The weighed leaf discs were allowed to float on distilled water in a Petridish and allowed to absorb water for four hours. After four hours, the leaf discs were taken out and their surface was blotted gently and weighed. This was referred to turgid weight. After drying in hot air oven at 72ºC for 48 hours, the dry weight was recorded and RWC was calculated by using the following formula and expressed in percent.

$$\text{RWC (\%)} = \frac{\text{Fresh weight-Dry weight}}{\text{Turgid weight-Dry weight}} \times 100$$

3.4 Yield and yield components

3.4.1 Yield of seed cotton per plant

Cotton was picked separately from the plant. The number of bolls picked during each picking were counted and weighed. From this mean yield of seed cotton per plant over all pickings was worked out.

3.4.2 Total number of good bolls per plant

At each picking, the total number of good bolls were counted from each plant. From this mean total number of good bolls per plant was worked out.

3.4.3 Total number of bad bolls per plant

At each picking, the total number of bad bolls were counted from each plant. From this mean total number of bad bolls per plant was worked out.

3.4.4 Boll weight

It was worked out by dividing the seed cotton yield per plant by the number of bolls harvested per plant.

3.5 STATISTICAL ANALYSIS

Data from the experiment was subjected to Factorial Completely Randomized Design using MS-EXCEL. Level of significance in ‘F’ and ‘T’ were at five per cent and critical differences were calculated whenever the ‘F’ test was found significant.

4. EXPERIMENTAL RESULTS

The pot experiment was conducted during the year 2006-07 to investigate the effect of seed priming with plant growth regulators (GA3, CK and CCC) and VAM (Vesicular Arbuscular Mycorrhizae) on LRA-5166 (*G. hirsutum)* cotton genotype under three moisture levels *viz.*, 100 %, 70 % and 50 % field capacity. The effect of these treatments on physiological, biochemical parameters and yield and yield components of cotton were analysed. The data recorded on these parameters were statistically analysed and are presented in this chapter.

4.1 Physiological parameters

4.1.1 Germination

Significant differences were observed in the germination per cent among the treatments, moisture stress level and interaction effects. (Table2).

The treatment VAM had significantly higher germination per cent of (86.3 per cent) followed by CCC (79.6 per cent), kinetin (73.8 per cent) and GA3 (73.8 per cent) and significantly lowest germination (63.9 per cent) was recorded in the control. .

With increase in the moisture stress, the germination per cent decreased. Higher moisture (100 % FC) significantly showed higher germination (95.0 per cent), where as, 50 % FC showed lowest germination (59.4 per cent). Among the interaction effects, the treatment VAM had significantly highest germination (97.8 per cent) at I_1 moisture stress level and significantly least was recorded in the control at I_3 moisture stress level (40.8 per cent).

4.1.1 Root characteristics

4.1.1.2 Root Length (cm)

Root length differed significantly among the treatments, moisture stress levels and its interaction effects at both the stages of crop growth were also significant (Table 3, 4).

The treatment VAM had the highest root length of 16.0 and 22.6 cm at 45 and 65 DAS respectively and least was recorded in the control (11.5 and 17.6 cm at 45 and 65 DAS respectively). The per cent increase in root length in VAM treatment was 39.13 and 28.4 per cent over control at 45 and 65 DAS respectively.

With an increase in moisture stress from I1 to I3 the root length decreased from 15 to 12 cm at 45 DAS and from 22.3 to 19.5 cm at 65 DAS respectively. Among the interaction effect VAM recorded significantly highest root length of 17.0 cm at I1 moisture level at 45 DAS as compared to all other treatments.

4.1.1.3 Shoot Length (cm)

Significant differences were observed in the shoot length among the treatments, moisture stress levels and interaction effects at both the stages of crop growth (Table 3, 4).

The treatment GA_3 at both the stages recorded significantly highest shoot length of 15.5 and 28.2 cm and in the VAM treatment it was 15.0 cm and 25.4 cm at 45 and 65 DAS respectively and least was observed in treatment CCC (11.8 cm and 17.0 cm at 45 DAS and 65 DAS respectively).

With increase in moisture stress levels, the shoot length was decreased from 19.4 to 13.57 cm (at I_1 to I_3 respectively) at 45 DAS and from 26.4 to 20.1 cm (at I_1 to I_3 respectively) at 65 DAS. Among the interaction effects, GA_3 recorded significantly highest shoot length of 31.7 cm at 65 DAS under lower level of moisture stress compared to all other treatments.

Table 2. Effect of plant growth regulators and VAM on germination (%) at different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	Germination %			
	I_1	I_2	I_3	Mean
T_1 : GA_3 (100 ppm)	96.0	71.2	54.3	73.8
T_2 : CK (10 ppm)	94.3	68.2	58.8	73.8
T_3 : CCC (200 ppm)	95.1	78.0	65.9	79.6
T_4 : VAM (5 g/kg of soil)	97.8	84.1	77.1	86.3
T_5 : Control	92.0	59.0	40.8	63.9
Mean	95.0	72.1	59.4	
For comparing	**S.Em ±**		**CD**	
Treatments (T)	0.07		0.27	
Irrigation levels (I)	0.04		0.16	
Interaction (T x I)	0.20		0.80	

I_1: 100% Field capacity I_2: 70% Field capacity I_3: 50% Field capacity

4.1.1.4 Root: Shoot Ratio

Significant difference was observed in the root: shoot ratio among the treatments, moisture stress levels and their interaction effects at both the stages of crop growth (Table 3, 4).

The treatment CCC had significantly higher root: shoot ratio of 1.09 and 1.17 at 45 and 65 DAS respectively, compared to all other treatments. The least root: shoot ratio (0.70 and 0.79) was observed in the control at 45 and 65 DAS respectively.

With an increase in moisture stress levels from I_1 to I_3 the root; shoot ratio increased from 0.78 to 0.92 at 45 DAS and from 0.85 to 1.02 at 65 DAS respectively. Among the interaction effects, CCC at I_3 levels of moisture stress had significantly higher root: shoot ratio 1.24 and 1.30 at 45 and 65 DAS respectively as compared to other treatments.

4.1.1.5 Seedling vigour

Significant differences were observed in seedling vigour among the treatments, moisture stress levels and interaction effects at both stages of crop growth (Table 5 and Plate 2).

The treatment VAM had significantly highest seedling vigour of 3021.1 and 4130.7 at 45 DAS and 65 DAS respectively followed by GA3 and kinetin. Significantly least seedling vigour was observed in the control at both stages of crop.

With increase in moisture stress from I1 to I3, the seedling vigour decreased from 3275.8 and 1529.9 at I1 to I3 at 45 DAS and from 4619.6 to 2320.2 at 65 DAS.

Among the interaction effects, the treatment VAM had significantly highest seedling vigour (3792.2 and 5136.4 at I1 moisture stress level at 45 DAS and 65 DAS respectively) and significantly least was recorded in control 999.5 and 1582.2 at I3 moisture stress level at 45 and 65 DAS respectively.

4.2 Biochemical Parameters

4.2.1 Chlorophyll content (mg g^{-1} fresh wt)

The data on chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents analysed at 45 and 65 DAS are presented in tables 6, 7, 8.

4.2.1.1 Chlorophyll 'a' content (mg/g fresh weight)

Significant differences were observed in the chlorophyll 'a' content among the treatments, moisture stress levels and interactions effects at both the stages (Table 6).

Irrespective of moisture stress levels, seeds treated with VAM had significantly higher chlorophyll 'a' (0.75 & 0.89 mg g^{-1}) and followed by CCC (0.70 and 0.84 mg g^{-1}) and least chlorophyll 'a' content was observed in control (0.57 and 0.67 mg g^{-1}) at 45 and 65 DAS respectively .

The chlorophyll 'a' content decreased from 0.76 to 0.55 mg g^{-1} at 45 DAS, from 0.87 to 0.69 mg g^{-1} at 65 DAS with increase in moisture stress level from 100 % to 50 % FC.

The treatment VAM had lower reduction in chlorophyll 'a' content compared to all other treatments, while, control had maximum reduction at both the stages. The percent increase of chlorophyll 'a' in treatment VAM was 31.57 and 32.83 % over control at 45 and 65 DAS respectively.

4.2.1.2 Chlorophyll 'b' content (mg g^{-1} fresh weight)

Significant differences were observed among treatments, moisture stress levels and interaction effects for chlorophyll 'b' at both the stages (Table 7).

At 45 DAS the treatment VAM had significantly higher chlorophyll 'b' content (0.84 mg g^{-1}) as compared to all other treatments and it was closely followed by the treatment CCC (0.78 mg g^{-1}).

At 65 DAS similar trend was followed, where the treatment VAM had significantly higher chlorophyll 'b' (0.91 mg g^{-1}) and followed by the treatment CCC (0.87 mg g^{-1}).

The chlorophyll 'b' content decreased from 0.84 to 0.64 mg g^{-1} with increase in moisture stress from I_1 to I3 at 45 DAS and at 65 DAS it was 0.89 g^{-1} at I_1 to 0.70 mg g^{-1}.The per cent increase in chlorophyll 'b' in the treatment VAM was 25.37 % and 28.16 % over control at both the stages.

The treatment VAM had lower reduction in chlorophyll 'b' content compared to other treatments, while control (T_5) had maximum reduction in chlorophyll 'b' under severe stress.

4.2.1.3 Total chlorophyll content (mg g^{-1} fresh weight)

Significant differences were observed in the total chlorophyll content among the treatments, moisture stress levels and interaction effects at both the stages (Table 8).

The treatment VAM had significantly higher total chlorophyll content of 1.59 mg g^{-1} and 1.89 mg g^{-1} at 45 and 65 DAS respectively and followed by the treatment CCC (1.48 and 1.71 mg g^{-1}) while, the control recorded least total chlorophyll content at both the stages of crop growth .

In general, as the stress level increased from 100% FC to 50% FC the total chlorophyll content decreased significantly from 1.61 to 1.19 mg g^{-1} at 45 DAS and from 1.78 to 1.40 mg g^{-1} at 65 DAS.

Table 3. Effect of plant growth regulators and VAM on root length (cm), shoot length (cm) and root: shoot ratio under 45 DAS at different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**Root length (cm)**				**Shoot length (cm)**				**Root : shoot ratio**			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	Mean
T_1 : GA_3 (100 ppm)	14.5	13.0	12.2	13.2	21.5	18.5	15.5	18.5	0.67	0.70	0.79	0.72
T_2 : CK (10 ppm)	15.7	15.0	12.7	14.5	19.0	16.7	15.0	16.9	0.82	0.89	0.85	0.86
T_3 : CCC (200 ppm)	15.0	12.8	10.5	12.7	14.7	12.4	8.5	11.8	1.01	1.04	1.24	1.09
T_4 : VAM (5 g/kg of soil)	17.0	16.8	14.2	16.0	21.7	18.9	15.0	18.5	0.78	0.89	0.95	0.87
T_5 : Control	12.8	11.0	10.6	11.5	20.0	15.7	13.85	16.5	0.64	0.69	0.77	0.70
Mean	**15.0**	**13.7**	**12.0**		**19.4**	**16.47**	**13.57**		**0.78**	**0.84**	**0.92**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	0.08		0.33		0.09		0.35		0.080		0.03	
Irrigation levels (I)	0.05		0.19		0.05		0.21		0.004		0.01	
Interaction (T x I)	0.25		0.99		0.27		1.06		0.024		0.09	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Table 4. Effect of plant growth regulators and VAM on root length, shoot length and root: shoot ratio at 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	Root length (cm)				Shoot length (cm)				Root : shoot ratio			
	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean
T_1 : GA_3 (100 ppm)	23.0	21.5	20.5	21.6	31.7	27.7	25.1	28.2	0.79	0.84	0.88	0.84
T_2 : CK (10 ppm)	22.0	21.5	19.8	21.1	27.1	25.5	18.0	23.5	0.77	0.82	0.99	0.86
T_3 : CCC (200 ppm)	22.5	22.2	20.5	21.7	20.0	16.5	14.7	17.0	1.02	1.20	1.30	1.17
T_4 : VAM (5 g/kg of soil)	25.5	22.0	20.5	22.6	29.0	25.5	22.5	25.6	1.00	1.02	1.07	1.03
T_5 : Control	18.5	17.8	16.5	17.6	24.4	22.0	20.2	22.2	0.69	0.81	0.87	0.79
Mean	**22.3**	**21.0**	**19.5**		**26.4**	**23.4**	**20.1**		**0.85**	**0.94**	**1.02**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	0.06		0.24		0.07		0.30		0.02		0.08	
Irrigation levels (I)	0.10		0.40		0.13		0.51		0.01		0.04	
Interaction (T x I)	0.31		1.22		0.39		1.54		0.06		0.24	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Table 5. Effect of plant growth regulators and VAM on seedling vigour at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**45 DAS**				**65 DAS**			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
T_1 : GA_3 (100 ppm)	3455.3	2245.2	1508.2	2402.9	5257.2	3508.8	2309.9	3692.0
T_2 : CK (10 ppm)	3277.6	2165.6	1633.1	2358.8	4637.7	3206.0	2126.7	3323.5
T_3 : CCC (200 ppm)	2829.4	1969.8	1252.1	2017.1	4041.4	3022.0	2322.8	3128.7
T_4 : VAM (5 g/kg of soil)	3792.2	3014.4	2256.7	3021.1	5136.4	3997.0	3258.8	4130.7
T_5 : Control	3024.6	1578.6	999.5	1867.5	4025.2	2707.0	1582.8	2771.7
Mean	**3275.8**	**2194.7**	**1529.9**		**4619.6**	**3288.1**	**2320.2**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	10.20		39.70		15.42		59.98	
Irrigation levels (I)	6.12		23.82		9.25		35.99	
Interaction (T x I)	30.62		119.11		46.27		179.95	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

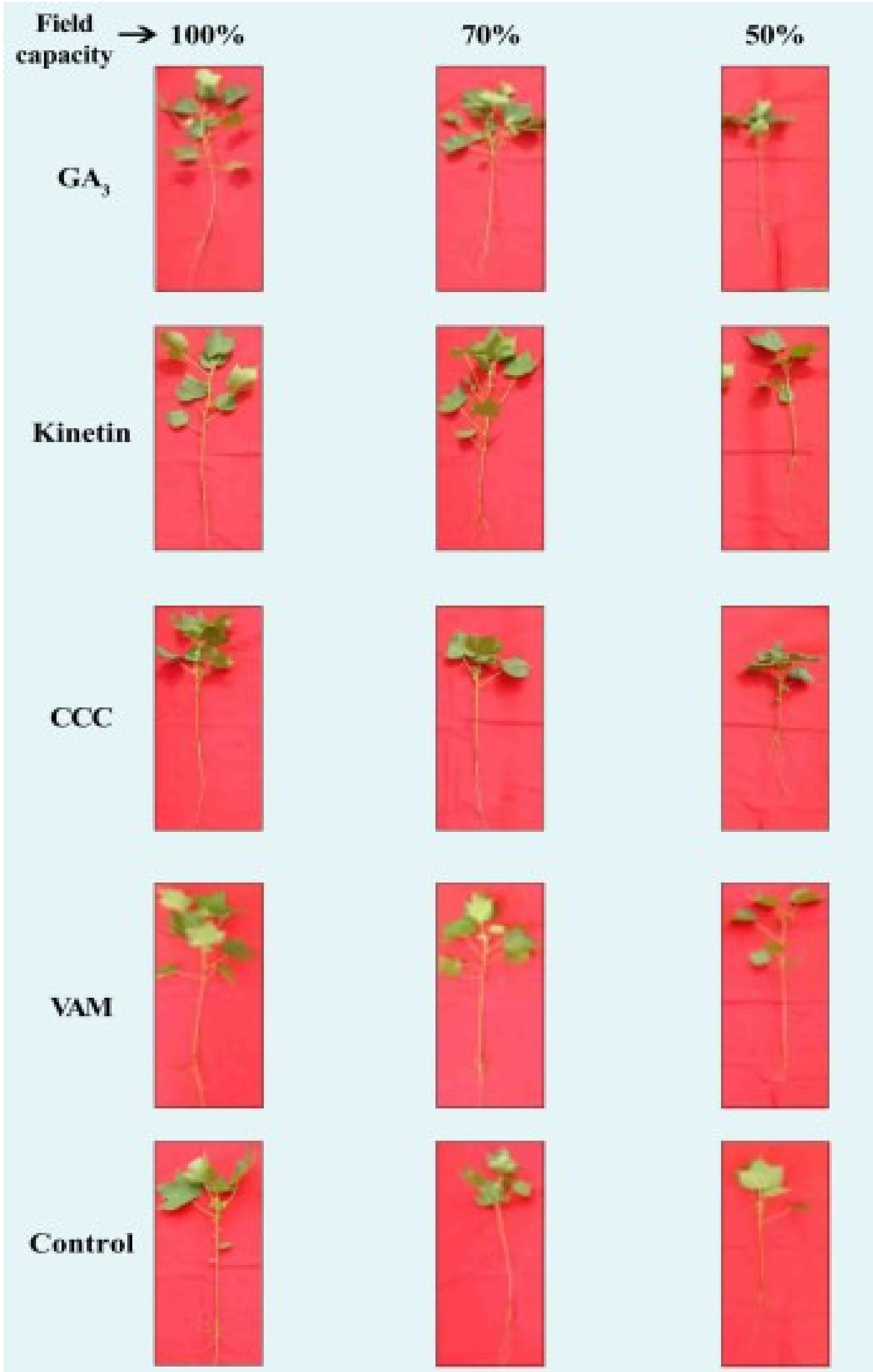

Plate 2. Seedling growth of cotton as influenced by plant growth regulators, VAM and moisture levels at 65 DAS

Table 6. Effect of plant growth regulators and VAM on Chlorophyll 'a' content (mg g^{-1} fresh wt.) in leaf at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**45 DAS**				**65 DAS**			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
T_1 : GA_3 (100 ppm)	0.72	0.61	0.52	0.62	0.80	0.71	0.65	0.72
T_2 : CK (10 ppm)	0.74	0.62	0.56	0.64	0.86	0.80	0.70	0.78
T_3 : CCC (200 ppm)	0.80	0.70	0.59	0.70	0.95	0.83	0.74	0.84
T_4 : VAM (5 g/kg of soil)	0.84	0.75	0.65	0.75	1.03	0.85	0.78	0.89
T_5 : Control	0.71	0.56	0.43	0.57	0.74	0.69	0.58	0.67
Mean	**0.76**	**0.65**	**0.55**		**0.87**	**0.77**	**0.69**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	0.008		0.03		0.008		0.03	
Irrigation levels (I)	0.005		0.01		0.005		0.01	
Interaction (T x I)	0.025		0.09		0.025		0.09	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Table 7. Effect of plant growth regulators and VAM on Chlorophyll 'b' content (mg g^{-1} fresh wt.) in leaf at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**45 DAS**				**65 DAS**			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
T_1 : GA_3 (100 ppm)	0.80	0.72	0.61	0.71	0.84	0.76	0.63	0.74
T_2 : CK (10 ppm)	0.81	0.75	0.64	0.73	0.88	0.82	0.70	0.80
T_3 : CCC (200 ppm)	0.89	0.81	0.65	0.78	0.95	0.88	0.79	0.87
T_4 : VAM (5 g/kg of soil)	0.95	0.86	0.73	0.84	0.97	0.92	0.83	0.91
T_5 : Control	0.79	0.69	0.55	0.67	0.83	0.71	0.58	0.71
Mean	**0.84**	**0.76**	**0.64**		**0.89**	**0.82**	**0.70**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	0.006		0.03		0.004		0.02	
Irrigation levels (I)	0.003		0.01		0.007		0.03	
Interaction (T x I)	0.019		0.08		0.021		0.08	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Table 8. Effect of plant growth regulators and VAM on total chlorophyll content (mg g^{-1} fresh wt.) in leaf at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**45 DAS**				**65 DAS**			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
T_1 : GA_3 (100 ppm)	1.52	1.33	1.14	1.33	1.64	1.47	1.28	1.46
T_2 : CK (10 ppm)	1.55	1.37	1.22	1.38	1.74	1.62	1.40	1.58
T_3 : CCC (200 ppm)	1.70	1.51	1.24	1.48	1.90	1.71	1.53	1.71
T_4 : VAM (5 g/kg of soil)	1.79	1.61	1.38	1.59	2.05	1.77	1.62	1.81
T_5 : Control	1.50	1.26	0.98	1.25	1.58	1.40	1.16	1.38
Mean	**1.61**	**1.41**	**1.19**		**1.78**	**1.59**	**1.40**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	0.011		0.04		0.013		0.05	
Irrigation levels (I)	0.007		0.02		0.008		0.03	
Interaction (T x I)	0.035		0.13		0.040		0.15	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Table 9. Effect of plant growth regulators and VAM on proline content (µg g^{-1} fresh wt.) in leaf at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**45 DAS**				**65 DAS**			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
T_1 : GA_3 (100 ppm)	104.2	130.1	212.8	149.0	113.2	176.1	225.4	171.6
T_2 : CK (10 ppm)	105.8	159.5	239.6	168.3	115.3	208.6	309.5	211.1
T_3 : CCC (200 ppm)	110.8	205.8	369.1	228.3	119.2	308.5	408.7	278.8
T_4 : VAM (5 g/kg of soil)	120.5	259.2	404.6	261.4	142.5	320.5	534.3	332.4
T_5 : Control	97.5	108.2	122.2	109.3	111.7	169.2	210.7	163.8
Mean	**107.8**	**172.6**	**269.6**		**120.4**	**236.6**	**337.7**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	0.40		1.56		0.28		1.09	
Irrigation levels (I)	0.24		0.93		0.16		0.65	
Interaction (T x I)	1.20		4.68		0.84		3.29	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Among the interaction effects, at both the stages the VAM at I_1 moisture stress level recorded highest total chlorophyll content (1.79 mg g $^{-1}$) at 45 DAS and 2.05 mg g $^{-1}$ at 65 DAS and least total chlorophyll was recorded in control at I3 moisture stress level (0.98 and 1.16 mg g $^{-1}$ at 45 and 65 DAS respectively). The per cent increase in total chlorophyll in the treatment VAM was 27.2 and 31.15 % over control at both the stage respectively.

4.2.2 Proline content (µg g $^{-1}$fresh weight)

Significant differences were observed among the treatments, moisture stress levels and interaction effects at both the stages (Table 9).

Irrespective of moisture stress the treatment VAM had higher accumulation of proline 261.4 µg g^{-1} and 332.4 µg g^{-1} fresh weight at 45 and 65 DAS and followed by CCC (228.64 µg g^{-1} and 278.8 µg g^{-1}) and least proline content accumulation was observed in control (109.3 µg g^{-1} and 163.8 µg g^{-1}) at 45 and 65 DAS respectively.

Moisture stress had a positive impact on proline accumulation and it increased from 107.8 µg g^{-1} at I_1 to 269.6 µg g^{-1} at I_3 at 45 DAS and from 120 µg g^{-1} at I_1 to 337.7 µg g^{-1} at I_3 at 65 DAS.

Among the interaction effects at both stages the treatment VAM at I_3 moisture stress level recorded significantly highest proline accumulation (404.6 µg g^{-1}) at 45 DAS and (534.3 µg g^{-1}) at 65 DAS and least proline content was observed in control at I_1 moisture level (97.59 and 111.7 µg g^{-1} at both 45 and 65 DAS respectively) and the per cent increase was 139 and 103 % in VAM over control at 45 and 65 DAS, respectively.

4.2.3 Reducing Sugar (µg g^{-1} fresh weight)

Significant differences were observed among the treatments, moisture stress and interaction effects at both the stages for reducing sugar content (Table 10).

Irrespective of moisture stress levels, the soil treated with VAM had significantly higher reducing sugar of 47.7 µg g $^{-1}$ and 88.6 µg g $^{-1}$ and followed by CCC (42.2 µg g $^{-1}$ and 83.2 µg g $^{-1}$ at 45 and 65 DAS respectively). Significantly least reducing sugar was observed in control at both stages.

As the soil moisture stress level increased from 100% FC and 50% FC, the reducing sugar accumulation increased significantly from 37.6 to 43.3 µg g $^{-1}$ at 45 DAS and from 72.8 to 87.9 µg g $^{-1}$ at 65 DAS. Significantly higher accumulation of reducing sugar was observed in VAM treatment as compared to other treatments and least was recorded in control at both stages. The sugar percent increase in accumulation in VAM treatment was 33.6 and 23.2 % at 45 and 65 DAS respectively over control. The highest sugar content was observed in VAM (110.0 µg g $^{-1}$) at 65 DAS at I_3 moisture stress level (50 % FC) as compared to all other treatments and differed significantly and least sugar was observed in control at 100 %FC at 45 DAS (34.6 µg g $^{-1}$).

4.2.4 Peroxidase Activity (∆ ODSec^{-1} mg^{-1} protein)

Significant differences were observed among the treatments, moisture stress levels and interaction effects at both the stages (Table 11).

The treatment VAM had significantly higher peroxidase activity of 1.32 and 1.38 ∆ OD/sec/mg and followed by CCC (1.13 and 1.32 ∆OD/sec/mg) at 45 and 65 DAS respectively and significantly least peroxidase activity was recorded in control at both the stages.

As moisture stress was increased, peroxidase activity was also increased significantly from 0.89 at I_1 and 1.30 at I_3 at 45 DAS and from 1.09 at I_1 and 1.45 at I_3 at 65 DAS.

With regard to interaction effect, both at 45 and 65 DAS, VAM had significantly higher peroxidase activity at I_3 level of stress (1.60 and 1.66 ∆OD/sec/mg at 45 and 65 DAS respectively) as compared to all other treatments, while control had least peroxidase activity at I_1 level of stress at both the stages (0.8 and 1.06 ∆OD/sec/mg at 45 and 65 DAS respectively). The percent increase in peroxidase activity in treatment VAM was 50.0 and 19.0 % over control at 45 and 65 DAS respectively.

Table 10. Effect of plant growth regulators and VAM on reducing sugar content (µg gm^{-1} fresh weight) in leaf at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	45 DAS				65 DAS			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
T_1 : GA_3 (100 ppm)	35.5	37.2	38.7	37.1	69.2	73.4	82.8	75.1
T_2 : CK (10 ppm)	37.5	38.4	41.9	39.2	73.5	80.2	86.6	80.1
T_3 : CCC (200 ppm)	38.8	42.3	45.6	42.2	76.7	83.5	89.5	83.2
T_4 : VAM (5 g/kg of soil)	40.0	50.2	53.0	47.7	78.9	87.0	100.0	88.6
T_5 : Control	34.6	35.3	37.1	35.7	65.5	69.7	80.5	71.9
Mean	**37.6**	**40.7**	**43.3**		**72.8**	**78.7**	**87.9**	

For comparing	**S.Em±**	**CD**	**S.Em±**	**CD**
Treatments (T)	0.10	0.41	0.158	0.61
Irrigation levels (I)	0.17	0.68	0.09	0.36
Interaction (T x I)	0.53	2.06	0.47	1.83

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Table 11. Effect of plant growth regulators and VAM on peroxidase activity in leaf (ΔOD sec^{-1} mg^{-1}protien) at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	45 DAS				65 DAS			
	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean
T_1 : GA_3 (100 ppm)	0.85	0.92	1.23	1.00	1.07	1.17	1.44	1.23
T_2 : CK (10 ppm)	0.87	0.94	1.27	1.03	1.08	1.20	1.31	1.20
T_3 : CCC (200 ppm)	0.95	0.98	1.44	1.13	1.09	1.31	1.58	1.32
T_4 : VAM (5 g/kg of soil)	1.00	1.35	1.60	1.32	1.14	1.37	1.66	1.38
T_5 : Control	0.80	0.91	0.94	0.88	1.06	1.13	1.30	1.16
Mean	**0.89**	**1.02**	**1.30**		**1.09**	**1.23**	**1.45**	
For comparing	**S.Em±**		**CD**		**S.Em±**		**CD**	
Treatments (T)	0.003		0.014		0.002		0.009	
Irrigation levels (I)	0.006		0.024		0.003		0.015	
Interaction (T x I)	0.018		0.070		0.012		0.045	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Table 12. Effect of plant growth regulators and VAM on osmotic potential in leaf (- bar) at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**45 DAS**				**65 DAS**			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	**Mean**
T_1 : GA_3 (100 ppm)	7.81	8.66	9.67	8.72	10.58	12.23	14.16	12.32
T_2 : CK (10 ppm)	7.50	8.15	9.44	8.36	9.90	11.75	13.33	11.66
T_3 : CCC (200 ppm)	7.06	7.60	9.13	7.93	9.58	11.00	12.05	10.87
T_4 : VAM (5 g/kg of soil)	6.26	7.55	8.40	7.40	8.41	9.83	10.75	9.66
T_5 : Control	8.63	10.08	11.14	9.95	11.81	13.91	15.58	13.72
Mean	**7.45**	**8.40**	**9.50**		**10.06**	**11.74**	**13.17**	
For comparing	**S.Em±**		**CD**		**S.Em±**		**CD**	
Treatments (T)	0.03		0.12		0.04		0.17	
Irrigation levels (I)	0.05		0.20		0.07		0.29	
Interaction (T x I)	0.15		0.60		0.22		0.87	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

4.2.5 Osmotic Potential (- bar)

Significant difference in the osmotic potential was observed among the treatments, moisture stress and interaction effects at both the stages of the crop (Table 12).

In general, OP decreased with increase in moisture stress levels. I_3 moisture stress levels recorded significantly lower OP than I_1 and I_2.

The treatment VAM had significantly lower (7.4 and 9.66 - bar) and followed by CCC 7.93 and 10.87 bar both at 45 and 65 DAS respectively. Moisture stress had a positive effect on osmotic potential and it decreased from 7.45 to 9.5 at 45 DAS and from 10.06 to 13.17 at 65 DAS with increase in moisture stress level from I1 to $I_{3.}$

Among the interaction effects, VAM recorded significantly lower osmotic potential at I_1 moisture stress in both stages, (6.26 and 8.41 at 45 and 65 DAS respectively) compared to all other treatments and significantly highest was recorded in control at I_3 level of stress (11.14 and 15.58 at 45 and 656 DAS respectively).

The per cent decrease of osmotic potential in VAM treatment was 34.5 and 42.0 % over control at 45 and 65 DAS respectively.

4.2.6 Relative water content (%)

A significant difference in the relative water content was observed among the treatments, moisture stress levels and interaction effect at both the stages of the crop (Table 13).

In general, RWC decreased with increase in moisture stress levels. I_3 moisture stress levels recorded significantly lower RWC than I_1 and I_2.

Irrespective of the moisture stress levels, the treatment VAM had significantly higher RWC 78.5% and 81.6% followed by CCC (76.3% and 79.2%) at 45 and 65 DAS respectively. Significantly least RWC was observed in the control at both the stages (71.1 and 68.9 percent at 45 and 65 DAS respectively).

Moisture stress had a negative effect on RWC and it decreased from 80.4 to 69.2% at 45 DAS and from 86.0 to 72.3% at 65 DAS at I_1 and I_3 level of stresses respectively.

Irrespective of irrigation levels and treatment effects, VAM recorded significantly higher RWC (84.5 and 88.9 % at 100 % FC (I_1)) as compared to all other treatments and least was recorded in control at I_3 levels of moisture stress (50 % FC) at both the stages of crop growth (65.8 and 68.9 % at 45 and 65 DAS respectively).

4.3 Yield and Yield Components

4.3.1 Number of good opened bolls

Significant differences in total number of good opened bolls per plant were found for treatments, moisture stress and its interaction effect. (Table 14).

The treatment of with VAM recorded significantly more number of good opened bolls (6.5 bolls plant $^{-1}$) over other treatments. The least number of good opened bolls were in control (4.5 bolls plant $^{-1}$).

Lower moisture stress level (I_1 – 100% FC) recorded higher number of good opened bolls plant $^{-1}$ (6.8) with increase in moisture stress levels number of good bolls per plant decreased to 4.2 bolls plant $^{-1}$ at 50% FC.

Among the interaction effects, the highest number of good opened bolls was observed in VAM treatment 7.3 at I_1 moisture stress level compared to all other treatments and least was recorded in the control (3.3 at I_3 moisture stress level). The per cent increase in good opened bolls in the treatment VAM was 44.4 % over untreated.

Table 13. Effect of plant growth regulators and VAM on relative water content (%) in leaf at 45 and 65 DAS under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**45 DAS**				**65 DAS**			
	I_1	**I_2**	**I_3**	**Mean**	**I_1**	**I_2**	**I_3**	Mean
T_1 : GA_3 (100 ppm)	78.7	72.0	66.9	72.6	85.3	75.2	70.8	77.1
T_2 : CK (10 ppm)	80.0	73.7	68.2	74.0	86.0	76.3	72.1	78.1
T_3 : CCC (200 ppm)	81.1	76.4	71.3	76.3	86.2	78.0	73.5	79.2
T_4 : VAM (5 g/kg of soil)	84.5	77.4	73.6	78.5	88.9	79.5	76.4	81.6
T_5 : Control	77.5	69.9	65.8	71.1	83.8	74.3	68.9	70.4
Mean	**80.4**	**73.9**	**69.2**		**86.0**	**76.6**	**72.3**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	0.08		0.33		0.14		0.58	
Irrigation levels (I)	0.14		0.56		0.08		0.34	
Interaction (T x I)	0.43		1.68		0.44		1.74	

I_1 : 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

4.3.2 Number of bad opened bolls

Treatments and moisture stress levels were found significant for total number of bad bolls per plant (Table 14).

Significantly higher number of bad opened bolls were observed in control (3.3) compared to all other treatments. The treatment VAM recorded least number of bad bolls per plant (2.4) followed by CCC (2.6).

Interaction effect was found to be significant. Significantly higher number of bad bolls per plant was observed in control (3.3) at I_3 moisture stress level and least was recorded in VAM and kinetin treatments (2.3) at I_3 moisture stress level. The per cent decrease in bad opened bolls in the treatment VAM (26.9 % over control).

4.3.3 Boll weight (g)

The data showed significant differences between moisture stress level, treatments and interaction effect (Table 14). Higher mean boll weight was observed in VAM (4.21) followed by CCC (3.91) and CK (3.71) as compared to control (3.38).

With increase in moisture stress levels, there was decrease in boll weight and I_3 moisture stress had lowest boll weight of 3.27 g and significantly differed with I_1 (4.28) and I_2 (3.69) moisture stress levels.

Among the interaction effects, VAM treatment had significantly highest boll weight of 4.75 g at I_1 moisture stress level compared to all other treatments and least was recorded in control (2.93) at I_3 moisture stress level. The percent increase in good boll weight in the treatment VAM was 25% over control.

4.3.4 Seed Cotton yield (g plant^{-1})

In general, seed cotton yield decreased significantly from 32.9 to 17.2 g plant^{-1} with increase in moisture stress level from I_1 (100% FC) to I_3 (50% FC) (Table 15).

Among the treatments, VAM recorded significantly higher yield of 31.6 g/plant as compared to other treatments followed by CCC (26.8 g/plant). The treatment control had the lowest seed cotton yield of 19.8 g plant^{-1}.

Among the moisture stress levels, I_1 had significantly highest yield of 32.9 g plant^{-1} and lowest yield was observed at I_3 moisture stress level (17.2 g plant^{-1}). The per cent increase of seed cotton yield in VAM treatment was 59.5 % over control. Among the interaction effects, the VAM had significantly higher seed cotton yield of 40.4 g plant^{-1} at I_1 moisture stress level and significantly least seed cotton yield was recoded in control (13.6 g plant^{-1}) at I_3 moisture stress level.

4.4 Correlation study

The correlation coefficient values were calculated among the biochemical parameters and seed cotton yield (Table 16).

RWC had strong and positive correlation with yield and total chlorophyll content indicating turgidity in the plant is an important factor for stabilized yield. RWC had strong negative correlation with osmotic potential, decrease in osmotic potential leads to increase in RWC under moisture stress situation in cotton. The peroxidase activity at 65 DAS positively correlated with proline content.

Table 14. Effect of plant growth regulators and VAM on number of good and bad opened bolls and boll weight (g) at harvest under different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	Number of good opened bolls				Number of bad opened bolls				Boll weight (g)			
	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean
T_1 : GA_3 (100 ppm)	6.3	4.6	3.6	4.8	2.6	2.6	2.6	2.6	4.08	3.40	3.00	3.51
T_2 : CK (10 ppm)	6.6	5.3	4.3	5.4	3.0	2.0	2.3	2.4	4.16	3.60	3.30	3.71
T_3 : CCC (200 ppm)	7.3	5.6	4.6	5.8	3.3	2.3	3.0	2.8	4.50	3.83	3.40	3.91
T_4 : VAM (5 g/kg of soil)	7.6	6.6	5.3	6.5	3.0	2.6	2.3	2.6	4.75	4.25	3.65	4.21
T_5 : Control	6.0	4.3	3.3	4.5	3.0	3.6	3.3	3.3	3.91	3.30	2.93	3.38
Mean	**6.8**	**5.3**	**4.2**		**3.0**	**2.6**	**2.7**		**4.28**	**3.69**	**3.27**	
For comparing	**S.Em ±**		**CD**		**S.Em ±**		**CD**		**S.Em ±**		**CD**	
Treatments (T)	0.09		0.38		0.07		0.30		0.04		0.16	
Irrigation levels (I)	0.05		0.22		0.04		0.18		0.02		0.09	
Interaction (T x I)	0.29		1.14		0.23		0.90		0.12		0.48	

I_1: 100% Field capacity I_2 : 70% Field capacity I_3 : 50% Field capacity

Table 15. Effect of plant growth regulators and VAM on seed cotton yield (g $plant^{-1}$) at different levels of moisture stress in cotton

Irrigation levels / seed priming treatments	**Seed cotton yield (g/plant)**			
	I_1	**I_2**	**I_3**	**Mean**
T_1 : GA_3 (100 ppm)	28.7	18.8	14.2	20.5
T_2 : CK (10 ppm)	31.2	21.6	16.8	23.2
T_3 : CCC (200 ppm)	36.9	24.2	19.3	26.8
T_4 : VAM (5 g/kg of soil)	40.4	31.9	22.4	31.6
T_5 : Control	27.1	18.6	13.6	19.8
Mean	**32.9**	**23.0**	**17.2**	
For comparing	**S.Em ±**		**CD**	
Treatments (T)	0.63		2.47	
Irrigation levels (I)	0.38		1.48	
Interaction (T x I)	1.90		7.41	

I_1: 100% Field capacity I_2: 70% Field capacity I_3: 50% Field capacity

Table 16. Correlation coefficients (r) between seed cotton yield and biochemical parameters at 65 DAS in cotton

At 65 days	Yield	Chl 'a'	Chl 'b'	Total chl	Proline	Peroxidase	Reducing sugar	Osmotic potential	RWC
Yield	1.00	0.942**	0.921**	0.948**	-0.34	-0.44	-0.20	-0.921**	0.943**
Chl 'a'		-	0.945**	0.990**	-0.188	-0.298	-0.172	-0.948**	0.856**
Chl 'b'			-	0.980**	-0.104	-0.263	-0.115	-0.976**	0.830**
Total chl				-	-0.513	-0.286	-0.148	-0.974**	0.874**
Proline					-	0.940**	-0.079	0.082	-0.430
Peroxidase						-	-0.089	0.214	-0.538*
Reducing sugar							-	0.08	-0.160
Osmotic potential								-	-0.870**
RWC									-

**.Correlation is significant at the 0.01 level (2 tailed)
*. Correlation is significant at the 0.05 level (2 tailed)

5. DISCUSSION

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 plants and induces anatomical and morphological changes resulting in reduced growth.

The use of plant growth regulators and VAM (Vesicular Arbuscular Mycorrhizae) is an alternative approach to ameliorate the effect of moisture stress on crops. The present investigation deals with the effect of plant growth regulators and VAM under varying levels of moisture and their relative performance to biochemical, physiological parameters and the cause differences in yield and attributes. The results obtained on these aspects in the present study are discussed in this chapter.

A pot experiment was carried out during the year 2006-07 to find out the ameliorative effects of plant growth regulators and VAM on different physiological, biochemical and yield and yield attributes in cotton.

5.1 Physiological parameters

5.1.1 Germination

Treatments showed variation in the percent 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 imbibition 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), Abugrab and Ebrahim, (1998) who reported increase in germination of onion due to growth regulators as compared to control.

5.1.2 Root Characteristics (Root length, shoot length and root: shoot ratio and seedling vigour)

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).

The present investigation revealed that, the treatment 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. Similarly, Davies *et al,* (1996) reported that mycorrhizae tend 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 posssessed 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.

5.2 Biochemical parameters

5.2.1 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 cytokinetin 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 (Fig. 1).

5.2.2 Free 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 osmolyte. 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

LEGEND

Irrigation levels

I_1	-	100% field capacity (Control)
I_2	-	70% field capacity
I_3	-	50% field capacity

Treatments

T_1	-	GA_3 @ 100 ppm
T_2	-	Kinetin @ 10 ppm
T_3	-	CCC @ 200 ppm
T_4	-	VAM @ 5 g/kg of soil
T_5	-	Control

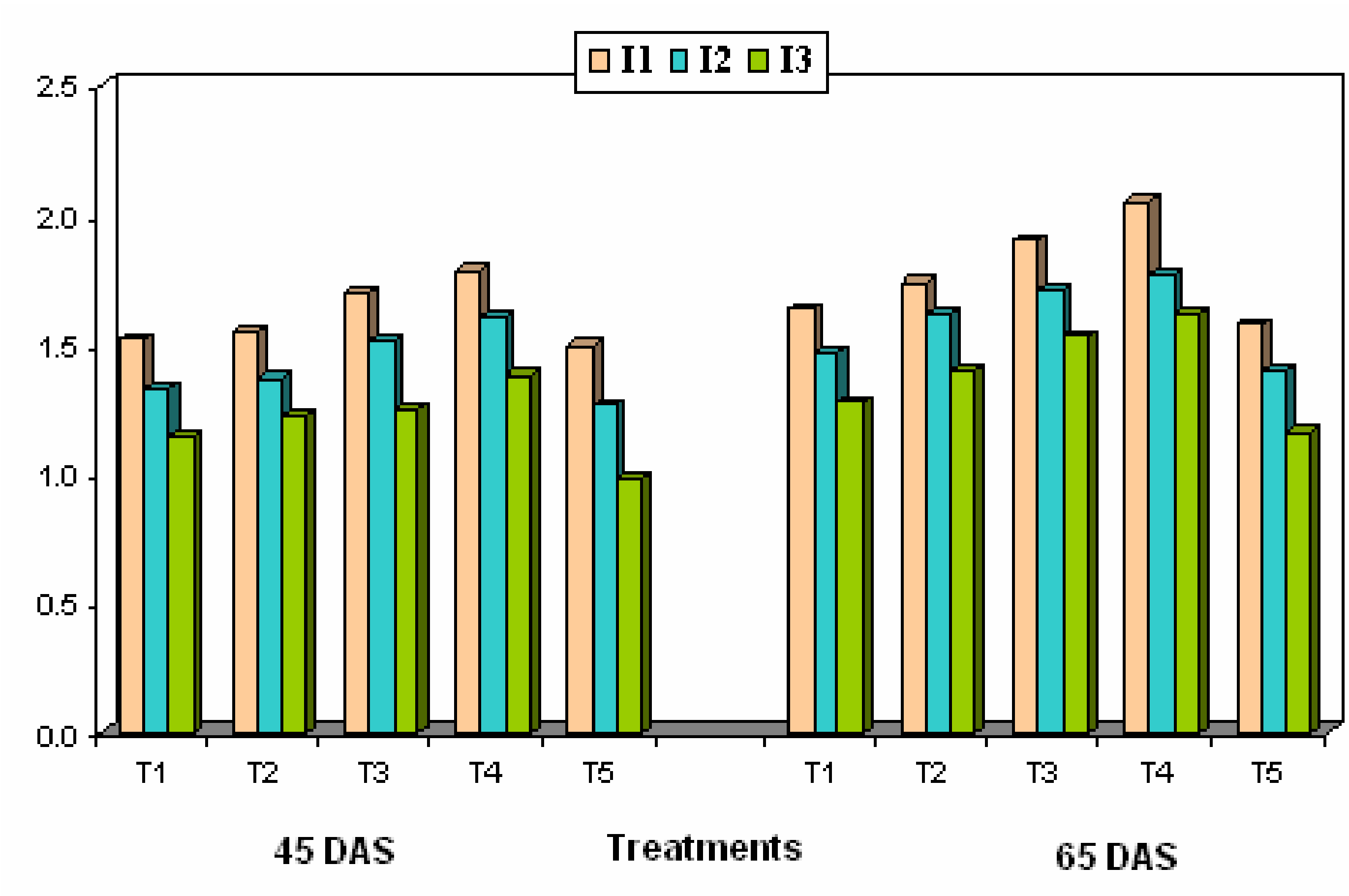

Figure 1. Effect of plant growth regulators and VAM on total chlorophyll content (mg/g fresh wt.) in leaf at 45 and 65 DAS under different levels of moisture stressing cotton

symbiosis enlarges absorption areas and improves the nutritional status of plant. The increase in amminoacid 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) (Fig. 2).

5.2.3 Reducing sugar

Increase in the sugar content in response to water stress has been reported by Bajji *et al,* (2001), Karimi *et al.* (2005) and Moradshahi *et al.* (2004) who explained that sugar tends to increase in cultivars of *Brassica napus* which is probably due to mobilization of reserved polysacchacides with decreasing water potential and Martinez *et al*. (2004), reported that sugar concentration increased in drought at a rate closely corresponding to the decrease in leaf RWC in *Atriplex halimus.*

The present investigation showed, that the treatment VAM had significantly higher reducing sugar accumulation of 88.6 µg due to water stress at 65 DAS and the results are in agreement with Abdel *et al.* (2002) and this was due to the increase in the content of fungal disaccharide in the roots of all mycorrhizal plants upon exposure to drought (Schellenbaum *et al.,* 1999). The accumulations of these solutes in mycorrhizal plants may serve as osmoregulators, which is an important adaptation of plants to drought stress. This was closely followed by the treatment CCC (83.2 mg at 65 DAS) CK and GA_3, these results are in agreement with Uday Burman and Kathju (2003) in cluster bean and Vora *et al.* (1975) in Bajra. Further Woodhams and Kozlowski, (1954) suggested that the increase in sugar content is due to oxidation through increased respiratory activity in germinating seeds. Fazeli *et al.* (2006) resulted that increase in sugar under drought may be due to decrease in total polysaccharides (Fig. 3).

5.2.4 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 H_2O_2 which is also toxic to plants. Catalase and peroxidase enzymes catalyze the breakdown of H_2O_2 (Chang *et al.,* 1984). Plants with high levels of antioxidants have greater resistance to oxidative damage by H_2O_2 (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.

5.2.5 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 Dumbuoff, 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

LEGEND

Irrigation levels

I_1	-	100% field capacity (Control)
I_2	-	70% field capacity
I_3	-	50% field capacity

Treatments

T_1	-	GA_3 @ 100 ppm
T_2	-	Kinetin @ 10 ppm
T_3	-	CCC @ 200 ppm
T_4	-	VAM @ 5 g/kg of soil
T_5	-	Control

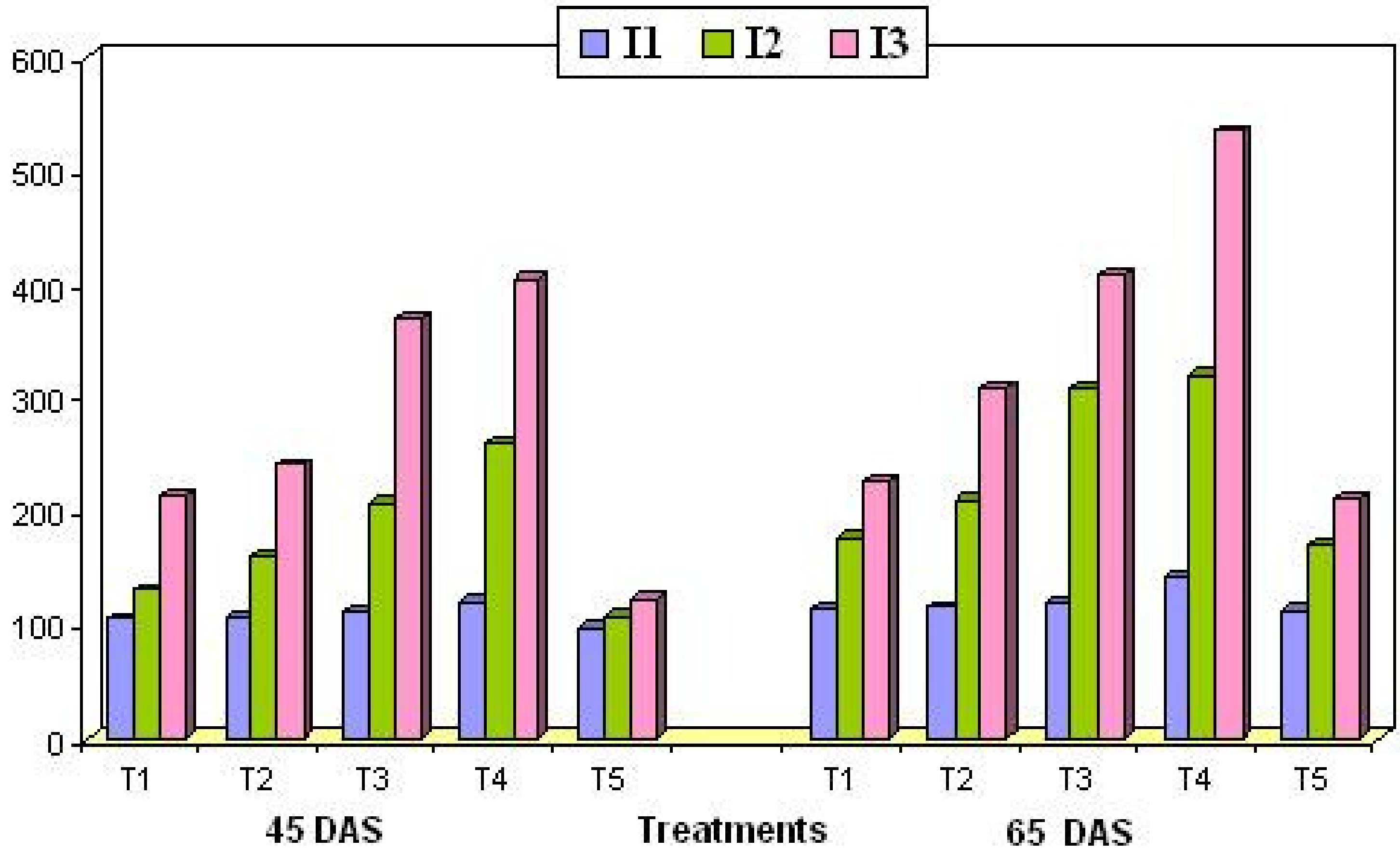

Figure 2. Effect of plant growth regulator and VAM on proline content (µg/gfresh wt.) in leaf at 45 and 65 DAS under Different levels of moisture stress in cotton

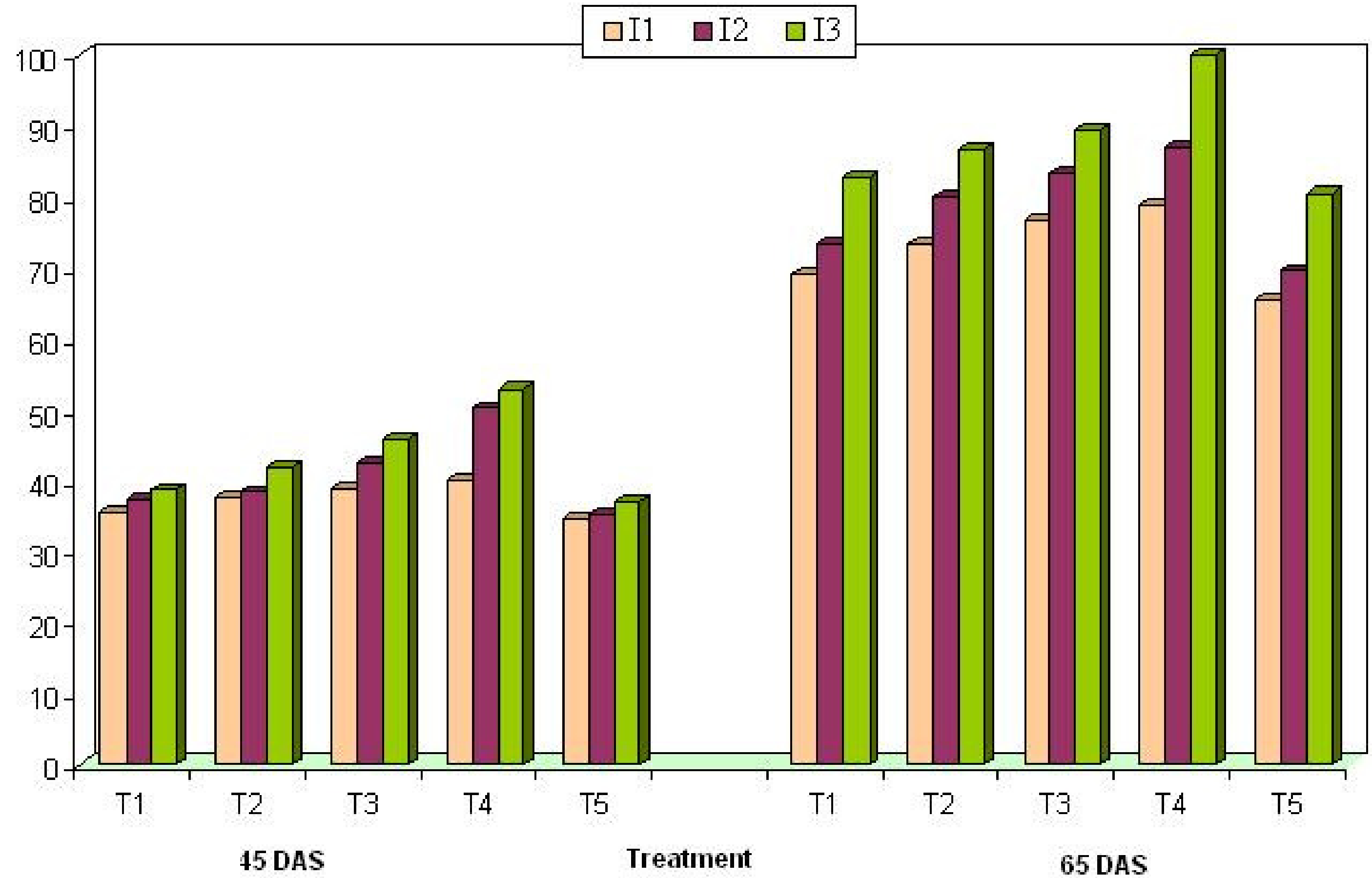

Figure 3. Effect of plant growth regulators and VAM on resucing sugar content (µg/g fr. Wt.) in leaf at 45 and 65 DAS under different levels of moisture stress in cotton

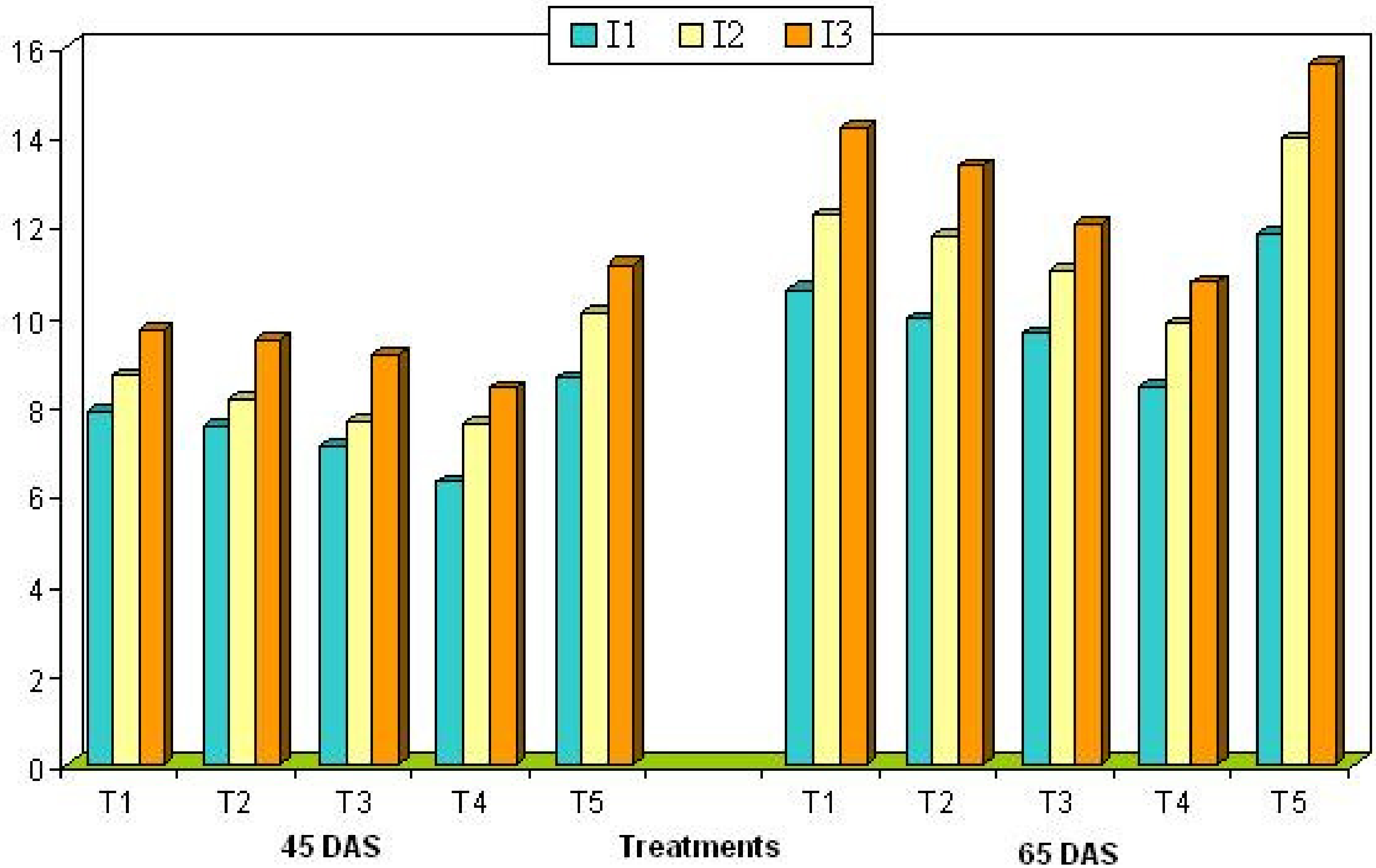

Figure 4. Effect of plant growth regulators and VAM on osmotic potential in leaf (-bar) at 45 and 65 DAS under different levels of moisture stress and cotton

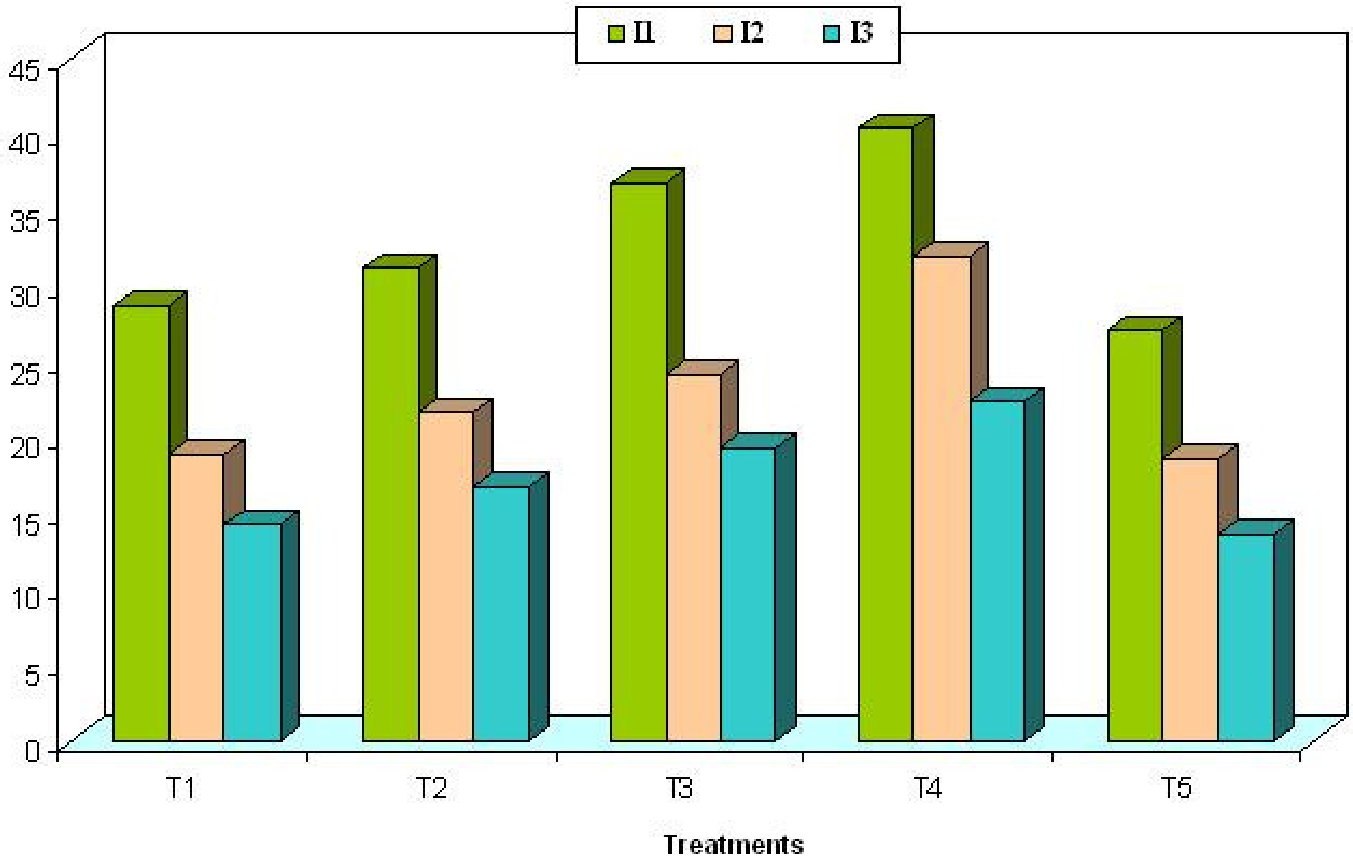

Figure 5. Effect of plant growth regulators and VAM on seed cotton yield (g/plant) under different levels of moisture stress in cotton

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) (Fig. 4).

5.2.6 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 the present study, 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 % and 15.9 % over control at both stages of crop growth.

5.3 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 radiate* (Fig. 5).

5.4 Practical utility of the results

Drought is a major constraint which affects cotton productivity and it can be mitigated using VAM and plant growth regulators.

Impact of moisture stress can be mitigated by treating VAM to the soil @ 5 g/kg of the soil or/ seed priming with CCC @ 200 ppm for enhancing the yield under moisture stress.

5.5 Future line of work

Drought is a common phenomenon in present day agriculture. Much work has been done on alleviation of problem with plant improvement and using physical and chemical methods. But they are highly expensive, time consuming and do not meet the objective. The present investigation has shown encouraging results with the use of PGR's and VAM culture in mitigating the drought stress to certain extent. However, there is a need to strengthen the research work on this aspect under rainfed conditions as mentioned below.

1. Testing of more number of PGR's and various concentrations of VAM on large number of cotton genotype under moisture stress conditions for studying genotypic response.

2. Study the effect of PGR's and VAM culture on antioxidant enzyme activity under moisture stress in cotton.

6. SUMMARY AND CONCLUSION

Environmental stresses are among the most limiting factors to plant productivity. Among these, drought is an important abiotic stress affecting the productivity of all rainfed crops. Moisture stress potentially limits the future of agriculture in most productivity areas of world. Even though cotton is recognized as the most drought tolerant of all crops, its productivity can still be enhanced through use of plant growth regulators and VAM as an alternate approach to mitigate the effects of moisture stress. Hence, the present investigation was carried out with seed priming through plant growth regulators (GA_3, CK, and CCC) and VAM (vesicular arbuscular mycorrhiza) tested under three levels of moisture stress i.e. 100 % field capacity, 70 % field capacity, 50 % field capacity in LRA 5166 cotton genotype belonging to *Gossypium hirsutum* during 2006-07 at Agriculture college, UAS, Dharwad. It was intended to study the ameliorative effect of PGR' s and VAM and changes in growth, physiological processes ,biochemical parameters and yield potential of cotton under moisture stress condition.

1. Under severe moisture stress condition in black cotton soil, germination decreased by 60 % and application of VAM enhanced germination by 25 %.
2. Root characteristics (root length, shoot length and root: shoot ratio) decreased with an increase in the moisture stress. The per cent decrease or inhibition was less in the treatment VAM and CCC compared to control with increase in moisture stress from 100 % FC to 50 % FC. Root: shoot ratio was less (19.5 %) in VAM treatment compared to other treatments.
3. Similarly, seedling vigour index reduced drastically with increase in moisture stress levels. The reduction was less in the treatment of VAM and highest was in control.
4. Total chlorophyll content and its fractions 'a' and 'b' decreased with an increase in moisture stress both at 45 and 65 DAS. In general, VAM treatment and seed priming with CCC showed less reduction in total chlorophyll and 'a' and 'b' fractions followed by the treatment kinetin and GA_3 where as control showed higher reduction among the treatments.
5. Free proline content increased in all treatments with an increase in moisture stress. The treatment VAM and CCC further enhanced the accumulation of more proline under increased moisture stress, while control (T_5) accumulated least proline in leaves.
6. The peroxidase activity was maximum at 65 DAS. With an increase in moisture stress, the peroxidase activity was increased. Among the treatments, VAM and CCC showed higher activity at both the stages followed by kinetin and GA_3 and least enzyme activity was observed in control.
7. The reducing sugar accumulation increased in all the treatments with an increase in the moisture. The treatment VAM and CCC accumulated more reducing sugar irrespective of moisture levels. While, control accumulated least reducing sugar in leaves. However, the accumulation was more conspicuous under moisture stress when soil was treated with VAM.
8. Osmotic potential decreased with increase in moisture stress. Lower osmotic potential was maintained at higher moisture stress levels in VAM and CCC and followed by kinetin and GA_3 and least was in control, which clearly indicates the higher maintenance of water status in VAM and CCC treatments.
9. Relative water content indicated significant differences due to plant growth regulators and VAM and it was higher in the treatment VAM and CCC followed by kinetin and GA_3 and least was in control. This is also a good indication of maintenance of higher turgidity under moisture stress which helps in higher metabolic activities.
10. Moisture stress reduced the seed cotton yield and among the treatment VAM treatment recorded highest seed cotton yield followed by CCC.
11. Yield components such as, number of bolls and boll weight was also affected by moisture stress. The treatment VAM and CCC had higher boll number of good open

bolls at all the moisture stress levels, while, control had least boll number. Similarly, boll weight was also more in VAM and CCC treatment and less in control.

Based on the information generated from the present investigation, it can be concluded that treatments differ in their response to moisture stress and different plant growth regulators and VAM have different ameliorative mechanism against moisture stress. Thus, information obtained would be useful in coming over the moisture stress by using PGR's and VAM in cotton. Based on this study, among the various seed priming treatments, CCC and soil treatment with VAM would be better in mitigating the moisture stress and enhancing seed cotton yield. Thus, it could be concluded that higher yield obtained in VAM and CCC treatment might be due to the mechanism of moisture stress tolerance by maintenance of higher water status in plants, higher chlorophyll, proline content, reducing sugar and higher peroxidase enzyme activity, lower osmotic potential and highest relative water content.

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MITIGATION OF DROUGHT STRESS THROUGH PLANT GROWTH REGULATORS AND VESICULAR ARBUSCULAR MYCHORRIZAE (VAM) IN COTTON (*Gossypium hirsutum*)

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ABSTRACT

The present investigation was carried out during kharif, 2006-07 with seed priming through plant growth regulators (GA_3, CK and CCC) and VAM (Vesicular Arbuscular Mycorrhiza) tested under three levels of moisture stress i.e. 100 %, 70 %, 50 % field capacity in *Gossypium hirsutum* cv. LRA 5166 to mitigate the drought stress.

Under severe moisture stress condition, germination decreased by 60 per cent while application of VAM enhanced germination by 25 per cent. Root and shoot length and seedling vigour index reduced with increase in moisture stress. These parameters enhanced considerably with VAM application to soil. While, root: shoot ratio was minimum (19.5%) in VAM treatment compared to other treatments.

Total chlorophyll content and its fractions decreased with an increase in moisture stress. In general, VAM treatment and seed priming with CCC showed lesser reduction in total chlorophyll and its fractions followed by the kinetin and GA_3 treatments. Free proline content increased in all the treatments with an increase in moisture stress. The treatment VAM and CCC further enhanced the accumulation of proline under increased moisture stress. The reducing sugar increased in all the treatments with an increase in the moisture stress. The treatment VAM and CCC accumulated more reducing sugar irrespective of moisture levels.

The peroxidase activity was maximum at 65 DAS and with increase in moisture stress the peroxidase activity increased. Among the treatments, VAM and CCC showed higher activity at both the stages followed by kinetin and $GA_{3.}$ Relative water content (RWC) indicated significant differences due to plant growth regulators and VAM and it was higher in the treatment VAM and CCC followed by kinetin and $GA_{3.}$

Moisture stress reduced the seed cotton yield and yield components (number of bolls and boll weight) and among the treatments, VAM recorded significantly higher seed cotton yield, higher number of good opened bolls and higher boll weight at all the moisture stress levels compared to other treatments.