EFFECT OF GLYCINE BETAINE IN THE ALLEVIATION OF ABIOTIC STRESSES IN GROUNDNUT GENOTYPES

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CERTIFICATE

Ms. Koti Sailaja has satisfactorily prosecuted the course of research and that the thesis entitled **"EFFECT OF GLYCINE BETAINE IN THE ALLEVIATION OF ABIOTIC STRESSES IN GROUNDNUT GENOTYPES ARACHIS HYPOGAEA. L.,"** submitted is the result of original research work and is sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF GLYCINE BETAINE IN THE ALLEVIATION OF ABIOTIC STRESSES IN GROUNDNUT GENOTYPES"** submitted in partial fulfilment of the requirements for the degree of 'Doctor of philosophy in Agriculture' of Acharya N. G. Ranga Agricultural university, Hyderabad is a record of bonafide research work carried out by Ms. K. Sailaja under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigations has been duly acknowledged by the author of the thesis.

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(SAILAJA KOTI)

DECLARATION

I, **Ms SAILAJA KOTI,** hereby declare that the thesis entitled **"EFFECT OF GLYCINE BETAINE IN THE ALLEVIATION OF ABIOTIC STRESSES IN GROUNDNUT GENOTYPES"** is the result of the original research work done by me. I further declare that the thesis of part thereof has not been published earlier in any manner.

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Abstract

Abiotic stresses occurring at critical growth stages in groundnut affect productivity by reducing the total dry matter, pod yield and quality. Present study investigates the role of glycine betaine in alleviating effects of the three major abiotic stresses i. e., drought, heat and salinity on selected groundnut genotypes. The investigation was conducted in 3 phases i.e., (a) effect of betaine on tolerance of groundnut seedlings to heat and salinity stress conditions, (b) effect of betaine on isolated plants growing in pots subjected to drought, heat and salinity, and (c) effect of betaine on tolerance of groundnut genotypes to simulated drought under field conditions. The experiments were conducted during 1996- 98 period at ICRISAT centre, Patancheru, in laboratory, glass house, plant growth chambers and field. To investigate betaine effect on seedling systems, the seedlings were subjected to high temperature and salinity stress conditions in laboratory with and without glycine betaine treatment. Under high temperature stress conditions, the seedlings with betaine treatment were able to produce greater root and shoot lengths 34 and 40% respectively than seedlings without betaine treatment. In the non induced treatments, there was a 122% greater growth of roots in betaine treated seedlings compared to untreated ones. The gel electrophoresis results indicated that betaine treatment was able to produce four new proteins with molecular weights of 76.4, 60.6, 54.6 and 16.5 kDa. Under salinity stress conditions, the betaine treatment was able to produce 30 and 32% more root and shoot growths than untreated

seedlings. The protein profiles indicated that betaine treatment was able to produce four new proteins with molecular weights of 65.4, 37.8, 35.4 and 16.5 kDa. These stress shock proteins which are produced under high temperature and salinity stress conditions were implicated as molecular mechanisms to enhance the adaptation of the tissues to stress conditions. In the pot culture experiments effect of glycine betaine on isolated plants growing under heat, drought and salinity stress conditions in glass house and growth chamber. Under heat stress conditions, seed treatment with glycine betaine could increase the root and shoot development by 150 and 32% and total dry matter by 20%. There was a relative increase in net photosynthetic rate and Fv/Fm ratios, decrease in leaf water potential. Under salinity stress conditions, the betaine treatment enhanced the growth in root, shoot and total biomass by 135%, 25% and 28% respectively when compared with untreated control. Correspondingly the net photosynthetic rate increased by 35% with betaine treatment. Similarly with high temperature stress conditions, the seed treatment with glycine betaine could increase the root and shoot development by 22 and 43% and total dry matter was increased by 23%. There was a relative increase in RWC by 10% and decrease in leaf water potential by 25%. The fluorescence (Fv/Fm) which is an index of PSII quantum yield was reduced in stressed plants without betaine when compared with betaine treated stressed plants. These results indicate that glycine betaine accumulation confers protection against the photochemical reaction of PS II *in vivo*. In field studies effect of glycine betaine at 3, 6 and 9 kg ha^{-1} under mid season and end season drought was examined whether its application could ameliorate the effects of drought on the yield of groundnut. The biomass production and pod dry matter were significantly reduced by 45 and 58% by drought. Exogenous application of betaine at 3 kg ha^{-1} resulted in alleviation of drought effects as evidenced by reduction in drymatter by 36% compared to control. The positive effects of glycine betaine treatment appear to be linked not only to its physiological role as a plant osmoticum that improves drought tolerance but also to a protective role for proteins and membranes even at low concentrations.

The results of the present study suggest that foliar application of glycine betaine may be used to improve stress tolerance and economic yield of groundnut. However, detailed biochemical studies need to be taken up to establish the metabolic engineering of glycine betaine biosynthetic pathway in higher plants.

Introduction

Chapter I

Introduction

Legumes are the important source of dietary proteins and fat in many developing countries including semi arid tropics (SAT). Even though legumes have greater ecological efficiency than live stock industry, their cultivation is mainly predominant in seasonally rainfed, low input marginal lands in SAT. Major abiotic stress factors that limit the productivity of legumes in SAT are drought, salinity and high temperature stresses. Among the grain legumes groundnut is the major cash crop of SAT and about 67 % of global groundnut production comes from the rainfed cultivation (Gibbons 1980).

India is the largest producer of groundnut in the world with a total production of 8.9 million tonnes. The crop is grown on 22.5 lakh ha area. The yield of groundnut crop is lower and erratic (900 Kg/ha) mainly due to drought, diseases and pests (The Hindu, Survey of Indian Agriculture). Drought remains as one dominant abiotic factor affecting groundnut production in India. Since availability of water for supplementary irrigation will be an increasingly scarce commodity, there is a need to explore genetic and managerial ways to enhance the tolerance of groundnut to water deficit conditions. The drought is often associated with high temperatures. It is well known that optimum temperature for germination of groundnut is 27 - 30° C and temperatures on the either side of the optimum range result in reduction in the rate of germination (Kelring 1984). There is no clear documentation of base and optimum temperatures for various phenophases of groundnut. For all practical purposes groundnut crop growth models (such as PEANUTGRO, QNUT and PARCHNUT) assume a base of 10^0 C and an optimum of 27^0C as threshold temperatures for the crop growth.

Temperatures of a range of 35 - 40^0 C are common in India during the drought period in rainy season as well as reproductive period in summer season.

Nageswara Rao *et al.*, (1989) has shown that reproductive development in groundnut is sensitive to high temperatures. Fertilisation has been shown to be the most sensitive to temperature above 35^0 C. Hence, identification of traits and development of management practices that impart tolerance to heat stress is having paramount importance particularly so, in view of the global warming (Schneider 1989) and this change coupled with increase in CO_2 concentration may substantially increase the need for tolerant genotypes all over the world (Hall 1992).

In India salinity is also a major factor limiting the crop production. About 10% of total cutivable soils in India suffer from salinity disorders. In view of growing population and growing demand for food and food crops the important legumes such as groundnut need to be expanded in hostile environments or non traditional areas.

Accumulation of osmoprotectants in higher plants and other organisms is a well known phenomenon representing metabolic adaptation to salinity, drought and high temperature stress. Osmoprotectants are small molecules that can benefit osmotically stressed cells in two ways i.e., by acting as nontoxic cytoplasmic osmolytes to raise osmotic pressure and by protecting enzymes and membranes against damage by salt levels (Wyn Jones 1984).

Osmoprotectants fall into two chemical classes : Polyols and their derivatives (Somero. 1986; Csonka and Hanson 1991). Glycine betaine is a polyol which occurs in small families of higher plants, particularly in species adapted to dry and saline environments (Rhodes and Hanson 1993). However, many higher plants do not accumulate glycine betaine or any other

osmoprotectant, and this has led to interest in the metabolic engineering of the glycine betaine biosynthesis pathway as an approach for enhancing stress resistance (Lerudulier *et. al.*, 1984; Mc Cue and Hanson 1990)

Higher plants synthesise glycine betaine in chloroplasts via the pathway :

Choline → betaine aldehyde → Glycine betaine (Rhodes and Hanson 1993). The first step is catalysed by choline monooxygenase (Brouquisse *et al.*, 1989), and the second step by betaine aldehyde dehydrogenase (BADH) (Weigel *et al.*, 1986).

The accumulation of glycine betaine (N, N, N - Trimethyl glycine) in water and salt stressed plants has been proposed to play an important role in osmotic adjustment which is widely considered to be an adaptive response to stress due to water stress and salinity (Hanson 1980, Wyn jones 1984, Yancey *et al.*, 1982). It appears that betaine functions as an compatible or protective solute in the cytoplasm and chloroplasts (Incharbensakdi *et al.*, 1986). Since it appears to be a relatively inert end product of metabolism that is not catabolised to any appreciable extent in plants (Hanson and Hitz 1982; McCue and Hanson 1990).

Alleviation of abiotic stress factors by enhancing the adaptation of the crop by genetic and management factors can substantially contribute to the yield improvement.

There have been studies on the use of chemical compounds to alleviate the effects of drought on plants and interest is increasing with better understanding of the physiological effects of stresses. For example, the foliar application of glycinebetaine to potato (Solanum tuberosum L.) and Tomato (Lycopersicum esculentum Mill.) indicates its possible use to reduce crop failures under conditions of osmotic stress in Sudan (Agboma *et al.*, 1997). In a green house

study of drought stressed tobacco (a non betaine accumulating model crop) the foliar application of glycine betaine significantly increased leaf area and leaf dry weight (Agboma *et.al.*,1997). The benefits of external application of glycine betaine on seedlings or plants have been demonstrated under vitro conditions on isolated enzymes (Paleg *et al.*, 1985) or on whole plants (Zao *et al.*, 1992). External application of glycine betaine on cotton enhanced seedling vigour, germination and yield in cotton (Naidu *et al.*, 1996).

Application of exogenous glycine betaine, timed to coincide with critical development stage at which a crop is especially susceptible to drought, could reduce yield losses under field conditions. Therefore studies were undertaken to evaluate the potential of exogenously applied glycine betaine.

The present investigation was undertaken with the following objectives:

1. To investigate the effect of glycine betaine in the alleviation of drought stress in groundnut genotypes.

2. To study the effect of glycine betaine on response of groundnut to high temperature stress.

3. To study the genotypic variation in response to and effect of glycine betaine under salinity stress in groundnut genotypes.

Review of literature

CHAPTER II

REVIEW OF LITERATURE

Food legume crops which constitute an important components of human diet and live stock feed are cultivated widely in arid, semi-arid, sub humid tropical regions of the world. The crops are chosen to suit the climate and soil type in different cropping systems. In semi-arid environments (SAT) where rainfall is low and growing season is short, crops like pigeonpea, groundnut, soybean, navybean etc., are sown in the rainy season either as a sole crop or as an intercrop, while crops such as chickpea, lentil and pea are grown either on residual moisture or during the post rainy season (Reddy and Willey, 1982; Papendick *et al.*,1988; Willey *et al.*,1986; Ali 1990; Wood and Myer 1986; Squire *et al.*,1986). Yield levels of food legume crops grown in SAT environments are generally low and erratic because they are grown under low inputand rainfed conditions (Carangal *et al.*,1986).

Groundnut is one of the important food legume crops grown in the semi arid tropical regions. About 2/3 of the world production of groundnut is utilized as an edible oil, making it one of the world's leading oil seed crops. India ranks first in the groundnut production in the world with about 30% share in the global production. The crop is grown on over 7.5 million hectares in the country and accounts for 53% of the oilseeds output and 59% in edible oil production. In India groundnut is grown predominantly as a rainfed crop with 82% of the crop production occurring in the rainy season. However, the yields of groundnut remained virtually stagnant at 890 kg/ha as against a world average of 1100 kg/ha.

The yields of groundnut grown under rainfed conditions are in general low and erratic because of combination of various biotic factors (pests and diseases) and abiotic factors (drought, high temperature, salinity and nutrient disorders). When biotic factors are controlled by integrated management factors drought remains to be the most important abiotic constraint affecting groundnut productivity under rainfed situations.

As the demand for food, feed and oil is increasing with increase in population in non traditional areas and hostile environments are being explored for crop production. Hence there is a need for development of novel ways of enhancing stress tolerance in important food legumes like groundnut.

Various reports have indicated large difference between potential and realized yield in food legume crops (Mc William & Dillon 1987) in general and groundnut in particular (Nageswara Rao 1992).

Classical genetic methods (involving crossing, and selection schemes) have already made enormous contributions towards crop improvement under non-limiting high input conditions (Acevado & Fereres 1993; Jones & Gorham 1986). However genetic enhancement for stress tolerance remains complex because of lack of thorough knowledge of traits contributing to the tolerance and lack of simple and economic tools for stress tolerance in large scale breeding programs. Hence, breeding for stress tolerance using classical selection schemes is not yet practiced.

This chapter deals with the review of research in groundnut in three major abiotic stress areas i. e., Drought, High Temperature and Salinity stresses.

2.1 DROUGHT STRESS:

In semi-arid environments, drought stress is a major factor responsible for low yield of groundnut (Simpson, 1981). The yield losses due to drought range from 5-75% depending on timing, intensity and duration of drought during crop growth. The intensity of drought also depends on water holding capacity of the soil and other environmental factors such as high temperatures. A thorough understanding of effects of drought on crop growth, yield formation and genotypic interaction is essential to make any progress in enhancing drought tolerance in groundnut. Effect of drought during different growth phases of groundnut has been investigated by many researchers. Drought during the vegetative stage has generally less effect on seed yield when subsequent environmental conditions are conducive for recovery compared to drought during reproductive stage (Turk *et al.*,1980; Hall and Grantz 1981). Nageswara Rao *et al.*,1985a found that moderate drought during pre flowering stage can intact increase in yield by 20% compared to irrigated control. Effects of drought in groundnut depend primarily on the pattern of drought and genotype variation is usually of secondary significance (Nageswara Rao *et al* 1991).

In groundnut, stress during the flowering stage can reduce number of flowers and delay flowering time (Boote *et al.*,1982). However, reduction in the number of flowers did not directly influence the pod yield (Nageswara Rao *et al.*,1992). Groundnut can compensate for reduced number of flowers by producing a new flush of flowers when stress has been relieved (Nageswara Rao

et al.,1988; Harris *et al.*,1988). Pod yield was significantly reduced by drought stress during pegging and pod set primarily because of reduction in pod number rather than kernel weight per pod (Boote *et al.*,1976; Pallas *et al.* 1979; Roy *et al.*,1988). Stress at pod filling phase was shown to reduce groundnut yield by 15-30% (Stansell & Pallas 1985; Nageswara Rao *et al.*,1985a; Chapman 1989; Wright *et al.*,1991). Pathak *et al* (1988) recorded a yield reduction of 62.7% compared to the control when stress was imposed at the pod filling stage. Late season drought has been shown to reduce pod yield more severely in long duration varieties than in early ones (Muchow & Sinclair 1986). mostly through reduction of pod number and seed size (Pallas *et al.*,1979; Nageswara Rao *et al.*,1985; Wright *et al*, 1991).

During 1980's substantial research had focussed on examining physiological basis of drought tolerance in groundnut. Although a number of studies have proposed phenomena related with biochemical basis for drought tolerance such as osmoregulation, proline, Abscissic acid etc. These results have found limited application in breeding programs mainly because of lack of consistency in the positive role of these traits in performance of genotypes under water deficit condition.

Recently, physiological models have been proposed to explain the performance of genotypes under a given environment. Passioura 1977 defined the yield as a function of transpiration (T), water use efficiency (WUE) and harvest index (HI). This physiological frame work of yield formation allowed to explain the performance of genotypes in different environments. This model has been recently evaluated for groundnut (Wright *et al*, 1994) and allowed selection of genotypes with high levels of each of these traits.

Transpiration:

Efficient water uptake requires the presence of roots in deeper soil layers, which enables the crop to explore a greater soil volume for water. The superior ability of groundnut to maintain favourable leaf water status during periods of soil water deficit was related to greater proliferation of roots in the deeper rooting zone (Bunting and Kassam 1988; Devries *et al.*,1989). Similarly, the higher root density in groundnut at lower soil depths conferred superior drought tolerance compared to soybean and mung bean (Pandey *et al.*,1984). The utilization of profile water from 120 cm depth which was reported by Stansell & Pallas (1985) suggests scope for exploiting groundnut germplasm for the ability to exploit water from deeper soil profile.

Efficient water uptake by roots was shown to be linked with osmoregulation occurring in root tips, (Subba Rao *et. al.* 1996, Davies *et al.*,1986). However, growth of roots into deeper soil layers under drought stress is a function of both genotype and environment (Gulmon & Turner 1978; Begg & Turner 1976; Malik *et al.*,1979, Sharp & Davies 1985).

Water use efficiency(WUE):

WUE is defined as the quantity of DM produced per unit of water transpired. Thus it is apparent that WUE is one of the most important factors influencing crop productivity, particularly under water limited conditions (Turner 1986; Uma 1987; Martin & Thortenson 1988). Reviews of the literature often concluded that the exploitable variations in transpiration efficiency (*TE*) among cultivars within a species is small and the potential for improvement by breeding is limited (Fischer and Turner, 1978); Fischer, 1981; Tanner and Sinclair, 1983).

Significant genotypic variations in WUE (upto 60%) between different groundnut genotypes have been reported in glass house and field experimental studies (Hubick

et al.,1986; Wright *et al.*,1988; Nageswara Rao *et. al*, 1993).

Variation in *TE* among cultivars was largely due to differences in biomass rather than to differences in water use. This result suggests that photosynthetic capacity, rather than leaf stomatal conductance, dominates the *TE* response in groundnut cultivars. Similar groundnut cultivar differences in *TE* have been reported in the field by Mathews *et al.* (1988a). In their study, cv. Kadiri-3 had the highest (2.17 g/kg) and cv. EC 76446(292) the lowest *TE* (1.71 g/kg). It is clear that considerable scope exists to improve *TE* and ultimately pod yield under water-limited conditions by selection for this trait in breeding programmes.

Sensitivity of leaf area expansion rate to water deficit is one of the mechanism for reducing water loss (Kowal & Kassam 1978), Turk & Hall 1980; Muchow 1985a). Leaf area development appears to be more sensitive to water deficit than either leaf senescence or leaf photosynthesis (Turner 1986a). For example, leaf expansion rate of soybean was significantly reduced when leaf water potential (LWP) decreased below –1.0 to –1.2 MPa whereas, leaf senescence and shedding occurred only when minimum LWP fell below –2.0 MPa (Constable & Hearn 1978).

Stomatal closure provides another mechanism for reducing water loss. Stomata of crop plants are sensitive to vapor pressure deficit which is an important mechanism for maximizing TE (Farquhar 1978). By reducing stomatal conductance during periods of maximum daily evaporative demand without a

significant reduction in total daily photosynthesis, WUE of the crop will be increased (Schulze & Hall 1982; Davies 1986). For example, partial stomatal closure of cowpea which was subjected to drought resulted in improved WUE (Hall & Schulze 1980). Reduced stomatal aperture can increase TE when the plant is subjected to moderate levels of water stress. The rate of photosynthesis is reduced proportionately less than the transpiration (Bradford *et al.*,1983; Morrison 1985).

HARVEST INDEX:

Attempts have been made to relate harvest index to the timing and severity of water stress in order to improve the prediction of ET by pod yield relationships (Slabbers *et al.*,1970; Stewart *et al.*,1977). Kanemasu (1983) reported that ET/pod yield relationships are not unique because of the complex interactions between development, assimilate partitioning and environment, and considers it is doubtful that an ET/pod yield relationship can be extended to climatically diverse regions.

Several models have been developed to explain dry matter production from climate and crop variables such as evapotranspiration and transpiration in a range crops (de Wit 1958; Arkley 1963; Bierhuizen and Slateyer 1965, Tanner and Sinclair 1983).

Molecular basis of drought resistance in groundnut:

Tolerance of dehydration is considered to arise at the molecular level depending on the ability of cell membranes to maintain integrity so that the critical metabolic activities are not inhibited due to stress (Gaff, 1980), and

physiological phenomenon such as osmotic adjustment (Hsiao *et al.*,1984), plant growth regulation (Levitt *et al.*,1980). Accumulation of osmoticums as a result of dehydration was known to maintain cell turgor, stomatal activity and photosynthesis at low leaf water potential (Turner *et al.*,1978; Ackerson,1983; Wright *et al*, 1983; Ludlow *et al.*,1985). Osmoregulation was implicated with maintenance of root growth, thus allowing greater exploration of soil by roots at low soil water potential (Sharp & Davies 1979; Hsiao *et al.*,1984). Yields were higher in those genotypes that had greater osmotically adjusted under water stress conditions compared to those that do not (Morgan 1983; Wright *et al.*,1983). The degree of osmotic adjustment varied with species and genotypes and with pattern of drought stress (Turner & Jones, 1980; Shackel & Hall, 1983; Morgan & Condon 1986; Flower & Ludlow 1987; Anderson & Aremu 1991).

2.2 SALINITY STRESS:

Salinity is a major factor limiting agricultural production in large areas worldwide. are affected by salinity. About 60 million ha of riceland in south and south east Asia are rendered non-arable by soil salinity (Akbar and Ponnamperuma, 1980). In India 15% of soils are affected by salinity, which limits production of crops such as chick pea and pigeon pea. Successful crop production on these soils depends on the possibility to overcome this problem is to change the optimum mix of genetic and soil amendment practices to alleviate soil toxicity. The salinity affect crop growth by creating osmotic imbalance in the cell. Sodium chloride influences membrane functions and induces ultrastructural changes in membrane. The maintenance of osmotic pressure inside the cell by

the accumulation of solutes has been documented as an adaptive mechanism to salinity stress. The principle role of osmotic adjustment, which can reduce some of the negative effects of water deficit, is to facilitate the maintenance of turgor (Morgan, 1984). Although management remains the most feasible means of improving crop yields on salt affected soils, there is a scope for genetic enhancement of salt tolerance in particular crops (Epstein, 1985; Epstein & Rains, 1987). To achieve an integrated approach towards economic utilization of saline soils, the traditional approach of drainage and reclamation should be supplemented with genetic improvements in salinity tolerance of crop plants (Epstein and Rains, 1987). However, this knowledge about the control of the physiological mechanisms involved in salinity tolerance, is essential for an efficient breeding strategy for improvement of salinity tolerance in crop plants (Tal, 1985). Attempts were made to assess the extent of genotypic diversity for salinity tolerance in food legumes such as chickpea and pigeonpea, (Chauhan, 1987) and cereal crops (Akbar, 1986, Flowers and Yeo 1981, Senadhira, 1987, Yeo and Flowers 1983). However, the heterogenous nature of saline soils presents a major factor confounding genotypic differences in the field (Richards, 1985).

Although, developing salt tolerant genotypes appeared to be practical and feasible approach, the salinity problem is a complex issue and it appears that no single process can account for this variation in the plants' response to salinity (Yeo *et al.*,1990). Thus knowledge about morphological, physiological and biochemical basis for salinity tolerance is essential to ensure better plant and crop development to this abiotic stress.

As discussed earlier, accumulation of salt in the soil poses a big threat to irrigated agricultural lands and the costs of engineering technologies, chemical treatments of overcoming salinity are economically impractical. Thus, with increasing demand for food, crop productivity in saline environment is envisioned to come from genetic modifications rather than environmental modifications.

2.3 HIGH TEMPERATURE STRESS

The frequency of extreme weather has been projected to increase in future due to global climatic change (Schneider, 1989) and this change coupled with increase in CO_2 concentrations may substantially increase the need for heat or cold tolerant genotypes all over the world (Hall, 1992).

In semi-arid environments, seasonal temperature often exceeds the optimum (30^0 C) for growth and high temperature during reproductive development of crops presents a major factor affecting crop production (McWilliam an Dillon, 1987).

High temperature is one of the major abiotic constraints in the adaptation of legumes in semi arid tropics. High temperatures occurring along with water deficits accentuate damaging effects of drought. Therefore, improvement in heat tolerance is considered vital to enhance the yield in many regions and cropping systems.

Thus, enhancement of heat tolerance in crop provides a scope for extending legume cultivation to previously unsuitable regions and seasons. For instance, development of heat tolerant pigeonpea enables its sowing early in

summer, thereby allowing timely sowing of wheat and leading to high yields in Pigeonpea - wheat rotation in north western India. Davies *et al.*,(1985) have reviewed the yield response of pea to heat stress. Mean maximum temperatures of 20 ^{0} C - 21^0 C appear optimum for pea yields and the stage most sensitive to heat stress is from 5-10 days after bloom.

Dreyer (1980) studied 5 different fruiting zone temperatures in groundnut and concluded that peg numbers and pod numbers increased linearly to harvest at 23 ^{0} C than that of 27, 30, 34, 37 ^{0} C because of slower pod growth rates and which resulted in higher pod and kernel yields.

High temperatures (>30°) limit growth and adaptation of legumes in many countries (Ketring 1984, Wery *et al.*,1994). Information is not mich available on the response of groundnut to high temperature (Ketring, 1984; Srinivasan *et al.*,1996). Experiments conducted at ICRISAT have shown that the base temperature (t_b) for germination range from 9-13^0 C for groundnut and the rate of germination increased lineally with increase in temperature upto 29^0 C. The optimum temperature range for germination was 29-30^0 C above which the germination rate reduced lineally. Optimum temperature range for growth stage is not clear from the literature. But the unpublished data indicate that temperature above 32^0 C might start damaging effect on crop growth and development.

Heat stress is, therefore, a major cause for the unstable and low seed yields (~1 T ha^{-1}) that are far below the potential yields of 7 – 8 T ha^{-1} in groundnut and soybean and 4 T ha $^{-1}$ in chickpea and pigeon pea.

Heat stress affects seedling growth since the portion of the stem close to the soil surface come in direct contact with wet soil thus causing damage in

seedling. Heat stress at flowering results in severe drop of reproductive structures in groundnut (Sutcliffe, 1977). Under severe water stress even the leaves can be damaged by high temperatures.

Crop species differ in their optimum temperature, for example, germination of chick pea and pea decreased when the temperature was >=35°C, that of lentil was impaired at > 30^0 C, and that of Faba bean at > 20^0 C. The temperature during germination affects germination rate as well as time for germination (Ellis *et al*, 1985). Covell *et al*, (1986) have shown that temperatures above 33 ^{0} C reduce the rate of germination in chick pea and lentil respectively.

The influence of temperature on groundnut is complex and disparity exists in the literature on peanut response to temperatures (Ketring 1984; Sanders *et al.*,1985). Optimum air temperature for vegetative growth of peanut plants under controlled environment have been reported to be 26^0 C (Cox 1979) to 31^0 C (Bagnall and King 1991). Similarly variable temperature optima are reported by various workers for different reproductive growth phases (flowering, pegging, pod formation and kernel growth). Previous studies have established that reproductive growth is more sensitive than vegetative in various crops including many grain legumes. Warragg and Hall (1983) reported that high temperature 6 days before anthesis causes male sterility and excessive flower abscission in cowpea.

Alarkon *et al.*,(1979) showed positive correlation exist between the fertility of pollen and proline content in pollen. It was speculated that proline acted as adaptive mechanism to protect pollen (Zhang and Croes 1983) and

several plant enzymes (Paleg *et al.*,1981) from heat injury. Mutters *et al.*,(1989) suggested that heat injury during floral development of sensitive cowpea genotypes might be attributed to inhibition of proline translocation from anther wall to pollen. Similar type of results have been reported in maize anthers (Palfi *et al.*,1981) and Tomato (Kuo *et al.*,1986) plants.

Limited efforts have been made in breeding heat tolerant legumes, perhaps because yield losses due to heat were not qualified and the damaging effects of heat remain more subtle than those due to disease or insect infestations (Summerfield *et al.*,1990). Plant responses to heat stress are diverse, and include cessation of cytoplasmic streaming (Alexandrov, 1964), Protein denaturation (Bernslam, 1978), changes in lipid composition (Suss and Yordanov, 1986), reduction in membrane stability (Shen & Li, 1982) and efficiency of photosynthesis (Bar-Tsur *et al.*,1985). The relative importance of each can vary with species. However, membrane dysfunction is a physiological process disturbed mostly by heat stress (Levitt, 1980, Quinn, 1989). Heat stress results in a disruption of membrane integrity leading to leakage of electrolytes, reduction in photosynthetic or mitochondrial activity, and the ability of plasmalemma to retain solutes and water (Lin *et al.*,1985). The electrolyte leakage test was used to examine variation for heat tolerance in common bean (Schaff *et al.*,1987) and soybean (Sapra & Anaek, 1991) but the relative tolerance of legumes under uniform growing conditions has not been assessed. It is well known that electron transfer from photosystem II (PS II) is extremely heat sensitive. Measurements of chlorophyll fluorescence has been used to quantify inhibition or damage to electron transfer (Baker *et al.*,1989) thus as a tool to assess heat tolerance of several crops (Chauhan and Senboku T.,1997)

The mechanism of injury due to high temperature stress have been described by Sutcliffe, 1977; Lawlor, 1979. Injury may occur indirectly if heat causes desiccation when transpiration rates increase. High temperatures may cause injury to plant metabolism by either directly (by desiccation) or by inhibiting a set of metabolic activities or sequence of enzyme reactions or by changing the balance between the components of a given system (Lawlor, 1979). Temperature of 35^0 C and above can result in rise of rates of photo-respiration and dark respiration rates in several crop plants, causing a rapid loss of assimilate reserves which leads to 'thermal death'. High temperature can also impair protein metabolism by affecting rate of protein synthesis due to a reduction in the rate of ATP production. High temperature effects on the structural integrity of proteins in cytoplasm and membrane protein denaturation has been shown and aggregation (Levitt, 1969).

Stress shock proteins :

At the molecular level, one of the most extensively characterized stress responses in higher plants is the synthesis of stress shock proteins (SSPs). These proteins are synthesized under a variety of stresses such as high temperature (Lindquist and Craig, 1988), desiccation (Chandler *et al.*, 1988), salinity (Singh *et al.*, 1985); Ramagopal, 1987; Esaka *et al.*, 1992), heavy metals (Lin Roberts and Key, 1984; Howarth, 1990), chilling (Tseng and Li, 1991) and anoxia (Czarnecka *et al.*, 1984). Many of these proteins are suggested to protect the cell against the adverse effects of stress. The significance and relevance of these stress proteins has been well characterized in several studies (Lin *et al.*, 1984; Bray, 1988; Krishnan, Nguyen and Burke, 1989). These proteins are shown to be synthesized when the organism is exposed to a mild non-lethal level of stress

often referred to as an induction stress. The ability of induced systems to tolerate severe levels of stress signifies the importance of stress proteins (Lin *et al.*, 1984;; Krishnan *et al.*, 1989; Vierling, 1991). Thermosensitive mutants that do not synthesize stress proteins when subjected to mild stress do not survive severe stress (McAlister and Finkelstein, 1980). Information on differential synthesis of stress proteins in genotypes differing in stress tolerance is however inconclusive (Fender and O'Connell, 1989; Krishnan *et al.*, 1989; Ristic, Gifford and Cass, 1991; Vierling and Nguyen, 1992).

In recent years, several workers have addressed the underlying mechanism of induction to these proteins by various stresses (Marcotte, Russel and Quatrano, 1989; Guiltinan, Marcorre and Quantrano, 1990; Skriver and Mundy, 1990; Gurley and Key, 1991; Hetherington and Quatrano, 191; Bray, 1993). In contrast to those induced by heat stress, the stress proteins synthesized due to desiccation, salinity and cold stress have been shown to be mediated by turgor-dependent gene expression (Bray, 1993).

Ashwani *et al.*, 1997 gave a detailed report on few salt regulated proteins including osmotin, late embryogenesis abundant (LEA) proteins; 16 kDa responsive to ABA (RAB) protein as well as dehydrins are covered in this chapter. Apart from these, protein responsive to dehydration 29 (RD 29), heat shock proteins of 70 and 90 kDa (HSP 70 and HSP 90) and 104 kDa stress associated protein (SAP 104) which represent some of the other examples of salt-induced proteins are as yet only partially characterized.

As discussed earlier, the productivity of plants is greatly affected by environmental stresses, therefore the genetic improvement of abiotic stress tolerance poses an important challenge to agricultural scientists.

Glycine betaine and abiotic stress tolerance:

Plants accumulate a variety of low molecular weight solutes as an adaptive mechanism which enables them to tolerate different stresses. Many prokaryotes and eukaryotes, including higher plants, accumulate low molecular weight organic solutes like glycinebetaine (N, N, N Trimethyl glycine), sorbitol, or proline, in response to environmental stresses (Kemble and McPherson 1954, Singh

et al.,1972, Storey and Wyn Jones 1975, Ahmad *et al.*,1979). It was postulated that the accumulation of the organic solutes as compatible cytoplasmic osmotica play an important adaptive value in several plant species (Stewart and Lee 1974; Wyn Jones *et al.* 1977). In this regard, the compatible solutes have been shown to protect to integrity of enzymes (Pollard and Wyn Jones 1979) and membranes (Jolivet *et al.*,1982) and to protect against free-radical-induced damage of (Smirnoff and Cumbes 1989) "in vitro" studies. The beneficial effects of accumulation of organic solutes has been demonstrated in various abiotic stresses such as high temperature (Paleg *et al.* 1981, Storey and Wyn Jones 1979, Shomer-Ilan and Waisel 1986)), salinity, cold stress (Shirahashi *et al.* 1978).

Beneficial effects of betaines (N methyl amino acids) in conferring resistance to drought, salinity, high and low temperatures have been demonstrated in a number of crop species (Wyn Jones and Storey, 1981; Zao *et.al.*, 1992, Naidu *et al.*,1996). Accumulation of organic acids such as proline

and glycine betaine, and their role under various abiotic stress conditions have been described in earlier studies (Ford 1984; Thomas *et al.*,1992; Vernon & Bohnert, 1992; Delauney & Verma 1993; Hanson *et al*, 1994). Accumulation of these compounds has been implicated with resistance of plants to various abiotic stresses (McCue & Hanson 1990).

Higher plants synthesize glycine betaine in chloroplasts via the pathway :

choline → betaine aldehyde → glycine betaine (Rhodes and Hanson 1993). The first step is catalyzed by choline monoxygenase (Brouquisse *et al.*,1989), the second by betaine aldehyde dehydrogenase (BADH) (Weigel *et al.*,1986). A survey conducted by Poljakoff-Mayber *et al.* (1987) reported high levels of proline analogues in ***Melaleuca*** species and trigonelline (*T*) in ***Zygophyllum aurantiacum***. These quaternary ammonium compounds are accumulated in the plants under water stress and salinity (Naidu *et al.* 1986).

There have been limited studies to examine external application of betaine on use of chemical compounds to alleviate tolerance to abiotic stresses in crops. The foliar application of glycinebetaine on potato (***Solanum tuberosum L.***) and Tomato (***Lycopersicon esculentum Mill.***) resulted in indicating possible role of betaines in alleviating damaging effects of droughts. Glycine betaine applied foliarly at 6 kg ha^{-1} could increase the grain yield by 18 %, dry matter content by 30% and number of grains/sq. m by 20% (Agboma *et al.*,1997). In a green house study the foliar application of glycinebetaine on tobacco (a non betaine accumulating model crop) significantly increased leaf area and leaf dry weight (Agboma *et.al.*,1996). The benefits of glycine betaine have been demonstrated under "in vitro" conditions on isolated enzymes (Paleg *et.al.*, 1985) and on whole

plants (Zao *et al.*,1992). External application of glycine betaine on cotton enhanced seedling vigour, germination and yield in cotton (Naidu *et al.*,1995).

Muthukumaraswamy and Paneerselvam (1997) observed that application of "triadimefon" a fungicide on groundnut growing under salinity stress, (genotype VRI-2), resulted in an increase in proline and glycinebetaine content. This study indicates that groundnut is able to accumulate glycinebetaine.

Under salinity stress conditions an accumulating metabolite would replace other compounds within the cell, thus sequestering sodium or other toxic compounds into the vacuole. For example, when mistletoe a parasite when it taps into the host phloem, polyol accumulation provides the parasite with a high osmotic pressure (Richter & Popp 1992). Metabolites accumulating during osmotic adjustment are compatible and non-inhibitory to cellular metabolism and their osmotic regulatory role might be exerted at high or moderately high concentrations. Osmoprotectants were known to act at even low concentrations by protecting specific structures or enzymatic processes, by exerting regulatory effects on ion or water uptake or transport, or by stabilizing multi-subunit enzyme complexes or membranes (Smirnoff & Cumbes 1989; Sommer *et al.*,1990; Smirnoff 1993).

Several workers had demonstrated that betaines and their sulfanio analogs can play important role in osmotic adjustment and/or osmoprotection in bacteria (Csonka and Hanson, 1991), cyanobacteria (Borowitke, 1986), marine algae (Blunden and Gordon, 1986) and mammals (Garcia – Perez and Burg, 1991). Yancey *et al*, 1982, and Robinson and Jones, 1986 reviewed the role of betaines and their sulfanio analogs as compatible solutes.

However, many higher plants do not accumulate glycine betaine and this has led to interest in the metabolic engineering of the glycine betaine biosynthesis pathway as an approach for enhancing stress resistance (Lerudulier *et al.*,1984; Mccue and Hanson, 1990).

Rathinasabapathi *et al.*,1994 reported that tobacco transgenic plants could convert externally supplied betaine aldehyde to glycine betaine at high rates, demonstrating that they were able to transport betaine aldehyde across both the plasma membrane and the chloroplast envelope. The glycine betaine produced in this way was not further metabolized and reached concentrations similar to those in plants which accumulate glycine betaine naturally. Betaine aldehyde was toxic to non-transformed tobacco tissues whereas, transgenic plants were able to resistant the toxicity by converting of betaine aldehyde to glycine betaine. Thus, betaine aldehyde dehydrogenase is of interest as a potential selectable marker, as well as in the metabolic engineering of osmoprotectant biosynthesis.

The accumulation of betaine in plants under abiotic stress conditions has been proposed to play an important role (Hanson, 1980, Wyn Jones, 1984; Yancey *et al.*,1982). It appears that betaine functions as a compatible or protective solute in the cytoplasm and/or in chloroplasts (Incharoensakdi *et al.*,1986; Maton *et al.*,1987; Robinson & Jones, 1986).

Unlike proline, which is a bi-product of stress metabolism betaine is an inert end product of metabolism (Hanson & Hitz, 1982; McCue & Hanson, 1990), thus betaine levels in the plant is dependent on the rate of its synthesis and the rate of dilution by growth (Hasegawa *et al.*,1994). In barley Ladyman *et al.*,(1983) and Grumet *et al.*,(1985) demonstrated genotypic variation

associated with the accumulation of betaine and its genetic control under water, salinity or low temperature stress condition. In this study, significant differences in levels of betaine among genotypes were observed. It has also been shown that betaine accumulation was a nucleus-encoded, with significantly high narrow-sense high heretability. Also been suggested in this study was a possible role of betaine in cold acclimation, protection against freezing injury (Kishitani *et al.*,1994).

However, high betaine isopopulations which concomitantly maintain a more negative solute potential than low betaine isopopulations exhibit a growth and yield disadvantage, resulting in a reduction in yield potential (Grumet *et al.*,1987). In maize, preliminary genetic studies have indicated that lack of accumulation of betaine under stress in certain inbreds is caused by a single encoded homozygous recessive gene (Rhodes and Rich, 1988). However, unlike barley, little is known concerning the betaine yield potential and yield stability (in drought prone environments) of genotypes differing with respect to capacity to accumulate glycine betaine.

The occurrence of significant genotypic variations of glycine betaine levels in grasses, and the report of a six-seven fold accumulation of glycine betaine in salt stressed sorghum (Grieve and Maas, 1984) has prompted preliminary studies of betaine levels in a range of sorghum cultivars grown under field water deficits.

Keeping the above described literature in view, the present study was conducted to examine the role of Betaines in alleviation of major abiotic stresses in groundnut.

Materials and Methods

CHAPTER III

Materials and methods

3. 1 Laboratory experiments : Glycine betaine and heat tolerance

Laboratory experiments were conducted to examine the influence of glycinebetaine on response of groundnut to high temperature and salinity stresses.

About 160 sound mature seeds of ICG 476 and TAG24 genotypes of groundnut were imbibed in either distilled water (B_0) or 25, 50, 100mM glycine betaine (B_{25}, B_{50}, B_{100}) for 12 hrs and then the seeds were transferred into petriplates to allow germination for 40 hours at 30^0C and 75% relative humidity.. From each of the treatments 3 sub treatments i. e., heat induction (HI), no induction (NI) and control (C) were created (Fig 3. 1. 1). Each of the sub treatments had 20 seeds / petriplate and there were 3 replications. Root and shoot lengths were recorded at 40 hrs after germination before imposing the temperature stress treatments. In the HI treatment the germinating seeds were subjected to increasing levels of temperatures in the order of 35^0C (1hr), 40^0C (2hrs) and 45^0C (1hr) in NI and C sub treatments, the germinating seeds were maintained at 30^0C for 4 hrs. At the end of 4^{th} hour the HI and NI sub treatments were subjected to a lethal stress of 50^0C for 2 hrs following which the seedlings were returned to 30^0C. The recovery growth of seedlings was observed at the end of 72 hours period. The C sub treatment was maintained at 30^0 C all along (Fig. 3.1.1). At the end of 72 hours, the root and shoot length of seedlings were recorded in all the sub treatments. The data was analysed using a split split plot design with 2 genotypes as main treatments, induction treatments (HI, NI and C) as sub treatments and betaine levels (0,25,50,100 mM) as sub sub treatments.

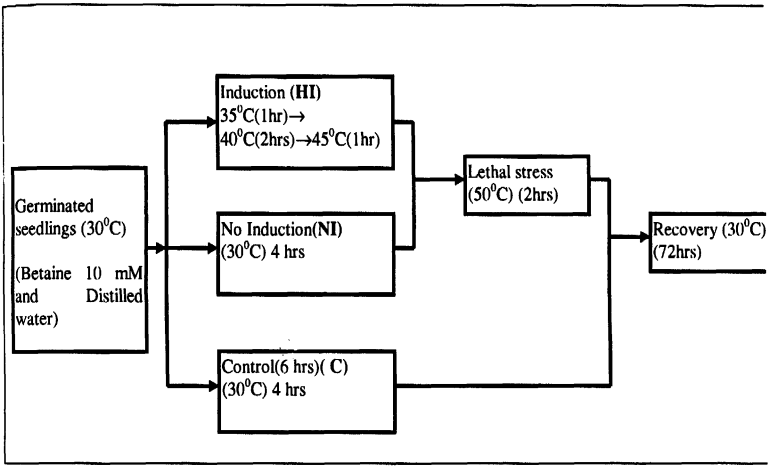


Fig : 3.1.1. Protocol followed to examine the influence of glycine betaine on response of groundnut genotypes (ICG 476 and TAG24) to high temperature stress.

3. 1. 1 Separation of proteins on Sodium Dodecyl Sulphate(SDS) - Poly Acrylamide Gel Electrophoresis (PAGE)

Known weight of tissue was sampled and frozen in liquid nitrogen. The tissue was ground in 1:4 (tissue weight : buffer volume) extraction buffer and the extract was centrifuged at 12,000g for 10 min at 4° C, and 100 µl of supernatant was used for protein analysis.

Reagents used in the extraction buffer were Tris buffer 8.0 pH (Tris 50 mM, NaCl 50 mM, EDTA 2 mM, 2- mercaptoethanol 5 mM, PMSF I mM, PVPP - 0.5%).

Protein was quantified by using the method as described by Brad Ford (1976) (Bradford dye binding technique).

A 100 µl of extracted aliquot was taken in to test tubes and 3 ml of Coomosie brilliant blue (CBB) reagent was added. After 5 minutes of adding the reagent, the absorbance was measured at 595 nm. Standard curve of protein is developed using a range of concentrations of using Bovine Serum albumin (BSA).

Reagents of CBB DYE : 10 mg of CBB-G-250 is dissolved in 5 ml of methanol, 10 ml of 80% ortho phosphoric acid is added and mixed well, the volume is added to 100 ml using distilled water and filtered to remove undissolved material.

The proteins were concentrated by trichloro acetic acid (TCA) precipitation. Known volume of the extract was taken in a centrifuge tube. TCA (100%) was added equal to 1/10 volume of extract and kept on ice for 1 hour and then centrifuged at 12,000g for 10 minutes, supernatant was then discarded and chilled acetone was added and centrifuged again at 12,000g for 10 min and acetone is decanted and the traces of acetone were removed by drying.

Sample containing 100 µl of total protein was dissolved in sample buffer containing 50 mM Tris - Hcl (pH 6.8), 1% (v/v) SDS, 2% (v/v) 2- mercaptoethanol, 12.5% Glycerol and 0.05% stracking dye. The protein samples were denatured in boiling water for 4 min. After cooling, 100µg of protein is used for loading into the wells.

Gels containing 12. 5 % resolving gel and 3 % Stacking gel were prepared from acrylamide stock containing bis. The Composition of 30 ml resolving gel was 12.5 ml of 30 % Acrylamide with bis, 0.3 ml of 10 % SDS, 7.5 ml of 1.5 M Tris HCl buffer (P^H 8.8) , 9.6 ml of water, 0.1 ml of 10 % Ammonium Per Sulphate. The contents were degassed for 2 min. The gels were chemically polymerised by the addition of 0.025 % TEMED by volume. The mixture was poured in gel moulds overlaid with water and was left undisturbed for an hour to get satisfactory polymerisation.

The stacking gel contained 1.67 ml of stock Acrylamide (30 %) with Bis, 1.25 ml 0.5 M Tris Hcl Buffer (P^H 6.8), 0.1 ml of 10 % SDS, 0.05 ml of 10 % Ammonium per sulphate and 6.9 ml of water. The gel was exactly polymerised like resolving gel after the addition of 0.025 % of TEMED. The combs were inserted on top of the resolving gel after removing the layer of water. Stacking gel was poured over resolving gel and left undisturbed for about half an hour. Then combs were removed and sample was loaded into the wells along with a standard mixture. Electrophoresis was carried out using LKB 2001 Vertical unit for 2 X 1.5 mm gels at a constant current of 60 milliamperes, until the bromophenol blue marker reached the bottom of the gel (approximately 5 Hrs). Gels were removed and fixed in 10% Acetic acid for 10 - 15 min. and stained overnight with 1 % Coomosie Brilliant blue dye and destained by repeated

washing with 7 % Acetic acid in 50 % Methanol. The gels were scored and the differences in protein banding patterns were noted.

3. 2 Laboratory experiment 2 (salinity stress):

About 160 sound mature seeds of ICG 476 and TAG24 genotypes of groundnut were imbibed in either distilled water (B_0) and 25, 50, 100mM glycine betaine (B_{25}, B_{50}, B_{100}) for 12 hrs and then the seeds were transferred into petriplates to allow germination for 40 hours at 30^0C and 75% relative humidity.. From each of the treatments 3 sub treatments i. e., Salinity induction (SI), no induction (NI) and control (C) were created (Fig 3. 2. 1). Each of the sub treatments had 20 seeds / petriplate and there were 3 replications. Root and shoot lengths were recorded at 40 hrs after germination before imposing the salinity stress treatments. In the SI treatment the germinating seeds were given a salinity induction at 150 mM NaCl (16 hours). In NI and C sub treatments, the germinating seedlings were maintained at 30^0C for 16 hours in distilled water. At the end of 16^{th} hour the SI and NI sub treatments were subjected to a lethal salinity stress of 300 mM for 48 hours following which the seedlings were returned to distilled water. The recovery growth of seedlings was observed at the end of 72 hours period. The C sub treatment was maintained in distilled water all along (Fig. 3.2.1). At the end of 72 hours, the root and shoot length of seedlings were recorded in all the sub treatments. The data was analysed using a split split plot design with 2 genotypes as main treatments, induction treatments (SI, NI and C) as sub treatments and betaine levels (0,25,50,100 mM) as sub sub treatments.

3. 2. 1 Separation of proteins on SDS - PAGE

The method followed was same as described in 3. 1. 1

3. 3 Glass house experiment 1 : Glycine betaine and salinity stress.

The seeds of the ICG 476, TAG 24 and CSMG 84-1 genotypes were imbibed in glycine betaine (10mM) and distilled water as control for 12 hrs. Before planting, the seeds were treated with captan and thiram to prevent seedling diseases. The seeds were

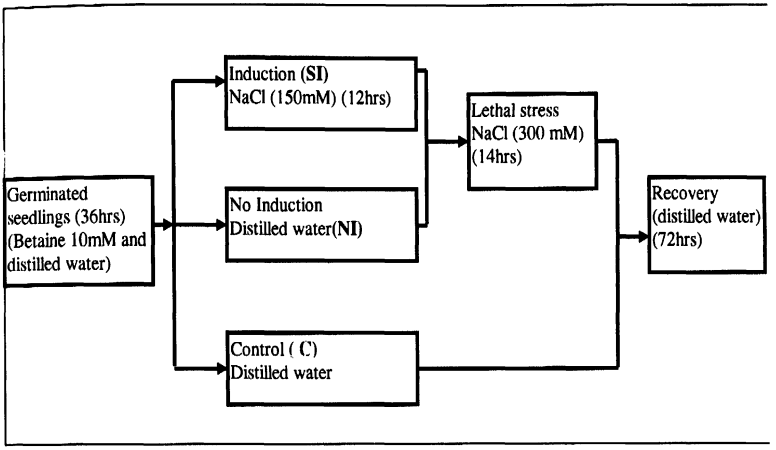


Fig : 3.2.1 Protocol to examine the influence of glycine betaine on response of groundnut genotypes (ICG 476 and TAG24) to salinity stress.

sown on 18th August '97 in 180 mm diameter plastic pots filled with acid washed and sterilised river sand. The soil surface was covered with gravel to minimise soil evaporation. The pots were randomised in three blocks containing 30 pots each. The pots were placed on bench tops in glass house. Temperatures during the experiment were around 28 / 22 ^{O}C (day / night) with daily mean relative humidity of 60 - 70 %.

3. 3. 1 Treatments

Three salinity levels i.e., 0, 6 and 8 ds m^{-1} were imposed from 15 days after sowing (DAS) using modified Hoagland solution as described below

A modified Arnon and Hoagland nutrient solution of 0.5 strength with 1.79 mM NH_4NO_3 amended with Nacl + $Cacl_2$ (1 : 1 w / w) was used to simulate the five different salinity treatments. The composition of the nutrient solution in mM was: 0.23 KH_2PO_4, 0.52 KCl, 0.25 $MgSO_4$, 0.37 $CaCl_2$, 0.0015 $MnSO_4$, 0.00023 $ZnSO_4$, 0.00025 $CuSO_4$, 0.001 H_3BO_3, 0.00005 Na_2MoO_4, and 0.04 NaFe EDTA . The electrolytic conductivity (EC) of the nutrient solution without salt treatment was 0.15 ds m^{-1}. Plants were irrigated with deionised water upto 15 DAS. Salinity treatments were imposed by irrigating pots with 1 litre of treatment solution on 15th DAS, following this the salinity treatments were maintained by irrigating the pots with 250 ml of treatment solutions at 4 day intervals. For the "0" Salinity treatment, 0.25 strength nutrient solution without salt amendment was used for all flushing operations. Pots were randomised every week to minimise spatial effects in the glass house, and the experiment was terminated at 30 DAS.

3. 3. 2 Observations and Measurements.

Growth Analysis

Three plants were sampled from each treatment for growth analysis at 30 and 60 DAS, root and main stem lengths were measured and the plants were separated into component parts as described in the Fig 3.3.2, and the plants were transferred to polyethylene bags and kept in a cold room at 5 ^{O}C until separation into component parts and the analysis was done.

Leaf areas were determined using an automatic leaf area meter (LICOR 3100), dry matter of the leaves, stem and root were determined after oven drying at 80^{0}C to a constant weight, various growth parameters were calculated as follows (Beadle 1993):

- Root Growth Rate (RtGR) (g plant^{-1}day^{-1}) = (In W_2 - InW_1) / (T_2 - T_1)

Where W_2 and W_1 are dry weights of the root at 30 and 15 DAS respectively, and T_2 and T_1 are 30 and 15 DAS.

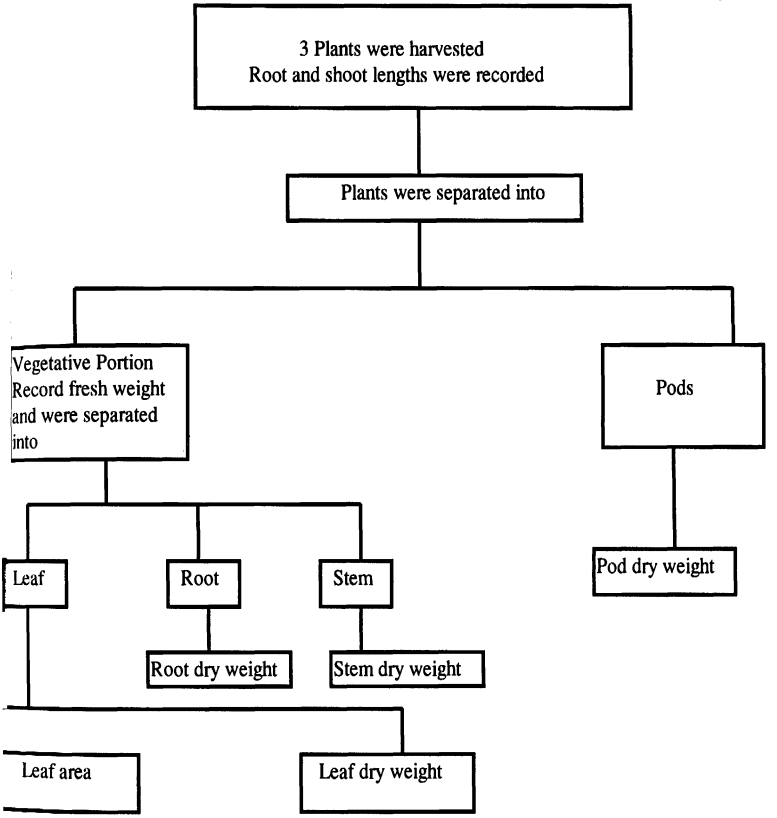
- Shoot Growth Rate (StGR) (g plant^{-1} day^{-1}) = (In W_2 - InW_1) / (T_2 - T_1)

Where W_2 and W_1 are dry weights of the shoot at 30 and 15 DAS respectively, and T_2 and T_1 are 30 and 15 DAS.

- Crop Growth Rate (CGR) (g plant^{-1} day^{-1}) = (In W_2 - InW_1) / (T_2 - T_1)

Where W_2 and W_1 are total dry matter of the plant at 30 and 15 DAS respectively, and T_2 and T_1 are 30 and 15 DAS.

- Net Assimilation Rate (NAR) (g m^{-2} day) =(W_2-W_1) / (T_2-T_1) x (In LA_2 -LA_1)/ (LA_2 - LA_1)



ig : 3.3.2 Procedure followed for analysing growth of plants sampled at each sampling ate in each treatment to know the effect of glycine betaine in the alleviation of salinity tress.

Where W_2 and W_1 are total dry matter of the plant at 30 and 15 DAS respectively, and T_2 and T_1 are 30 and 15 DAS and LA_2 and LA_1 are the leaf areas at 30 and 15DAS.

- Specific Leaf Area (SLA) ($cm^2\ g^{-1}$) = leaf area / leaf dry weight

Gas exchange measurements

The measurements of gas exchanges were measured at weekly intervals along with water relation measurements in order to interpret results in a cohesive manner. Measurements for gas exchange and water relations were made from 11:00 to 13:00 hrs in 3 leaves per plot. Gas exchange measuremnts were made using a LCA4 (Leaf chamber analyer). Second or third fully expanded leaf from the apex on the main axis was used for the measurement. LCA4 provides an on-spot measurement of stomatal conductance, photosynthesis, leaf temperature and transpiration in addition to incoming photosynthetically active radiation (PAR) at the time of measurement .

Relative leaf water content

The 2nd or 3rd leaf from the apex on the main axis was sampled from 2-3 plants /plot and placed in zip lock bags in an ice box. Fresh weight of sampled leaves was determined within 15min of excision in the laboratory first and then turgid weight was obtained. Leaves were kept in distilled water for 6-8 hrs at room temperature. After soaking, leaves were quickly and carefully dried with tissue paper prior to determining turgid weight . Dry weight was obtained after oven drying the leaf samples to a constant weight . The RWC was calculated by the equation:

RWC % = (fresh weight-dry weight) / (Turgid weight-dry weight) X 100

Osmotic potential

The 2 nd or 3 rd leaf from the apex on the main axis were sampled to measure the osmotis potentials (OP). Samples were placed in polyethylene bags and dipped in liquid nitrogen and transferred to a deep freezer (-40° C) until further processing. At the time of measurement the leaf samples were removed from the freezer and allowed to thaw for 2 min. The samples were placed in a 1.5 ml 'eppendorf' tubes and centrifuged tube for 5 minutes at 12,000g to extract the cell sap. The OP of the cell sap was determined using an automatic micro-osmometer (Roebling Automatic Freezing Point Osmometer by Cryo Scopic Method). The osmometer was calibrated before each set of measurements with a standard solution of 300 milli osmoles and distilled water to get the zero point . Cell sap of 25 µl was used to measure Osmotic potential .

Osmotic potential at full turgor (OP_{100}) was calculated according to the formula of assuming that apoplastic water content is negligible (Wilson *et al.*, 1979):

$$OP_{100} = OP \times RWC / 100$$

Osmotic adjustment (OA) was calculated as the difference between the OP_{100} of stressed and non-stressed, betaine treated and not treated leaves.

$$OA = OP_{100} (C) - OP_{100} (T)$$

Where C was control and T was treatment.

Total betaine content

The leaf samples were collected and were frozen in liquid nitrogen and kept in a freezer (-80^{O}C) . The frozen samples were lyophilised to a dry powder in a Lyophiliser (Vertis company ltd., New York) and stored until further processing. Total betaine content was measured calorimetrically according to the method of Wynjones & Storey (1976)

The tissue is homogenised in 10 ml of methanol /chloroform/water (12:5:3) extraction media in a large glass centrifuge tube . The tube was kept in an ice bath during extraction to counteract heat generation by the ultraturrax , since excessive heat can cause break down of the chloroform with the production of HCl . After extraction 10ml of distilled water was placed in a glass centrifuge tube and used to wash the grinding head. The resulting emulsion was added to first homogenate. The homogenate was centrifuged in a bench centrifuge at 12,000g/10'/20°C. The supernatant(MeOH / H_2O) was removed and stored for analysis of betaine by non-specific periodide method in which quaternary ammonium compounds (QACS) & betaine are precipitated at different P^H 's. The acid potassium triodide solution (for total QACS) was prepared by dissolving 7.5 g I_2 and 10 g KI in 1M HCl and filtered while the same reagents were dissolved in a 0.4 M KH_2 PO_4 - NaOH buffer pH (8.0) provided the alkaline reagent will determine betaine. Precisely 0.2 ml of either acid or alkaline potassium triiodide reagent was added to the sample. The mixture was shaken and left for atleast 90 minutes in an icebath with intermittent shaking. 2ml ice-cooled H_2O was added rapidly to the mixture to reduce the absorbance of the blank. This was quickly followed by 20 ml of

1,2- dichloroethane at -10° C and the 2 layers are mixed by a constant stream of air bubbles for 5 minutes while the temperature was maintained at 4° C. The absorbance of the lower organic layer was measured at 365 nm.

The standard curve was prepared by different concentrations of glycine betaine of 10 mM to 100 mM.

Statistical analysis

Experimental data were subjected to analysis of variance as described by Gomez and Gomez (1984) and using a Genstat for windows package at ICRISAT center.

3. 4 Glass House experiment 2 : Glycine betaine and Water stress

The experiment was conducted in glass house with 3 genotypes (ICG476, TAG24, CSMG84-1). The seeds of these genotypes were imbibed with glycine betaine 10 mM and distilled water as control for 12 hrs, before planting the seeds were treated with captan and thiram to prevent seedling diseases. The seeds were sown on 29th April '98 in 180 mm diameter plastic pots filled with river sand, soil and vermiculite in the ratio of 2:1:1. A b asal dose of fertilizer (18N : 40P) was mixed on the top soil at the time of sowing. The pots were randomised with in each of the three replications and arranged on bench tops in a glass house. Temperatures during the experimental period were maintained at 28/22° C (Day/Night) and relative humidity was 60-70% (Mean Day/Night).

3. 4. 1 Treatments

Plants were adequately irrigated daily upto 30 DAS after which the following irrigation regimes were imposed.

1. 100% field capacity (I_1)

2. 50% field capacity (I_2)

At 30 DAS all pots were satuarated with water and any excess water was allowed to drain through a drain hole in the base of the pots. When water leakage stopped, the drainage holes were blocked to prevent any further seepage of water from the pots. The pots were arranged in split - split plot design with the two irrigation regimes as main treatments, 3 genotypes as sub treatments, and the betaine treatments as sub - sub treatments. There were 3 replicates for each treatment.

The initial weight of the pots before irrigation was taken (W_1) and these pots were flushed with water completely and excess water was let to drain from the holes at the bottom of the pots, and the final weight of the pot was measured (W_2), and here the pots were said to be at 100% field capacity at this point.

Water stress treatment I_1 received irrigation as such to maintain the soil at its field capacity (calculated by initial soil measurements) , the plants in I_2 received 50% of the water given to the plants in I_1 . The amount of water loss was determined by weighing the pots,1 for every treatment daily by a Mettler balance (20 kg capacity). Three pots with soil, but without plants were maintained in each treatment to monitor soil evaporation. The experiment was terminated at 60 DAS.

3. 4. 2. Observations and measurements

Growth Analysis

Three plants were sampled from each plot for growth analysis at 30 and 60 DAS and the plants were transferred to polyethylene bags and kept in a cold room at 5 ^{o}C until separation into component parts and the analysis is done as described in Fig : 3.3.2.

Gas exchange measurements

Gas exchange measurements were done as described in the chapter 3.3.2.

Relative water content

Relative water content was measured as described in the chapter 3.3.2.

Total betaine content

Total betaine content was estimated as described in the chapter 3.3.2

Leaf water potential

Leaf water potential was determined using a pressure chamber as described by Turner (1988). Second or third leaf from top of the plant was sampled and the sampled leaflet was placed in a pressure chamber (Model B, soil moisture equipment Corp., Santa Barbara, CA, USA) with the cut end of the leaf petiole protruding through a gas tight seal of the chamber. The pressure in the chamber was gradually increased until the xylem sap just began to exude out at the cut surface. This point at which water was held in the leaf cells and the first drop of xylem sap was seen was recorded as water potential of the leaf cells.

Chlorophyll flouroscence

Chlorophyll fluorescence was measured using a modulated fluorimeter (Hansatech Electronics Ltd., UK) on the abaxial surface of fully expanded leaflets. Second or third leaf from the top of the main axis was used for the measurement. The leaflets were placed in dark for 45 minutes at room temperature, after which the dark adapted leaflets were placed into a leaf clip to which modulated light probe and a detector probe were attached. The leaflets were exposed to actinic light and saturating

light pulses through the fibre optic cables connected to Bjorkman lamp (1800 μ mol m^{-2} s^{-1} Photosynthetically photon flux ; Hansatech Electronics Ltd., UK). The Fluorescence signal at 700 nm, read directly to the computer was used to calculate the initial fluorescence (Fo) and maximum fluorescence (Fm) were recorded.

Variable flouroscence Fv = Fm - Fo.

The Fv / Fm ratio is the measure of efficiency with which light is utilised for photosynthesis.

Transpiration

During the experimental period transpiration was estimated as:

$T = I - (E_s + V_w)$, where

I is Cumulative water applied during the experimental period.

E_s is Soil evaporation

V_w is unused water left in the pot at the end of the treatment period. E_s was estimated from the water loss from empty pots in the absence of plants. Water - use efficiency (WUE) (g / kg) was estimated as the ratio of dry matter produced between 30 - 60 DAS to transpiration (T) during the same period.

Statistical analysis

Experimental data were subjected to analysis of variance as described by Gomez and Gomez (1984) and using a Genstat for windows package at ICRISAT center.

3. 5 Growth chamber experiment: Effect of glycine betaine in the alleviation of high temperature stress in groundnut genotypes

The experiment was conducted in a environmentally controlled growth chamber of dimensions (0.75m (width) X 1.82m (length) X 1.4m (height))and with 2 genotypes (ICG 476, TAG 24) in a completely randomised block design.

3. 5. 1 Treatments

The seeds of the two genotypes (ICG 476 and TAG 24) were imbibed with glycine betaine 10mM (B_{10}) and distilled water as control (C) for 12 hrs. Sowing was done on 5th June '98 after treating the seeds with captan and thiram to prevent seedling diseases. The seeds were sown in plastic pots with 101 mm diameter, filled with riversand, soil and vermiculite mixed in the ratio of 2:1:1. Two sets of pots were grown in glass house upto 15DAS at 28 / 22^{0}C (day / night) with daily mean relative humidity of 60 - 70%, and the pots were shifted to growth chambers at 15DAS The pots were arranged in 3 randomized blocks (replications)in each of the 2 Growth chambers. As described in the Fig : 3.3 the first Growth Chamber was programmed to maintain the temperature at 30° C and relative humidity at 60-70% throughout the growing period which serves as control (HT_1). The second Growth Chamber was programmed to simulate the naturally occuring diurnal rhythm of the temperatures such that starting from 30^{0}C, the temperatiures would rise gradually (4^{0}C hr^{-1}) to reach 45°C by 12:00 Noon. The high temperature of 45° C was reduced gradually to reach 30^{0}C by 6:00 PM. This high temperture stress treatments were imposed from 15DAS to 45 DAS (HT_2) and the experiment was terminated at 45 DAS.

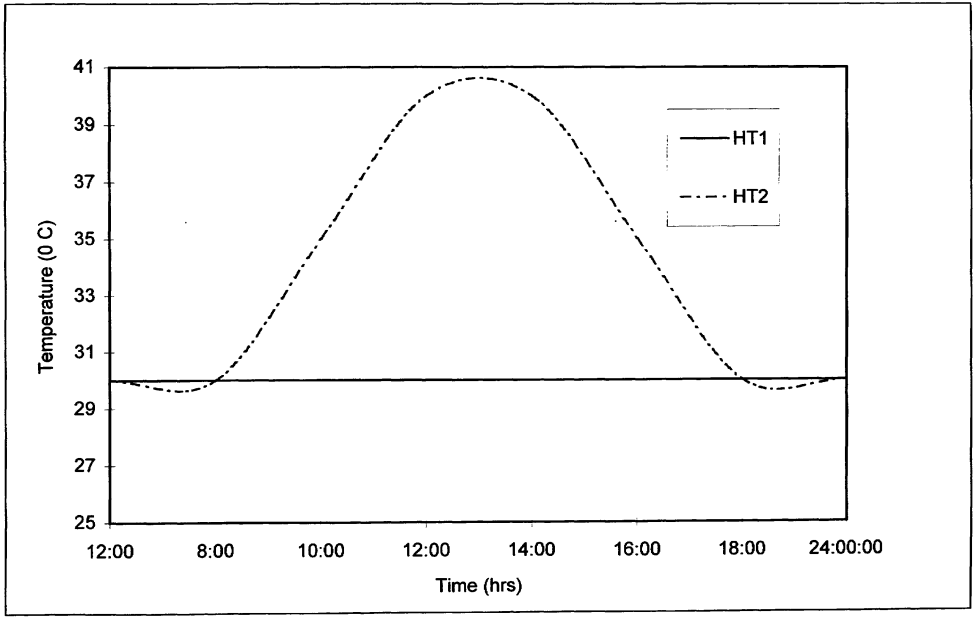


Fig : 3.5.2 Tempaerature regimes maintained in the growth chamber to study the effect of seed treatment of glycine betaine in the alleviation of high temperature stress.

3. 5. 2 Observations and measurements

Plant Growth Analysis

Three plants were sampled for growth analysis at 15 DAS and 30 DAS. Plant heights, leaf areas, root, shoot and leaf dry weights., aerial, subterranean peg number and all other growth analysis parameters were calculated by the same method as described in Fig : 3.3.2.

Other Observations

Photosynthetic rates, osmotic potentials and chlorophyll flouroscence were recorded as described in chapter 3. 3. 2 and 3. 4. 2.

3. 6 Field experiment :

A field experiment was conducted at ICRISAT center, Patancheru, near Hyderabad, Andhra pradesh, INDIA during the rainy season 1996 (Field experiment 1) to investigate role of betaines in the alleviation of drought stress in groundnut.

3. 6. 1 Crop management

Experimental block was disc ploughed to attain a fine tilth and a basal dose of 100 kg ha^{-1} Di ammonium Phosphate (DAP) (18 % N and 20 % P) was incorporated into the top soil. The field was prepared into broad beds of 1.5 m width with furrows of 30 cm on either side were established . Sowing of the experiment was done on 26th of June 1996. Before sowing, the seeds were treated with Thiram and Captan @ 3 g Kg^{-1} of seeds to prevent seedling diseases. A seed rate of 110 kg $ha^{-1}$was used and sowing was done by hand in shallow furrows which were 30 cm apart on the broad beds with a seed to seed distance of 10 cm within each row. After sowing, the field was uniformly irrigated to field capacity using sprinklers so that soil moisture was sufficient for seed

germination and good crop establishment. Plants were thinned at 20 -25 DAS to achieve a plant population of 33plants m^2. The crop was maintained pest and disease free by following all prophylactic measurements. There were no major problems of weeds, diseases and pests during the growing season. Gypsum @ 250 - 500 kg ha^{-1} is applied during pegging to favour pod filling.

3. 6. 2 Treatments

Design followed for this experiment was a split split plot design with water stress treatments as main treatments, genotypes as sub treatments and betaine levels as sub sub treatments.

Main Treatments: There were two main treatments i. e., irrigated (IRR) and mid season drought (MSD) imposed by operating portable rain out shelter (ROS). The mid season drought spanning from 40 to 80 DAS was imposed by using portable ROS (Chauhan *et al.*, 1997). The shelters were hand operated only during the period of treatment. Two border strips along the ROS were covered by a polythene sheet to prevent infiltration of water to plots. Thus water captured by the ROS during rainfall events was diverted into drains dug at two ends of the shelter and which led away from the ROS areas.

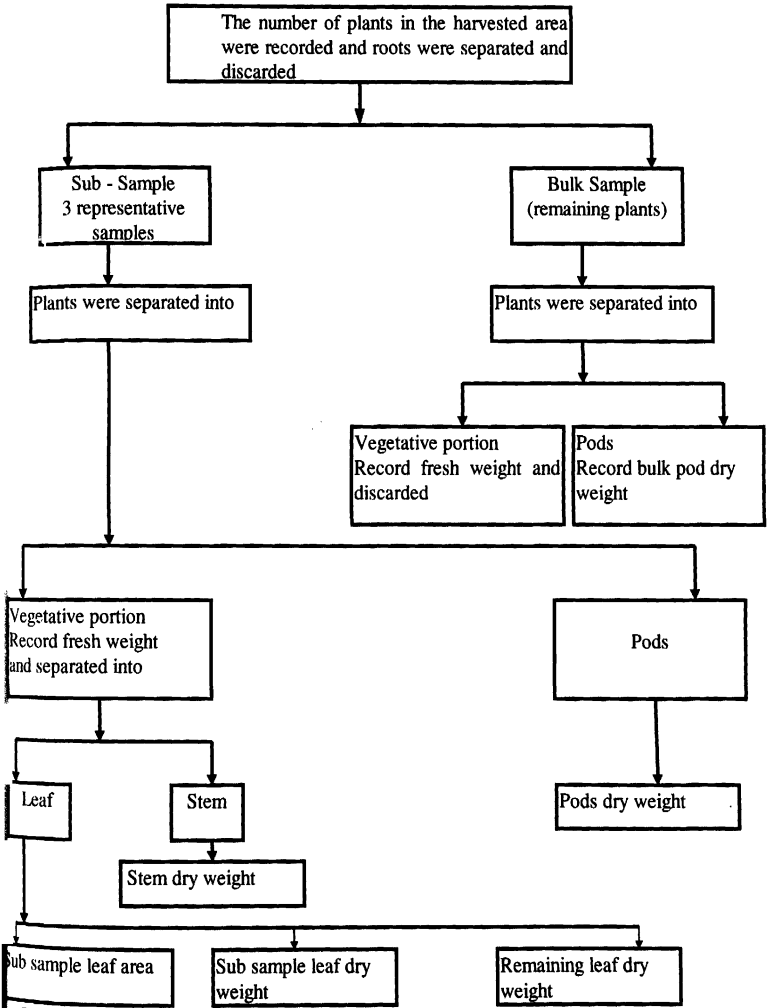
The adequately irrigated control treatment received irrigation through sprinkler irrigation system to avoid water deficit.

Sub treatments : The following 5 groundnut genotypes were assigned to sub plots.

1. ICG 476 (Spanish bunch germplasm)
2. TAG 24 (Spanish bunch breeding line developed at BARC Trombay and released for cultivation in India).

Plate 1 : The rain out shelter used in the rainy season experiment of 1996 to impose mid season drought.

Plate 2 : Overall view of the field during kharif '96 experiment



: 3.6.3 Procedure followed for analysing growth of plants sampled at every sampling e in each treatment to know the effect of glycine betaine in the alleviation of mid son drought stress during kharif'96.

Light interception

Canopy light interception (LI) was measured at mid-day by using a ceptometer (Degagon Instruments Washington, USA) at 20, 40, 60, 80, 100 DAS. The ceptometer readings were recorded by placing the sensor above the canopy (I_0) and placed across the rows below the canopy (I_1)The fractional radiation intercepted (LI) by the canopy at a given time was calculated using the following equation.

$LI(\%) = [(I_0 - I) / I_0] \times 100$

where, LI % is light interception %

I_0 is total incoming radiation (measured above the canopy)

I is radiation transmitted to the ground (measured below the canopy)

Osmotic potential

The 2 nd or 3 rd leaf from the apex from 4-5 plants/plot on the main axis were sampled to measure the osmotis potentials (OP). Samples were placed in polyethylene bags and dipped in liquid nitrogen and transferred to a deep freezer (-40° C) until further processing. At the time of measurement the leaf samples were removed from the freezer and allowed to thaw for 2 min. The samples were placed in a 1.5 ml 'eppendorf' tubes and centrifuged tube for 5 minutes at 12,000g to extract the cell sap. The OP of the cell sap was determined using an automatic micro-osmometer (Roebling Automatic Freezing Point Osmometer by Cryo Scopic Method). The osmometer was calibrated before each set of measurements with a standard solution of 300 milli osmoles and distilled water to get the zero point . Cell sap of 25 µl was used to measure Osmotic potential .

Osmotic potential at full turgor (OP_{100}) was calculated according to the formula of assuming that apoplastic water content is negligible (Wilson *et al.*, 1979):

OP_{100}= OP X RWC / 100

Osmotic adjustment (OA) was calculated as the difference between the OP_{100} of stressed and non-stressed, betaine treated and not treated leaves.

$OA = OP_{100}$ (C) - OP_{100} (T)

Where C was control and T was treatment.

Total Dry Matter at Harvest and Pod yield.

At final harvest a net plot area of 2.5 X 1.2 m^2 was harvested. The roots were separated and discarded . After picking of the pods, the shoots and pods were oven dried at 80 ^{0} C before recording of the dry weights. The total dry matter (TDM) was computed after adjusting the pod weights for the high energy content using a factor of 1.65. The TDM was calculated as follows

TDM = Shoot dry weight + (Pod dry weight X 1.65)

TDM was expressed per hactare basis.

Statistical Analysis

Experimental data were subjected to analysis of variance using a standard split-split plot design analysis as described by Gomez and Gomez (1984) and using the GENSTAT Package (Genstat manual, 1983) in a VAX mainframe Computer system at ICRISAT Center.

3. 7 Field experiment 2

Another field experiment was conducted during the post-rainy season 1996-97. The field preparation and crop management was done as described in Experiment 1. Sowing of the experiment was done on December 22nd 1998.

3. 7. 1 Crop management

Crop management was done as described in the chapter 3. 6. 1

3. 7. 2 Treatments

The design followed in this experiment was a split split plot design with different levels of water deficit as main treatments, genotypes as sub treatments, and betaine levels as sub sub treatments.

Main treatments

The stress was created as different water deficit % levels by the line source sprinkler irrigation. The line source sprinkler technique (Hanks *et al.*, 1976) results in the development of systematic gradient of soil moisture (drought intensities) as a function of distance from the source pipe line. This system is regularly used at ICRISAT for screening groundnut genotypes for drought tolerance (Nageswara Rao R C *et al.*,1985)

The line source sprinkler system consisted of a line(s) of overhead sprinklers with 1/8 " and 5/32"nozzles with an output of about 9.3 gal/min and were operated at a pressure of 275 kilo pascals (40PSI). They were operated during the periods when the wind velocity was minimal (less than 3 km/hr), usually at night. The water applied during each irrigation was measured in catchcans placed perpendicularly to the sprinkler line in each of the 8 beds at 4 different locations for a given bed as furnished in Fig: 3.7.2 .The volume of water collected in each of the catch cans was measured and averaged over 4 locations for a given bed to estimate the amount of water applied to the bed. The field layout of line source shown in plate : As shown from the figure Bed 1 & 2 from the sprinkler received almost similar amounts of water while bed 10 received

virtually nil. The test entries are planted from bed 2-9, in paired rows of 12m length each with a spacing of 30cm between rows and 10 cms between plants within a row, thus each plot of length 12m length consists of 8 beds (2- 9) perpendicular to the sprinkler line.

The crop was adequately irrigated to provide uniform irrigation to all the beds until seedlings were established. Uniform irrigation is given by arranging sprinkler lines at 15m intervals. The drought treatments are imposed using line source from 80 DAS to FH.

Out of the different levels of water deficit, 9.64, 27.65 and 67.54 % water deficits were taken as main treatments and analysed.

Sub treatments :

The same 5 genotypes used in the previous year were used in this year also.

Sub sub treatments:

The betaine spray solutions @ 3 and 6 kg/ha (B2 and B3) and water (B1) as a control spray were prepared in the same manner as that of the previous year. The betaine treatments were applied twice during the growing season. First application was made as soil application to 15 day old seedlings. The solution was applied in the planting rows to ensure the uptake of the chemical by the emerging seedling. The second application of betaine was made as a foliar spray at 45 DAS. The method of application was same as described in the Field Experiment 1.

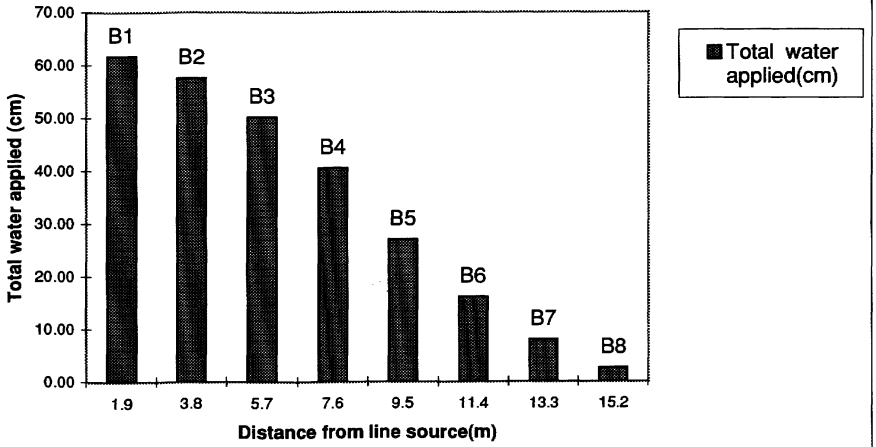


Fig: 3.7.2 Total water applied(cm) across the beds with line source sprinkler irrigation during the imposition of end season drought in rabi '96 - '97

3. 7. 3 observations and measurements

Growth analysis

Growth analysis was done as described in the chapter 3.6.3.

Gas exchange measurements, osmotic potential, RWC, and total betaine content were recorded as described in Chapter 3. 3. 2.

Light interception (LI) (%)

Light interception measurements were taken as described in the chapter 3. 6. 3.

Total Dry Matter at Harvest and Pod yield.

Total dry matter at harvest and pod yield was done as described in the chapter 3. 6. 3.

Statistical Analysis

Experimental data were subjected to analysis of variance using a standard split-split plot design analysis as described by Gomez and Gomez (1984) and using the GENSTAT Package (Genstat manual, 1983) in a VAX mainframe Computer system at ICRISAT Center.

Plate 3 : LCA4 in use during the glasshouse study of glycine betaine effect on salinity stress

Results

CHAPTER IV

Results

4. 1 Glycinebetaine and Heat tolerance

4. 1. 1 Effects of betaine on the sensitivity of germinating seeds to heat stress

Effect of betaine on sensitivity of two groundnut genotypes to heat stress was studied by subjecting seedlings to heat stress in the laboratory. The pregerminated seeds treated with (B_0, B_{25}, B_{50}, B_{100}) or without betaine were exposed to a lethal stress of 50^0 C for 2 hours and returned to 30^0C. There was also an imposition of heat induction by subjecting the seedlings to gradually raising temperatures before the lethal stress, so as to simulate natural conditions. The recovery growth of the seedlings from stress was studied. The details of the treatment imposition were given in materials and methods section (3.1.1) and Fig 3. 1. 1.

It was apparent from the results that at 40 hours after germination, the root and shoot lengths of seedlings were about 1 cm with no genotypic difference at this stage. However, at 72 hours after recovery, significant differences due to heat induction treatments (HI and NI), betaine treatments, as well as interaction of genotype x betaine treatments were apparent (Table 4.1.1). It was clear from Fig. 4.1.1a and 4.1.1b that in the B_0 treatment, the recovery growth of root and shoot in NI sub treatments was significantly less in both the genotypes than that in HI treatments. Whereas with HI treatment, the root and shoot growth were 157 and 44% higher than NI sub treatment.

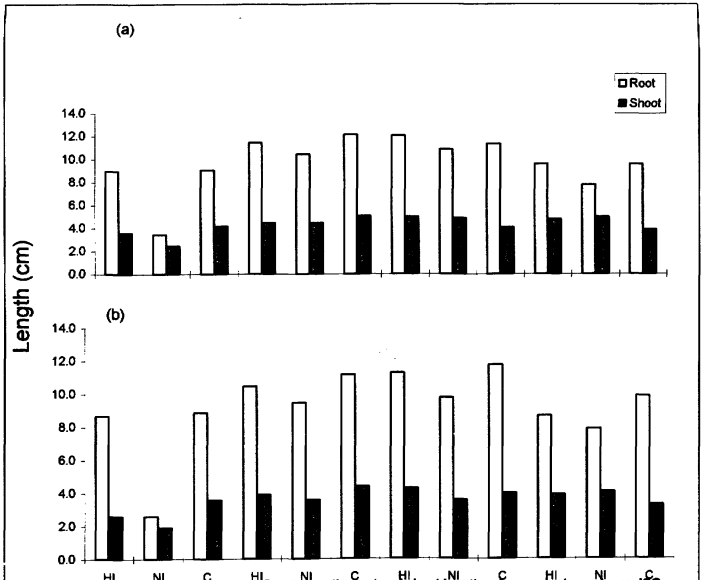


Fig : 4.1.1 Effect of seed treatment ofbetaine on the root and shoot lengths of groundnut genotypes, ICG 476(a) and TAG24 (b) under the influence of high temperature stress treatments

The root and shoot growth in HI sub treatment were on par with control treatment where there was no temperature stress in both the genotypes. Interestingly the seeds treated with 25 mM (B_{25}) or 50 mM (B_{50}) of glycine betaine have shown significantly greater growth of roots even under non induced (NI) treatment. Seedlings with HI + B_{25} treatments have resulted in greater root growth in comparison to NI + B_{25} treatments in both the genotypes. Similar responses were observed with B_{50} treatment. With 100 mM betaine treatment (B_{100}) there was 20% reduction in recovery growth compared to B_{50} . It was apparent that NI + B_{100} seedlings recorded 122% greater growth than NI + B_0 seedlings.

It was also clear from these results that the roots were more sensitive to high temperature stresses with the root growth ranging from 3 cm (NI + B_0) to 13 cm (HI B_{25}). Whereas, the response in the shoot growth due to the treatments ranged from 2 cm (NI + B_0) to 4 cm (HI + B_{25}). The B_{25} and B_{50} treatments have resulted in 20 - 26% greater growth of seedlings (root and shoot) compared to B_{100} treatment in both the genotypes (Fig. 4.1.1 a and 4.1.1b).

4. 1. 2 Variations in protein profiles in groundnut seedlings

It is well known that several physiological and biochemical changes play a major role in enhancing adaptation of plants to heat stress. In the present study influence of betaines and heat stress interaction treatments on possible changes in protein metabolism has been investigated by studying the protein profile in the seedlings using gel-electrophoresis technique. The methodology of the electrophoresis was given in materials and methods section 3.1.1.

It was apparent from the gel analysis that there were significant qualitative and quantitative differences between genotypes (ICG 476 and TAG 24), heat induction treatments (HI and NI) and betaine treatments (B_0 and B_{25}) (Fig 4. 1. 1a and 4. 1. 1b). Analysis of the protein banding pattern revealed that genotypes have responded differently to heat induction and betaine treatments. Heat induction treatment (B_0) alone resulted in production of 2 additional protein bands in the two genotypes, although the molecular weight of the proteins produced varied with genotypes. In ICG 476, the two proteins were of 85 and 54.5 kDa, while in TAG 24, they were 76.4 and 45.6 kDa. (Table 4.1.2).

B_{25} treatment has resulted in production of four additional bands in both NI (i.e., 76.4; 60.6; 54.6; and 16.5 kDa) and HI (i.e., 76.4; 54.6; 39.8; 16.5 kDa) treatments. However three out of the four new bands produced by B_{25} in NI and HI treatments had similar molecular weight. With combination of HI B_{25} treatment, two additional bands (35.6 and 34.8 kDa) than NI B_{25} treatment. In TAG 24 under HI as well as NI treatments B_{25} had resulted in 3 additional bands compared to B_0. Amongst 3 additional bands produced due to B_{25} treatment, two bands had same molecular weights except one band, wherein it was 75.6 kDa with B_{25} HI combination while under B_{25} NI it was 54.6 kDa. Genotypic differences were apparent with the total number of bands produced were being more in ICG 476 in any given treatment compared to TAG 24 . In ICG 476 B_0 treatment resulted in production of 16 bands under HI and 14 bands in NI treatments, whereas in TAG 24 there were 15 and 13 bands in HI and NI treatments respectively under B_0, the additional one band noticed in ICG 476 was of 66.2 kDa. In B_{25} treatment, ICG 476 had shown 2 additional bands compared to TAG 24 under both HI and NI treatments. It was

interesting to note that the molecular weight of one of the two proteins produced was 66.2 kDa. However with B_{25} treatment there was an additional protein with 56.4 kDa produced in ICG 476 only.

4. 2 Glycinebetaine and salinity tolerance

4. 2. 1 Effects of glycinebetaine on the sensitivity of groundnut to salinity stress.

Effect of betaine on sensitivity of two groundnut genotypes to salinity stress was studied by subjecting seedlings to salinity stress in the laboratory. The pregerminated seeds treated with (B_0, B_{25}, B_{50}, B_{100}) or without betaine were exposed to a lethal salinity stress of 300mM NaCl for 48 hours and returned to distilled water. There was also an imposition of a gradual salinity induction stress by subjecting the seedlings to 150 mM NaCl for 16 hours before transferring to lethal stress of 300 mM salinity. The recovery growth of the seedlings from salinity stress was studied. The details of the treatment imposition were given in materials and methods (3. 2. 1) and Fig 3. 2. 1.

It was apparent from the results that at 40 hours after germination, the root and shoot lengths of seedlings were about 1 cm with no genotypic difference at this stage. However, at 72 hours after recovery, significant differences due to salinity induction treatments (SI and NI), betaine treatments, as well as interaction of genotype x betaine treatments were apparent (Table 4.2.1). It was clear from fig. 4.2.1a and 4.2.1b that in the B_0 treatment, the recovery growth of root and shoot was significantly less in SI sub treatments in both the genotypes. Whereas with SI treatment, the root and shoot growths were 106% and 72% higher than NI sub treatment. The root and shoot growth in SI sub

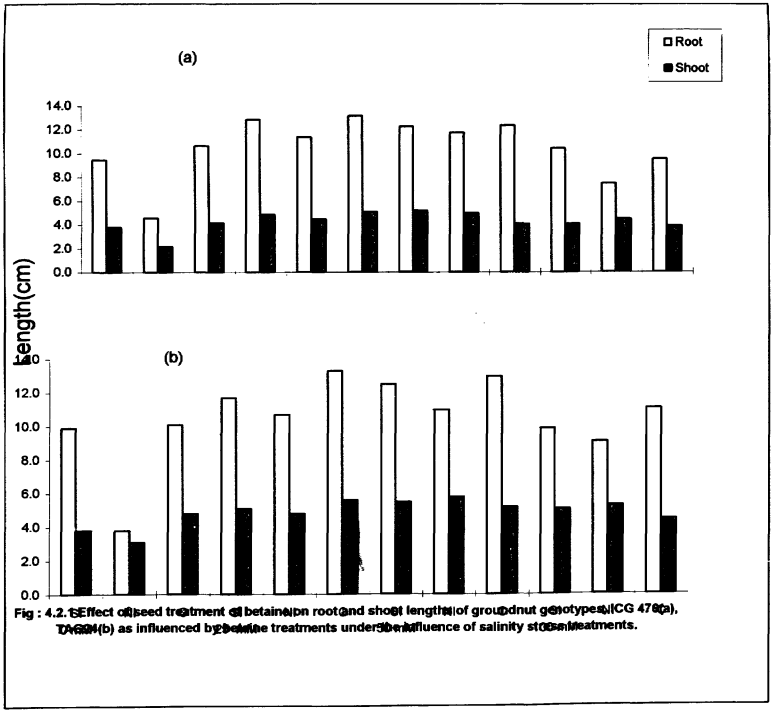


Fig : 4.2.1 Effect of seed treatment on retention root and shoot length of groundnut genotypes, ICG 476 (a), TAG 24 (b) as influenced by the treatments under influence of salinity stress treatments.

treatment were on par with non-stressed control treatment in both the genotypes. Interestingly the seeds treated with 25 mM or 50 mM of glycine betaine have shown significantly positive growth of roots even in non induced seedlings. Seedlings with combination of SI and B_{25} treatments have resulted in greater growth of roots in comparison to NI + B_{25} treatment combination in both the genotypes. Similar responses were observed with 50 mM betaine treatment. Whereas, with 100 mM betaine treatment, there was reduction in recovery growth by 17% compared to 50 mM betaine. Seedlings NI B_{100} recorded 38% greater growth than NI B_0 seedlings treated with NI + B_{100} treatment combination.

Similar to observations made under heat stress, these experiments also have shown that it was also clear from these results that the roots were more sensitive to salinity stresses with the recovery growth in NI B_0 ranging from 4 cm to 12 cm in SI B_{25}. Whereas, the variation in the shoot growth due to the treatments was from 2 cm NI B_0 to 5 cm SI B_{25}. The B_{25} and B_{50} treatments have resulted in 17 - 22% greater growth of seedlings compared to B_{100} treatment in both the genotypes (Fig. 4.1.1 a and 4.2.1b).

4. 2. 2 Variation in protein profiles in groundnut seedlings

In the present study influence of betaines and salinity stress treatments on possible changes in protein metabolism has been investigated by studying the protein profile in the seedlings using gel electrophoresis technique. The methodology of the electrophoresis was given in materials and methods section 3.2.1

It was apparent from the gel analysis that there were significant qualitative and quantitative differences between genotypes (ICG 476 and TAG 24), salinity induction

treatments (SI and NI) and betaine treatments (B_0 and B_{25}) (Fig 4.2.1). Analysis of the protein banding pattern revealed that genotypes have responded differently to salinity induction and betaine treatments. Salinity induction treatment (B_0) alone resulted in production of additional protein bands in the two genotypes, the molecular weight of the proteins produced varied with genotypes. In ICG 476, the two proteins were of 45.1 and 36.4 kDa, while in TAG 24, there were 4 proteins with molecular weight of 65.4, 37.8, 35.4, and 16.5 kDa. (Table 4.2.2). B_{25} treatment has resulted in production of four additional bands at SI (i.e., 45.4; 32.6; 24.8; and 18.4 kDa) and 2 additional bands at NI (i.e.,46.2 and 18.5 kDa) treatments. However three out of the four new bands produced by B_{25} in NI and SI treatments had similar molecular weight. With combination of SI and B_{25} treatment, two additional bands were produced (36.4 and 34.8 kDa) than NI B_{25} treatment. In TAG 24 under SI as well as NI treatments B_{25} had resulted in three (at 76.2, 35.6 and 18.8kDa) and one (at 27.8 kDa) additional bands compared to B_0. Genotypic differences were apparent with the total number of bands produced were being more in ICG 476 in any given treatment compared to TAG 24 . In ICG 476 B_0 treatment resulted in production of 18 bands under SI and 16 bands in NI treatments, whereas in TAG 24 there were 17 and 15 bands in SI and NI treatments respectively under B_0, the additional one band noticed in ICG 476 compared to TAG 24 was of 66.2 kDa. In B_{25} treatment, ICG 476 had shown two additional bands compared to TAG 24 under both SI and NI treatments. It was interesting to note that the molecular weight of one of the two proteins produced was 66.2 kDa. However with B_{25} treatment there was an additional protein with 56.4 kDa produced in ICG 476 only.

Table 4.1.1 : Root and shoot lengths (cm) of groundnut seedlings as influenced by heat stress and betaine treatments

Betaine (mM)	Treatment	ICG 476		TAG24	
0	HI	9.0	3.6	8.7	2.6
	NI	3.5	2.5	2.6	1.9
	C	9.1	4.2	8.9	3.6
25	HI	11.5	4.5	10.5	3.9
	NI	10.5	4.5	9.5	3.6
	C	12.2	5.1	11.2	4.4
50	HI	12.1	5.0	11.3	4.3
	NI	10.9	4.9	9.8	3.6
	C	11.4	4.1	11.8	4.0
100	HI	9.6	4.8	8.7	3.9
	NI	7.8	5.0	7.9	4.1
	C	9.6	3.9	9.9	3.3

Analysis of variance

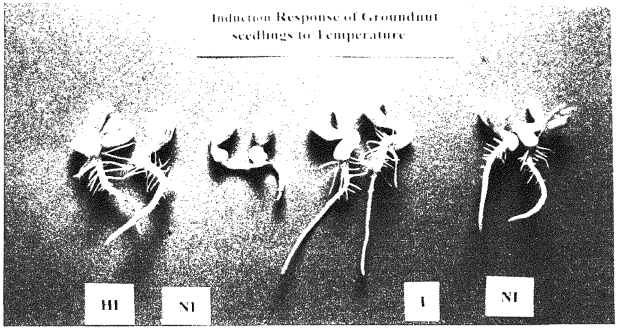
Source of variation	df	Root	Shoot
MT (genotypes(G)	1	NS	NS
ST(Treatments)(S)	1	**	**
G x S	1	*	*
SST(Betaine levels)(B	3	**	*
G x B	3	*	*
G x S x B	3	NS	NS

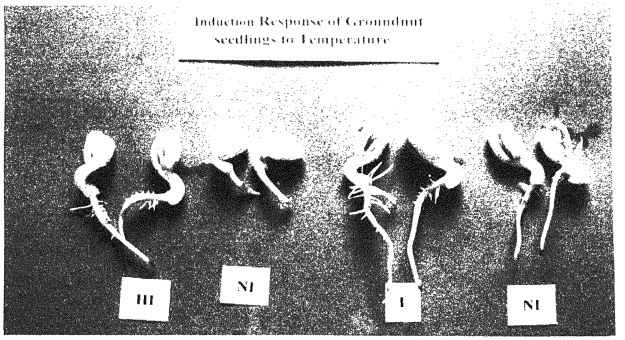
Table 4.2.1 : Root and shoot lengths (cm) of groundnut seedlings as influenced by salinity stress and betaine treatments

Betaine (mM)	Treatment	ICG 476		TAG24	
0	HI	9.5	3.8	9.9	3.8
	NI	4.6	2.2	3.8	3.1
	C	10.7	4.2	10.1	4.8
25	HI	12.9	4.9	11.7	5.1
	NI	11.4	4.5	10.7	4.8
	C	13.2	5.1	13.3	5.6
50	HI	12.3	5.2	12.5	5.5
	NI	11.8	5.0	11.0	5.8
	C	12.4	4.1	13.0	5.2
100	HI	10.5	4.1	9.9	5.1
	NI	7.5	4.5	9.1	5.3
	C	9.6	3.9	11.1	4.5

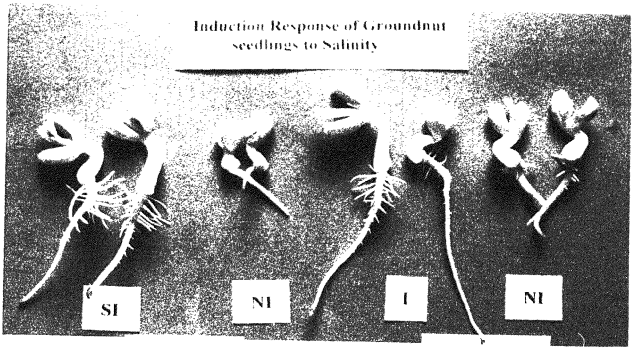
Analysis of variance

Source of variation	df	Root	Shoot
MT (genotypes(G)	1	NS	NS
ST(Treatments)(S)	1	**	**
G x S	1	*	*
SST(Betaine levels)(B	3	**	*
G x B	3	*	*
G x S x B	3	NS	NS





Plates 4 & 5 : Effect of glycine betaine on root and shoot lengths of groundnut seedlings (ICG 476 - above and TAG 24 below) as influenced by high temperature stress treatments



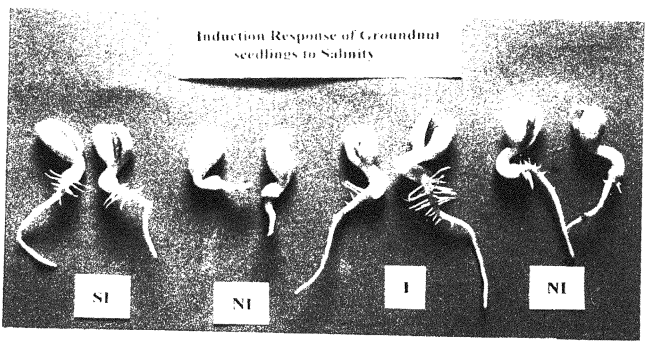


Plate 6 & 7 : Effect of glycine betaine on root and shoot lengths of groundnut seedlings (ICG 476 - above and TAG 24 - below) as influenced by salinity stress treatments

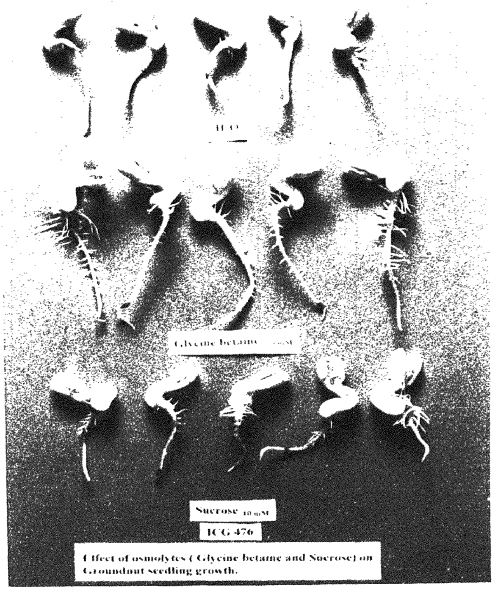
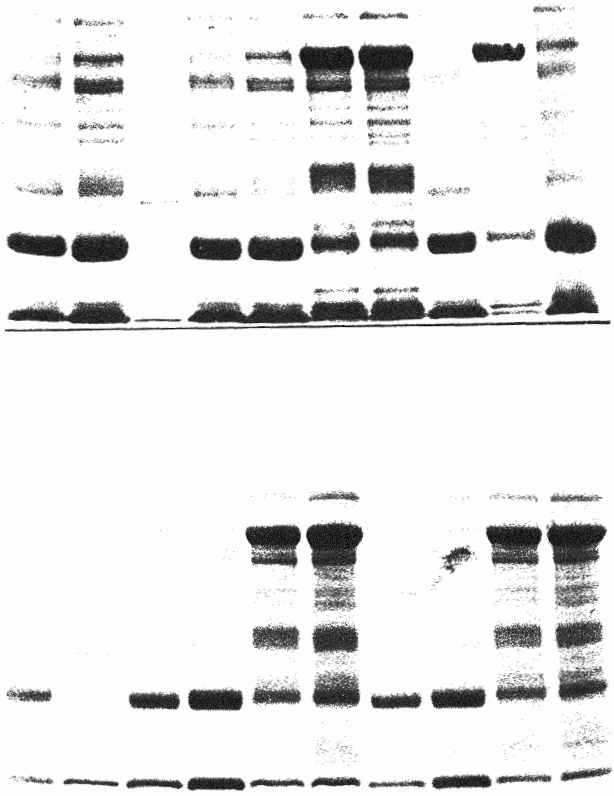


Plate 8 : Response of growth of groundnut seedlings to osmolytes like glycine betaine and sucrose and H_2O as control



Plates 9 & 10 : Protein profiles of groundnut seedlings (G_1 - ICG 476) (G_2 - TAG 24), subjected to high temperature (above) and salinity stress (below) as influenced by betaine treatments

Table: 4.1.2 Protein profile of the groundnut seedlings as influenced by betaine and heat stress treatments

	ICG 476			TAG24		
	Heat induction treatments			Heat induction treatments		
Betaine levels	HI	NI	New proteins due to HI treatments(kDa)	HI	NI	New proteins due to HI treatments(kDa)
B0	16	14	2 (85;54.5)	15	13	2 (76.4; 45.6)
B25	20	18	2 (35.6;32.4)	18	16	2 (35.6; 32.4)
New proteins due toB25 treatments (kDa)	4 (76.4;54.6; 39.8; 16.5)	4 (764;60.6; 54.6; 16.5		3 (75.8; 40.6; 18.4)	3(54.6; 40.8; 19.5)	

Table: 4.2.2 Protein profile of the groundnut seedlings as influenced by betaine and salinity stress treatments

	ICG 476			TAG24		
	Salinity induction treatments			Salinity induction treatments		
Betaine levels	SI	NI	New proteins due to SI treatments(kDa)	SI	NI	New proteins due to SI treatments(kDa)
B0	18	16	2 (45.1; 36.4)	17	15	2 (46.7; 85.2)
B25	22	18	4 (65.4; 37.8; 35.4; 16.5)	20	16	4 (80.7; 50.6; 29.8; 18.4)
New proteins due to B25 treatments (kDa)	4 (45.4; 32.6; 24.8; 18.4)	2 (46.2; 18.5)		3 (76.2; 35.6; 18.8)	1 (27.8)	

4. 3 Glycine Betaine and Salinity Stress

As explained in materials and methods section, (3.3), the seeds of three genotypes (ICG 476, TAG 24 and CSMG 84-1) were either treated with distilled water (B_0) or 25 mM of glycine betaine (B_{25}). The seeds after priming with treatment solutions, were planted in pots of size 180 mm diameter filled with acid washed sand. The pots were adequately irrigated with 0.25 strength nutrient solutions until 15 DAS after which, 3 salinity stresses (0,6 and 8 ds m^{-1}) (S_1, S_2 and S_3) were imposed from 15 to 30 DAS, the pots were randomised within each of the three replications. Observations on root and shoot growth, photosynthetic rate, osmotic potential and relative water content were made as described in the materials and methods section 3.3.1.

4. 3. 1. Root and main stem lengths (cm)

At 15 DAS, before the start of salinity stress, root lengths in B_0 treatment varied from 9.9 to 11cm while with B_{25} treatment the root lengths were 23% higher than the B_0 treatment representing a significant increase over control ($P < 0.01$), however there is no genotypic difference, neither there was a G x B interaction at this stage. (Table 4.3.1).

The main stem lengths under B_0 treatment ranged from 13 to 13.9 cm, whereas with B_{25} treatment the main stem length increased by 20% over control representing a significant difference ($P < 0.01$). As observed for root lengths, main stem lengths did not differ significantly among genotypes and G x B interaction was also not significant. (Table 4.3.1).

Table : 4. 3. 1 Effect of seed treatment with betaine on root (RL) and main stem length (SL) of three groundnut genotypes at 15 DAS.

	RL (cm)		MSL (cm)	
	B0	B25	B0	B25
ICG 476	9.9	12.4	13.0	15.0
TAG 24	10.8	13.1	12.8	15.0
CSMG 84-1	11.0	13.6	13.9	15.8
G mean	10.6	13.0	13.2	15.3
SeM	± 0.428		± 0.48	
CV %	6.5		6.8	

		Analysis of variance	
Source of variation	df	RL	MSL
MT (genotypes)(G)	2	NS	Ns
ST (betaine levels)(B)	1	**	**
G X B	2	NS	NS

4. 3. 2 Root and main stem development (mm day^{-1})

Salinity stress imposed from 15 to 30 DAS resulted in a significant reduction in the rate of root and main stem development in all the 3 genotypes. The rate of root development was reduced by 80% under salinity stress in B_0 and by 35% under B_{25} treatment. Genotypic variation was not observed and the G x S, G x B, G x S x B interactions were also not significant. S x B interaction was significant, there was a 125% increase in root development with B_{25} treatment in S_2 and a 200% increase with B_{25} treatment in S_3 level, whereas the S_1 differences were marginal with betaine levels in root development (Table 4.3.2; Fig 4.3.3(a)). Seed treatment with B_{25} resulted in a overall increase of root development which was 0.76mm day^{-1} in B_0 and 1.03 mm day^{-1} in B_{25} representing a significant effect of B_{25} treatment. S x B interaction was significant for example, the rate of root development was 0.97 mm day^{-1} in B_{25} treatment in S_2 and it was only 0.47 mm day^{-1} in B_0 treatment in S_2 representing the betaine effect in specificity for a positive response under salinity stress conditions.

The mean rate of shoot development was 1.1 mm day^{-1} in B_0, whereas it was 2.6 mm day^{-1} in B_{25} treatment representing a significant effect of B_{25} treatment on stem expansion, in B_{25} treatment there was a 137% increase over control. Imposition of salinity stress (S_3) resulted in an overall reduction in the main stem development by 78% in B_0 and 58% in B_{25} treatment. CSMG 84-1 had 1.84 mm day^{-1} mean shoot development compared to 0.86 mm day^{-1} (TAG 24) and 1.2 mm day^{-1} (ICG 476), representing a significant genotypic variation ($P < 0.05$) for main stem development. G x S interaction was significant, the salinity stress (S_3) reduced by 76% (ICG 476), 81% (TAG 24), whereas in CSMG 84-1 the reduction is only 54% with the imposition of S_3 treatment. S

Table : 4. 3. 2 Influence of salinity stress and seed treatment with betaine on root (RD) and main stem (MsD) development of three groundnut genotypes.

		RD (mm/day)		MsD (mm/day)	
		B0	B25	B0	B25
ICG 476	S1	1.6	1.4	1.6	2.8
	S2	0.4	1.5	0.8	1.0
	S3	0.4	1.5	0.6	0.5
	Mean	0.8	1.5	1.0	1.4
TAG 24	S1	1.4	1.3	0.8	2.4
	S2	0.5	1.2	0.3	1.0
	S3	0.3	0.5	0.3	0.3
	Mean	0.7	1.0	0.5	1.2
CSMG 84-1	S1	1.5	1.4	2.6	4.0
	S2	0.5	0.2	0.5	0.8
	S3	0.2	0.7	0.2	2.9
	Mean	0.7	0.8	1.1	2.6
	G mean	0.8	1.1	0.9	1.7
	SeM	± 0.250		± 0.025	
	CV %	34.5		29.7	

		Analysis of variance	
source of variation	df	RD	SD
MT (stress levels)(S)	2	**	**
ST (genotypes)(G)	2	NS	*
G X S	4	NS	**
SST (betaine levels)(B)	1	*	**
S X B	2	*	**
G X B	2	NS	NS
G X S X B	4	NS	NS

x B interaction was also found to be significant, for example, the reduction in the main stem development due to salinity stress was 80% in B_0 and only 60% in B_{25} treatment.

The G x B, G x S x B interactions were not significant.

4. 3. 3 Seedling growth and development

At 15 DAS, the root weights ranged from 0.76 to 0.77 g plant^{-1} in B_0 and 0.76 to 0.80 g plant^{-1} in B_{25} treatment. There was no significant difference between root weights when observed for betaine levels as well as for genotypes and also no significant G x B interaction was observed (Table 4.3.3).

The average leaf weights with B_0 treatment was 0.4 g plant^{-1} and 0.8 g plant^{-1} with B_{25} treatment representing a significant 100% increase ($P < 0.01$) with B_{25} treatment. Significant genotypic differences ($P < 0.01$) were observed for leaf weights , where in CSMG 84-1 had leaf weights of 0.74 g plant^{-1} compared to 0.47 g plant^{-1} in ICG 476 and 0.58 g plant^{-1} in TAG 24 (Table 4.3.3).

Stem weights differed significantly between genotypes ($P < 0.01$) , where in ICG 476 recorded 0.82 g plant^{-1} compared to 0.69 g plant^{-1} in TAG 24 and 0.55 g plant^{-1} in CSMG 84-1. Betaine treatment (B_{25}) had no significant effect on stem weights (Table 4.3.3).

The average shoot weight with B_0 treatment was 1.1 g plant^{-1} and 1.5 g plant^{-1} with B_{25} treatment representing a significant effect ($P < 0.01$) and a 87% increase with B_{25} treatment was observed. Genotypic variation was not significant.

Table : 4. 3. 3 Effect of seed treatment with betaine on root and shoot dry matter, leaf area (LA) and total dry matter (TDM) at 15 DAS.

	Rwt (g/plant)		Lwt (g/plant)		Stwt (g/plant)		Swt(g/plant)		RS (g/g)		LA (cm2/plant)		TDM (mg/plant)	
	B0	B25	B0	B25	B0	B25	B0	B25	B0	B25	B0	B25	B0	B25
ICG 476	0.76	0.80	0.23	0.71	0.86	0.79	1.09	1.50	0.697	0.533	95.8	151.8	1.8	2.3
TAG 24	0.77	0.79	0.36	0.79	0.50	0.89	0.87	1.69	0.887	0.467	112.8	172.5	1.6	2.5
CSMG 84-1	0.77	0.76	0.60	0.89	0.60	0.50	1.20	1.39	0.642	0.547	125.9	150.8	2.0	2.2
G mean	0.8	0.8	0.4	0.8	0.7	0.7	1.1	1.5	0.7	0.5	111.5	158.4	1.8	2.3
SeM	± 0.023		± 0.032		± 0.052		± 0.045		± 0.065		± 9.2		0.05	
CV %	5.5		10.5		14.2		9.9		12.9		14.6		15.2	

ANALYSIS OF VARIANCE

Source of variation	df	Rwt	Lwt	Stwt	Swt	RS	LA	TDM
MT (genotypes)(G)	2	NS	**	**	NS	NS	*	NS
ST (betaine levels)(B)	1	NS	**	NS	**	*	**	**
G X B	2	NS	NS	**	**	*	NS	**

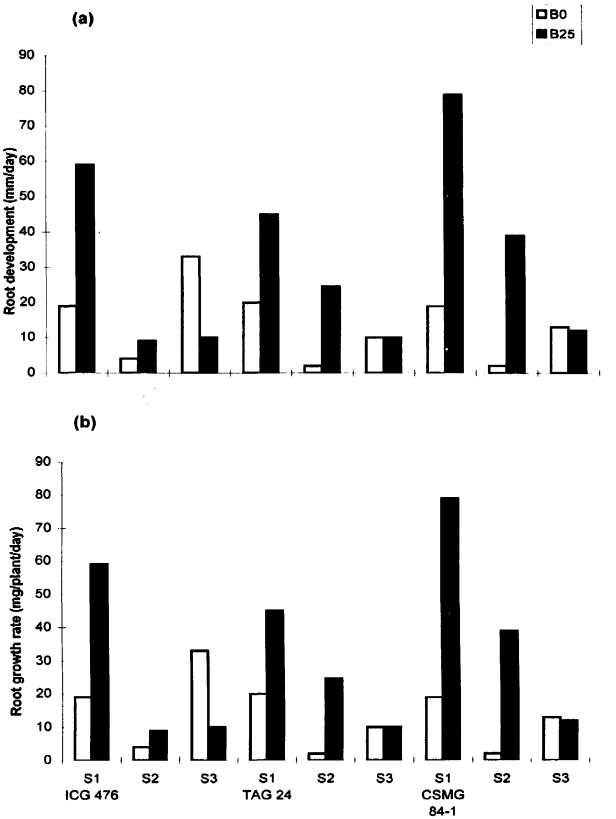


Fig : 4.3.3(a) Effect of seed treatment of betaine on root development(a) and root growth rate (b) in 3 groundnut genotypes under salinity stress conditions.

Root and shoot ratios were significantly different ($P < 0.05$) between betaine levels and there was no significant difference between genotypes. The R/S ratios ranged from 0.64 to 0.88 in B_0 treatment and 0.46 to 0.54 in B_{25} treatment showing a significant decrease in R/S ratios by 28% with B_0 treatment, this indicated that higher shoot weights in B_{25} treatment caused a significant reduction in R/S ratios. This shoot growth was contributed by an increase in leaf area under B_{25} treatment (Table 4.3.3) .

The leaf areas ranged from 95 to 125 cm^2 $plant^{-1}$ in B_0 and 150 - 172 cm^2 $plant^{-1}$ in B_{25} treatment (Table 4.3.3)There was a significant genotypic variation ($P < 0.01$), where as in ICG 476 the leaf areas ranged from 95 - 151 cm^2 $plant^{-1}$ and in TAG 24 112 - 172 cm^2 $plant^{-1}$ and 175 - 150 cm^2 $plant^{-1}$ in CSMG 84-1.

The significant positive effect of B_{25} ($P < 0.01$) resulted in a higher dry matter accumulation in B_{25} treatment compared to that in B_0 . TDM was about 1.8 g $plant^{-1}$ in B_0 and 2.3 g $plant^{-1}$ in B_{25} treatment. There is an overall increase of 28% with B_{25} treatment in TDM (Table 4.3.3).

4. 3. 4 Growth components

Root growth rate (RGR) (mg $plant^{-1}$ day^{-1})

RGR differed significantly ($P < 0.01$) for genotypes, CSMG 84-1 recorded highest RGR 27.3 mg $plant^{-1}$ day^{-1} when compared to 22.3 mg $plant^{-1}$ day^{-1} in ICG 476 and 18.6 mg $plant^{-1}$ day^{-1} in TAG 24 (Table 4.3.4; Fig4.3.3 (a). In B_{25} treatment there was a 135% increase in RGR over B_0 representing a significant ($P < 0.01$) positive effect of B_{25} treatment. RGR decreased by 65% with S_3 treatment when compared with S_1 treatment showing a significant effect of ($P< 0.01$) of salinity stress on RGR.

G x S interaction was significant ($P < 0.01$), the decrease in RGR differed significantly , for example in ICG 476 the % decrease was 84% when compared to 69% in TAG 24 and 75% in CSMG 84-1. G x B interaction was significant ($P < 0.01$) and the % increase with B_{25} treatment differed significantly between genotypes, for example, the B_{25} treatment increased RGR by 280% in CSMG 84-1 compared to only 39 and 150% in ICG 476 and TAG 24 respectively. S x B interaction was significant ($P < 0.01$), for example the RGR with B_{25} treatment increased by 200% in S_1 treatment whereas with S_2 and S_3 treatments there was only a marginal difference (Table 4.3.4).

Shoot growth rate (SGR) (mg plant^{-1} day^{-1})

SGR differed significantly ($P < 0.01$) for genotypes, with TAG24 showing the greatest SGR with 156.9 mg plant^{-1} day^{-1} and the CSMG 84-1 had the least (65.7 mg plant^{-1} day^{-1}). Imposition of a salinity stress resulted in a reduction in SGR i.e., 205 mg plant^{-1} day^{-1} in control to 57.2 and 30.7 mg plant^{-1} day^{-1} in S_2 and S_3 respectively (Table 4.3.4; Fig 4.3.4). G x S interaction was also significant with SGR ($P < 0.01$), where in TAG 24 recorded a greatest decrease in SGR by 50% representing a significant ($P < 0.01$) effect of betaine on SGR. G x B interaction was significant ($P < 0.01$), for example, CSMG 84-1 had a 154% increase in SGR with B_{25} treatment where as it was only 30% in ICG and TAG 24 respectively.

Rate of expansion of leaf area (LAER) (cm^2 plant^{-1} day^{-1})

Leaf area expansion ranged from 1.5 to 6.5 cm^2 plant^{-1} day^{-1} in B_0 treatment and 1.2 to 4.8 cm^2 plant^{-1} day^{-1} in B_{25} treatment (Table 4.3.4). Overall, genotypes differed significantly ($P < 0.01$), and the greatest LAER was in ICG 476 (4.09 cm^2 plant^{-1} day^{-1}

)and the least in TAG 24 (2.35 cm^2 $plant^{-1}$ day^{-1}). LAER reduced from 4.04 cm^2 $plant^{-1}$ day^{-1} in S_1 to 1.59 cm^2 $plant^{-1}$ day^{-1} in S_3 treatment showing a significant effect ($P < 0.01$) of salinity stress on LAER. G x S interaction was significant ($P < 0.05$) with ICG 476 showing a significant reduction in the leaf area expansion (64%) whereas the reduction in leaf area expansion was only 55 and 58% in TAG24 and CSMG 84-1 respectively. However the effects of betaine on LAER were marginal and not significantly different. S x B interaction was not significantly different whereas G x B interaction was significant ($P < 0.01$) showing ICG 476 had a 40% decrease in LAER with B_{25} treatment whereas in the other two genotypes the % increase was only 4%. G x S x B interaction was not significant (Table 4.3.4).

Crop growth rate (CGR)

CGR differed significantly ($P < 0.01$) for genotypes which ranged from 12-288 mg $plant^{-1}$ day^{-1} in B_0 treatment and 21 - 470 with B_{25} treatment (Table 4.3.4). TAG 24 had greater CGR (204 mg $plant^{-1}$ day^{-1}), and the least in CSMG 84-1 (98 mg $plant^{-1}$ day^{-1}). Salinity stress imposition reduced the CGR by 88% showing a significant negative effect ($P < 0.01$) of salinity stress on CGR. G x S interaction was significant ($P < 0.01$) for example, in TAG 24, S_3 treatment reduced the CGR by 93% compared to S_1 treatment whereas in ICG 476 and CSMG 84-1 the reduction was 82%. On an average B_{25} treatment increased CGR significantly ($P < 0.01$) by 60% showing a positive effect of betaine on CGR. S x B interaction was also significant ($P < 0.01$). B_{25} treatment resulted in a 72% increase over B_0 in ICG 476 and 62% in TAG 24 and in CSMG 84-1 the differences were marginal. G x B interaction was significant ($P < 0.05$) ,

Table : 4. 3. 4 Effect of seet treatment with betaine on root growth rate (RGR), shoot growth rate (SGR), leaf area development (LA),crop growth rate (CGR) during salinity stress imposed from 15 - 30 DAS.

		RGR (mg/plant/day)		SGR (mg/plant/day)		LA (cm2/day)		CGR (mg/plant/day)	
		B0	B25	B0	B25	B0	B25	B0	B25
ICG 476	S1	19.0	59.0	126.0	210.0	6.5	4.8	145.0	269.0
	S2	4.0	9.0	55.0	13.0	6.1	3.2	59.0	141.0
	S3	33.0	10.0	3.0	17.0	2.7	1.3	36.0	27.0
	Mean	**18.7**	**26.0**	**61.3**	**80.0**	**5.1**	**3.1**	**80.0**	**145.7**
TAG 24	S1	20.0	45.0	269.0	425.0	2.8	3.0	288.0	470.0
	S2	2.0	24.6	133.0	97.0	2.6	3.0	135.0	121.0
	S3	10.0	10.0	8.0	9.7	1.5	1.2	26.0	21.0
	Mean	**10.7**	**26.5**	**136.7**	**177.2**	**2.3**	**2.4**	**149.7**	**204.0**
CSMG 84-1	S1	19.0	79.0	89.0	113.0	4.2	3.0	109.0	192.0
	S2	2.0	39.0	9.7	36.0	2.4	4.0	12.0	76.0
	S3	13.0	12.0	13.7	133.0	1.5	1.4	27.0	26.0
	Mean	**11.3**	**43.3**	**37.5**	**94.0**	**2.7**	**2.8**	**49.3**	**98.0**
	G mean	**13.6**	**32.0**	**78.5**	**117.1**	**3.4**	**2.8**	**93.0**	**149.2**
	SeM	± 2.50		± 4.80		± 0.57		± 4.60	
	CV %	16.9		9.2		39.2		8.9	

		ANALYSIS OF VARIANCE			
source of variation	df	RGR	SGR	LAER	CGR
MT (stress levels)(S)	1	**	**	**	**
ST (genotypes)(G)	2	**	**	**	**
G X S	2	**	**	*	**
SST (betaine levels)(B)	1	**	**	NS	**
S X B	1	**	**	NS	**
G X B	2	**	**	*	*
G X S X B	4	**	**	NS	**

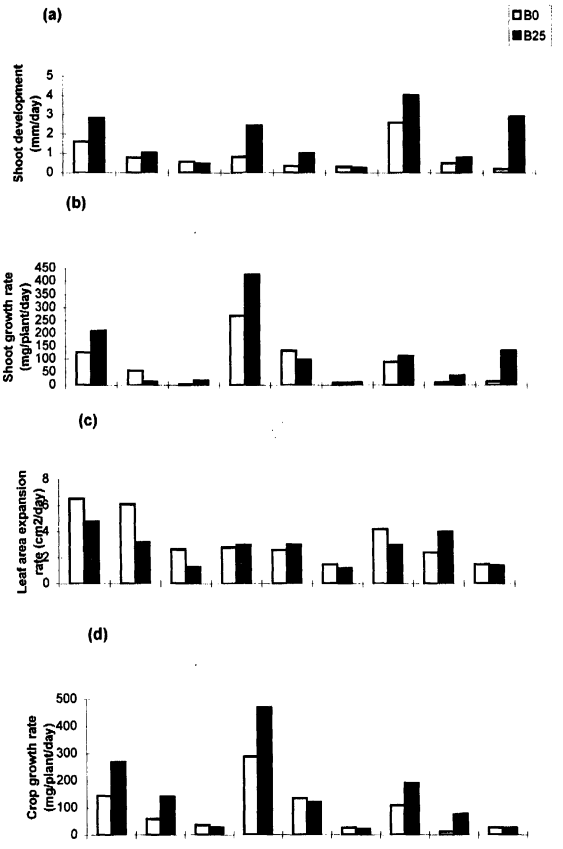


Fig ; 4.3.4 Effect of seed treatment of betaine on shoot development(a), shoot growth rate (b), leaf area expansion rate (c), and crop growth arte (d) as influenced by salinity stress treatments imposed during 15 - 30 DAS.

in CSMG 84-1 the % increase was 100 with B_{25} treatment, whereas in ICG 476 and TAG 24 it was 81 and 36% respectively.

4. 3. 5 Water relations, photosynthetic rate and total betaine content

Photosynthetic rate (Pn) (μmol $m^{-2}sec^{-1}$)

Salinity stress levels, genotypes, G x S interaction were not significant for the photosynthetic rates, whereas with betaine levels were found to be significantly different ($P < 0.01$). Overall, the B_{25} treatment increased the photosynthetic rates by 28% and the Pn rates ranged from 6.56 to 9.51 μmol $m^{-2}sec^{-1}$ in B_0 and 9.42 to 11.52 μmol $m^{-2}sec^{-1}$ in B_{25} treatment (Table 4.3.5).

S x B interaction was also significantly different ($P < 0.01$) for example, in S_1 and S_2 treatments the B_{25} treatment was able to increase Pn rates by 31 and 38% respectively, whereas in S_3 the B_{25} treatment was able to increase by Pn by only 18%. G x B interaction was also significant different ($P < 0.01$) , in ICG 476 and TAG 24 the B_{25} treatment was able to increase the Pn rates by 30 and 35% respectively, whereas in CSMG 84-1 the % increase was only 23%. G x S x B interaction was not significant.

Relative water content (RWC)

There were no significant differences in RWC for salinity stress levels, genotypes , G x S interaction, betaine levels, G x B, G x S , G x S x B interactions were found to be not significantly different (Table 4.3.5).

Table : 4. 3. 5 Effect of seed treatment with betaine on photosynthetic rates(Pn), relative water content (RWC), osmotic potentials(OP) and total betaine content (TB) during salinity stress imposed during 15 - 30 DAS.

		Pn (umol/m2/sec)		RWC(%)		OP(mosmoles)		TB(mM)	
		B0	B25	B0	B25	B0	B25	B0	B25
ICG 476	S1	8.92	9.42	97.2	98.5	481	372	10.8	65.8
	S2	7.41	10.51	97.6	97.8	356	289	12.6	70.1
	S3	7.61	11.25	96.6	99.9	629	391	10.9	67.9
	Mean	7.98	10.39	97.1	98.7	489	351	11.4	67.9
TAG 24	S1	7.41	10.12	97.2	97.3	589	317	12.6	79.0
	S2	6.56	11.52	98.1	99.9	681	371	14.8	67.9
	S3	9.56	9.74	97.3	97.9	412	319	13.5	60.3
	Mean	7.84	10.46	97.5	98.3	561	336	13.6	69.1
CSMG 84-1	S1	6.59	10.54	97.3	98.8	619	289	14.7	70.3
	S2	9.51	10.51	97.3	98.2	599	395	13.4	69.4
	S3	9.51	10.56	98.8	98.9	569	282	12.8	69.0
	Mean	8.54	10.54	97.8	98.6	596	322	13.6	69.6
	G mean	8.1200	10.4633	97.5	98.6	548	336	12.9	68.9
	SeM	±1.567		± 9.987		± 21		± 6.98	
	CV %	12.54		23.5		20.9		18.8	

	ANALYSIS OF VARIANCE				
source of variation	df	Pn	Rwc	op	TB
MT (stress levels)(S)	2	NS	NS	NS	NS
ST (genotypes)(G)	2	NS	NS	*	NS
G X S	4	NS	NS	**	**
SST (betaine levels)(B)	1	**	NS	**	**
S X B	2	**	NS	**	NS
G X B	2	**	NS	NS	NS
G X S X B	4	NS	NS	NS	NS

Osmotic potentials (OP)

Genotypic differences were significan ($P < 0.05$) with OP, wherein CSMG 84-1 recorded a highest OP of 460 milliosmoles compared to 420 in ICG 476 and TAG 24 (Table 4.3.5). Salinity stress imposed at 15 - 30 DAS was found to be marginal. On an average OP was 444 in S_1 treatment and 433 in S_3 treatment showing no significant differences in OP. G x S interaction was found to be significant ($P < 0.01$) , whereas in ICG 476 the OP increased from 426 to 510 milliosmoles with an imposition of salinity stress in TAG 24 and 454 - 497 milliosmoles in CSMG 84-1. Betaine levels were found to be significantly different ($P < 0.01$) for OP, overall the B_{25} treatment decreased OP by 38%. S x B interaction was significantly different ($P < 0.01$), the decrease in OP were 45% in B_{25} treatment in S_1, whereas it was 35 and 38% in S_2 and S_3 respectively. G x B, G x S x B interactions were not significant (Table 4.3.5).

Total betaine content (TB) (mM)

Neither genotypic differences, nor salinity stress differences were significant for TB content. G x S , S x B, G x B, G x S x B interactions were found to be not significantly different (Table 4.3.5). Whereas the B_{25} treatment increased the total betaine content on an average by 430%showing a significant positive increase ($P < 0.01$) of TB with B_{25} treatment. The TB content ranged from 10 to 14mM in B_0 treatment and 65 - 79 in B_{25} treatment (Table 4.3.5).

4. 3. 6 Correlations

Correlations of all the parameters with the total betaine content were studied, a significant positive correlations were observed between total betaine content and

Table: 4.3.6 Correlation coefficients

TB : RGR	0.471*
TB : SGR	0.244^{NS}
TB : CGR	0.306^{NS}
TB : Pn	0.772**
TB : RWC	0.572**
TB : OP	-0.798**

photosynthetic rates (0.772), total betaine content and RGR (0.471), total betaine content and RWC (0.572) and a significant negative correlation was observed between total betaine content and osmotic potentials, all the other parameters ha no correlation with total betaine content.

4.4 Glycine betaine and water stress

As explained in materials and methods section (3.4), the seeds of 3 genotypes (ICG 476, TAG 24 and CSMG 84-1) are either treated with distilled water (B_0) or 25 mM of glycine betaine (B_{25}). The seeds after priming with treatment solutions were planted in pots of size 180 mm diameter filled with sand and soil in the ratio of 2:1. The pots were adequately irrigated until 30 DAS after which 2 watering regimes, 100% field capacity (FC), (I_1), and 50% FC (I_2) were imposed from 30-60 DAS, the pots were randomised within each of the three replications. Observations on root, shoot growth, transpiration, specific leaf area (SLA) and water relations were made as described in materials and methods section (3.4).

4.4.1 Root and Main stem lengths (cm)

At 30 DAS, before the start of watering regimes, root length in B_0 treatment varied from 12.2 to 13.4 cm while with B_{25} treatment the root lengths were 25% higher than B_0 treatment representing a significant increase over control, however there is no genotypic difference neither there was G x B interaction at this stage (Table 4. 4. 1).

The main stem length under B_0 treatment ranged from 15-16 cm whereas with B_{25} treatment, the main stem length increased 15% over control representing a significant

Table : 4. 4. 1 Effect of seed treatment with betaine on root (RL) and main stem length (SL) of three groundnut genotypes at 30 DAS.

	RL (cm)		MSL (cm)	
	B0	B25	B0	B25
ICG 476	12.3	14.8	15.1	16.6
TAG 24	12.2	15.6	15.0	17.2
CSMG 84-1	13.4	16.0	16.1	18.3
G mean	12.6	15.4	15.4	17.3
SeM	± 0.423		± 0.48	
CV %	7.7		9.8	

		Analysis of variance	
Source of variation	df	RL	MSL
MT (genotypes)(G)	2	NS	NS
ST (betaine levels)(B)	1	**	**
G X B	2	NS	NS

difference ($P<0.05$). As observed for root lengths shoot lengths did not differ significantly among genotypes.

4.4.2 Root and Main stem development (cm day^{-1})

Water stress imposed from 30-60 DAS (I_2) resulted in significant reduction in the rate of root and shoot development in all the genotypes, the rate of root development reduced by 124% under I_2 treatment in B_0 and by 80% under B_{25} treatment, although genotypes showed significant variation in their response to water stress in terms of root development for example, the reduction in root development due to water stress was 10% in ICG 476, 7% in TAG 24 and 86% in CSMG 84-1 (Fig 4.4.2 (b)). Seed treatment with B_{25} resulted in an overall increase of rate of root development, which was 0.14 cm day^{-1} in B_0 and 0.17 cm day^{-1} in B_{25} representing a significant effect of B_{25} treatment (Fig 4.4.2(a). The G x B treatment interaction was significant for example, in ICG 476. The rate of root development increased from 0.16 cm/day in B0 and 0.14 cm day^{-1} with B_{25} treatment representing genotypic specificity for positive effects of betaine. Imposition of water stress treatment resulted in 56% reduction with B_0 treatment, whereas with B_{25} treatment, the reduction in root growth was only 45%.

The shoot development was 0.28 cm day^{-1} in B_0 treatment whereas it was 0.35 cm day^{-1} in B_{25} treatment representing effect of betaine on stem expansion(Fig 4.4.2(a). Imposition of water stress treatment resulted in an overall reduction in rate of main stem development of 35% under B_0 and with B_{25} the reduction was 32% (Fig 4.4.2(b)). Overall, it was clear that effect of water stress was more in root growth (55%) compared to that in shoot growth (35%). Further, it was clear that positive effects of betaines are

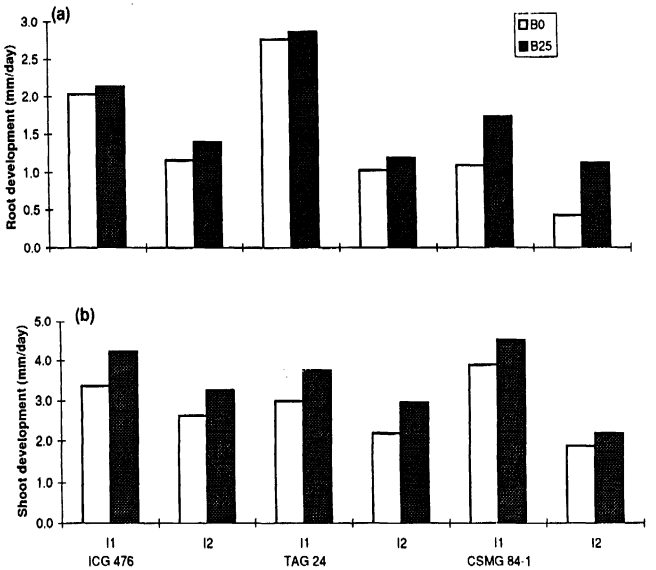


Fig : 4.4.2(a) Effect of seed treatment with betaine on the rate of root (RD) and main stem (MSD) development of three groundnut genotypes during water stress imposed from 30 - 60 DAS.

		Analysis of variance	
source of variation	df	RD	MSD
MT (stress levels)(S)	1	**	**
ST (genotypes)(G)	2	**	NS
G X S	2	**	**
SST (betaine levels)(B)	1	**	**
S X B	1	NS	NS
G X B	2	*	NS
G X S X B	4	NS	NS

seen in alleviating water stress on root development compared to the main stem, this observation supports the earlier findings observed in the germinating seedlings.

4.4.3. Effect of betaine on seedling growth and development.

At 30 DAS, the root weight ranged from 0.93 - 0.95 among genotypes under with B_0 reatment, with B_{25} seed treatment, the root growth increased by 10% in B_{25} compared to B_0. Genotypes had no significant difference for root weights, betaine levels and interaction were found to be significant (Table 4.4.3).

The average stem weight with B_0 treatment was 1.4 g plant^{-1} with no significant genotypic differences. In seed treatment with B_{25} the stem weight ranged from 1.7 g in CSMG 84-1 to 2.1 g in TAG 24 representing an overall increase of 25% in B_{25} compared to B_0. In general there was higher root shoot ratios in B_0 treatment than that in B_{25} treatment, the higher root shoot ratios was apparently manifested by greater shooter growth with betaine treatment, it was also clear that the increase in the shoot growth was contributed by an increase in leaf area under B_{25} treatment. The leaf areas plant^{-1} ranged from 200 cm^2 to 216 cm^2 with B_0 whereas the leaf areas plant^{-1} ranged from 240 in ICG 476 to 268 cm^2 in representing an overall increase of 18% in B_{25} treatment. The positive increase of seed treatment with B_{25} resulted in higher dry matter accumulation in B_{25} compared to that in B_0. It was apparent that total day matter plant^{-1} was about 2.3 g in B_0 treatment whereas, the TDM ranged from 2.6 to 3g in B_{25} treatment, there is an overall increase of 12% with B_{25} treatment in total dry matter (Table 4.4.3).

Table : 4. 4. 3 Effect of seed treatment with betaine on root and shoot dry matter, leaf area (LA), total dry matter (TDM) at 30 DAS.

	Rwt (g/plant)		Lwt (g/plant)		Stwt (g/plant)		Swt(g/plant)		RS (g/g)		LA (cm2/plant)		TDM (mg/plant)	
	B0	B25	B0	B25	B0	B25	B0	B25	B0	B25	B0	B25	B0	B25
ICG 476	0.93	0.98	0.41	0.89	1.01	0.97	1.43	1.86	0.652	0.532	215	239	2.3	2.8
TAG 24	0.95	1.06	0.54	0.97	0.68	1.07	1.22	2.04	0.786	0.519	200	260	2.2	3.0
CSMG 84-1	0.95	0.94	0.78	1.07	0.78	0.68	1.56	1.66	0.652	0.586	213	268	2.4	2.6
G mean	0.94	1.00	0.58	0.98	0.82	0.91	1.40	1.85	0.697	0.546	209	256	2.3	2.8
SeM ±	0.022		0.032		0.055		0.044		0.008		8.7		0.08	
CV %	10.9		10.8		19.3		20.9		20.7		21.8		22.9	

ANALYSIS OF VARIANCE

Source of variation	df	Rwt	Lwt	Stwt	Swt	RS	LA	TDM
MT (genotypes)(G)	2	NS	**	**	NS	*	NS	NS
ST (betaine levels)(B)	1	*	**	NS	**	**	**	**
G X B	2	Ns	NS	**	**	*	NS	**

4.4.4 Effect of betaine on sensitivity of groundnut to water stress.

Genotypes differed significantly for root and shoot growth rates and other physiological parameters such as leaf area development, crop growth rate and net assimilation rate (Table 4.4.4).

Root growth rate:

CSMG 84-1 showed greatest root growth rate 45mg plant^{-1} day^{-1} followed by TAG 24 (40 mg plant^{-1} day^{-1}) and ICG 476 (20 mg plant^{-1} day^{-1}). Imposition of water stress resuted in an overall reduction of 43% in root growth rate in B_0 treatment whereas in B_{25} treatment, the mean reduction in root growth rate due to water stress treatment was 39% suggesting alleviating effects of betaine on root growth and development in the water stress (Table 4.4.4). The genotype x betaine interaction was significant with 2 genotypes i.e., ICG 476 and CSMG 84-1 showing a significant 50% increase in the root growth rate with B_{25} treatment.

Similarly G x S interaction was significant in root growth rate with ICG 476 showing the 70% reduction in root growth rate under water stress, the reduction in root growth rate under water stress was 27% in case of CSMG 84-1and 22% in case of TAG 24.

Shoot growth rate:

Genotypes differed significantly in shoot growth rates with TAG 24 having the greatest shoot growth rate 280 mg plant^{-1} day^{-1} and CSMG 84-1 having the least shoot growth rate (60 mg plant^{-1} day^{-1}). Imposition of water stress resulted in reduction of

shoot growth rate in 150 mg plant-1 day-1 in control to 100mg plant-1 day-1 in water stress. G x S interactiion was also significant with CMG 84-1 showing very little effect of water stress on shoot growth rate (60 in both I_1 and I_2 treatments), whereas ICG 476 and TAG 24 showed significant reduction in shoot growth rate due to water stress (Table 4.4.4).

Seed treatment with betaine showed increased shoot growth rate (176 g/plant/day) compared to 124 in B_0 treatment under control conditions. A similar increase in shoot growth rate was also observed under the water stress treatments, the mean shoot growth rate was 110 in B_0 treatment whereas it was 154 with betaine treatment.

G x B interaction was significant with CSMG 84-1 showing an increase 60% due to B_{25} treatment whereas the increase due to betaine treatment 39% in TAG 24 and 36% in ICG 476. However, G x S x B interactioin was significant at 5% suggesting the sensitivity of betaines to G x S interaction.

Rate of expansion of leaf area (cm^2 day^{-1})

Leaf area expansion rate ranged from 1.5 to 2.5 cm^2 day^{-1} in B_0 treatment and 1-8 to 2.7 cm^2 day^{-1} in B_{25} treatment. Overall genotypes differed significantly with ICG 476 having the greatest leaf area development (2.35) compared to 1.99 cm^2 day^{-1} in CSMG 84-1 and 1.71 in TAG 24. The rate of leaf expansion reduced from 2.2 in I_1 to 1.9 with I_2 representing a significant effect of water stress on leaf expansion. Genotype x water stress interaction was significant with ICG 476 showing significant reduction in the rate of leaf expansion (21%) whereas, the reduction in leaf expansion due to water stress was only 4% in TAG 24 and 12% in CSMG 84-1. However, the effects of betaine on leaf

area expansion were marginal. The rate of leaf expansion was 2 cm^2 day^{-1} in B_0 compared to 2.03 in B_{25}. The genotype x betaine interaction was not significant. Similarly G x S x B interaction was not significant on leaf area expansion (Table 4.4.4)

Crop Growth Rate (mg plant $^{-1}$ day $^{-1}$)

Genotypes differed significantly in Crop growth rates which ranged from 110 to 280mg $plant^{-1}$ day^{-1} in B_0 and 190 to 300 with B_{25} treatment. CSMG 84-1 had greatest CGR (250). Imposition of water stress resulted in a reduction of 33% in Crop growth rate representing by effects of water stress. The G x S interaction was significant with ICG 476 and CSMG 84-1 showing greatest reduction in water stress (34%) compared to 28.5% in TAG 24. The B_{25} treatment resulted in an increase (27% to 33%) in all the three genotypes. However, G x B interaction was not observed (Table 4.4.4 and Fig : 4.4.4)

Net assimilation rate (g m^{-2} day^{-1})

NAR ranged from 3.6 - 7.3 representing a significant variation due to the treatments. The genotypes differed significantly with CSMG 84-1 having the greatest CGR (6.1) followed by ICG 476 (5.3) and TAG 24 (4.6). Imposition of water stress resulted in an overall reduction of 30% in NAR genotypes have shown marginal differences however, the reduction in NAR due to water stress in ICG 476. Seed treatment with betaine resulted in a overall increase of 15% in NAR. Howeverr, the effect of betaines on NAR varied with genotypes. For example, the increase in NAR was of the order of 3.78, in CSMG, 15.8 in TAG 24 and 28.4 in ICG 476 (Table 4.4.4 and Fig 4.4.4).

Table : 4. 4. 4 Effect of seet treatment with betaine on root growth rate (RGR), shoot growth rate (SGR), leaf area development (LA),crop growth rate (CGR) during water stress imposed from 30 - 60 DAS.

		RGR (mg/plant/day)		SGR (mg/plant/day)		LA (cm2/day)		CGR (mg/plant/day)		NAR(g/m2/day)	
		B0	B25	B0	B25	B0	B25	B0	B25	B0	B25
ICG 476	I1	40.0	60.0	128.0	180.0	2.5	2.7	217.0	250.0	5.7	6.9
	I2	10.0	20.0	101.0	130.0	2.3	1.9	115.0	193.0	3.6	5.0
	Mean	**25.0**	**40.0**	**114.5**	**155.0**	**2.4**	**2.3**	**166.0**	**221.5**	**4.6**	**5.9**
TAG 24	I1	45.0	40.0	195.0	280.0	1.7	1.8	203.0	260.0	4.8	5.9
	I2	30.0	30.0	143.0	190.0	1.6	1.8	137.0	194.0	3.8	4.0
	Mean	**37.5**	**35.0**	**169.0**	**235.0**	**1.7**	**1.8**	**170.0**	**227.0**	**4.3**	**4.9**
CSMG 84-1	I1	40.0	70.0	50.0	69.0	2.4	1.8	278.0	320.0	7.0	7.3
	I2	30.0	50.0	43.0	80.0	1.5	2.3	159.0	237.0	4.9	5.0
	Mean	**35.0**	**60.0**	**46.5**	**74.5**	**2.0**	**2.0**	**218.5**	**278.5**	**5.9**	**6.2**
	G mean	**32.5**	**45.0**	**110.0**	**154.8**	**2.0**	**2.0**	**184.8**	**242.3**	**4.9**	**5.7**
	SeM ±	1.09		2.45		0.14		25.42		0.65	
	CV %	21.8		9.9		20.1		18.7		19.7	

		ANALYSIS OF VARIANCE				
source of variation	df	RGR	SGR	LA	CGR	NAR
MT (stress levels)(S)	1	**	**	**	*	*
ST (genotypes)(G)	2	**	**	**	NS	*
G X S	2	**	**	NS	NS	*
SST (betaine levels)(B)	1	**	**	Ns	*	**
S X B	1	**	NS	NS	NS	NS
G X B	2	**	**	NS	*	*
G X S X B	4	*	**	NS	NS	NS

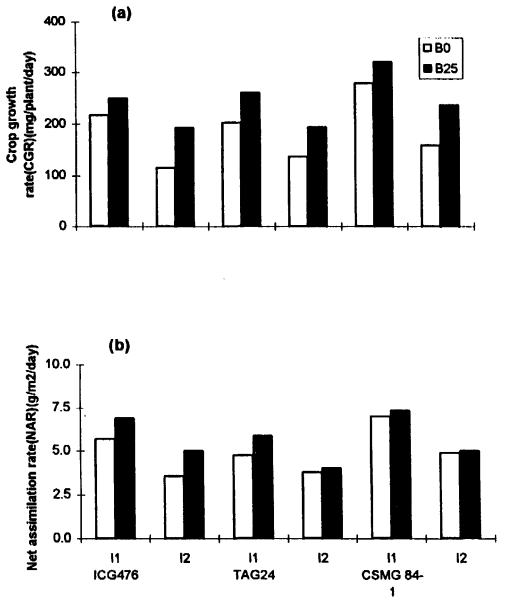


Fig : 4.4.4 Effect of seed treatment of betaine on crop growth rate(a) and net assimilation rate (b) during water stress imposed from 30 - 60DAS

4. 4. 5 Dry matter production and water use efficiency

Effect of betaine treatment has been examined by quantifying traits (transpiration (T), and water use efficiency (WUE)) that contributed to dry matter production.

All the genotypes irrespective of betaine and stress levels used similar quantities of water (2.7 -4.6 kg in I_1 and 2.4 to 3.1 kg in I_2) but were significantly different in dry matter production which ranged from 8.9 - 10.9 g plant^{-1} in I_1 and 5.6 -9.9 g plant^{-1} in I_2 during the treatment period resulting in a significant variability in WUE between genotypes (2.2 -4.7 g kg^{-1} in I_1 and 2.2 to 3.7 g kg^{-1} in I_2). Genotype TAG 24 with B_{25} treatment had the highest WUE in treatment I_1 (4.7g kg^{-1}) and the same genotype had a WUE of 3.8 g kg^{-1} in water limited conditions (I_2). Genotype ICG 476 recorded lowest WUE in both irrigation treatments.

Correlation of water use efficiency with transpiration and dry matter produced during 30 - 60 DAS were studied, there was a negative correlation (-0.33) between WUE and transpiration, water use efficiency and SLA (-0.512) had a significant negative correlation. A significant positive correlation (0.77) between transpiration and dry matter produced was observed, all the other correlations were not significant (Table 4.4.7).

4. 4. 6 Water relations

Relative water content

Betaine levels were found to be significantly different for RWC. The genotypic and betaine differences were not significant for relative water content and G x B, G x S, S x B

Table : 4. 4. 6 Effect of seed treatment with betaine on relative water content (RWC), leaf Water potential(ψp), total betaine (TB) (mM) and fluoroscence ratio (Fv/Fm) during water stress impose during 30 - 60 DAS.

		RWC %		ψp (Mpa)		Total betaine (mM)		Fv/Fm	
		B0	B25	B0	B25	B0	B25	B0	B25
ICG 476	I1	93.7	94.6	-2.1	-1.9	11.5	62.4	0.89	1.00
	I2	74.7	78.6	-4.5	-2.9	10.7	56.3	0.67	0.79
	Mean	84.2	86.6	-3.3	-2.4	11.1	59.4	0.67	0.89
TAG 24	I1	92.8	97.2	-2.5	-1.9	12.6	60.4	0.86	0.95
	I2	75.8	78.8	-3.1	-2.6	13.0	63.6	0.65	0.88
	Mean	84.3	88.0	-2.8	-2.3	12.8	62.0	0.65	0.95
CSMG 84-1	I1	90.7	92.6	-2.8	-1.9	11.6	52.4	0.88	0.91
	I2	75.3	77.7	-3.2	-2.3	15.6	86.5	0.56	0.91
	Mean	83.0	85.1	-3.0	-2.1	13.6	69.5	0.72	0.91
	G mean	83.8	86.6	-3.0	-2.3	12.5	63.6	0.68	0.92
	SeM	± 10.90		± 0.58		± 1.45		± 0.10	
	CV %	19.5		18.3		21.4		23.9	

ANALYSIS OF VARIANCE

source of variation	df	Rwc	LWP	Tot bet	Fv/Fm
MT (stress levels)(S)	1	**	**	NS	*
ST (genotypes)(G)	2	NS	NS	NS	NS
G X S	2	NS	NS	NS	NS
SST (betaine levels)(B)	1	NS	*	**	*
S X B	2	NS	NS	*	*
G X B	2	*	*	NS	*
G X S X B	4	NS	*	*	*

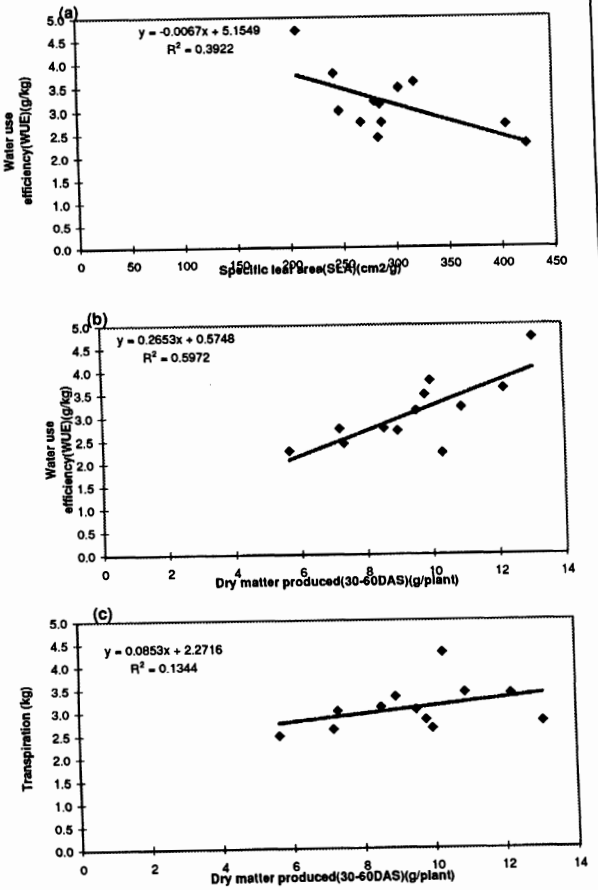


Fig : 4. 4. 5 Relationship between water use efficiency (WUE) with mean specific leaf area (a), dry matter produced during the water stress period(b) and transpiration (c) of three groundnut cultivars under two drought treatments.

and G x S x B interactions were also not significant. In I_1 treatment the RWC ranged from 90 - 97% in all the genotypes and in I_2 RWC ranged from 74 - 78% (Table 4.4.6).

Leaf water potential:

Leaf water potentials differed significantly ($P<0.05$) among stress levels, genotypic differences were not significant, where as leaf water potential differed significantly ($P<0.01$) with betaine treatments, the G x S , S x B interactions were not significant and the G x B, G x S x B interactions were found to be significant.

In I_1 the mean ψW was -2.2Mpa, whereas with I_2 treatment the mean LWP was -3.1Mpa. ψW ranged from -2.4 to -3.6 Mpa B_0 and -1.9 to -2.6 MPa in B_{25} treatments. ICG 476 with B_0 treatment had the lowest ψW (-3.3) and the highest ψW (-2.1) was recorded in CSMG 84 - 1 genotype (Table 4.4.6).

Total betaine content (mM)

Genotypic and stress differences were not observed for total betaine content, betaine differences were significant for the total betaine content ($P<0.01$), G x S, G x B interactions were not significant while the S x B and G x S x B interactions were significant (Table 4.4.6). Total betaine content ranged from 11.8 to 13.1 mM in B_0 treatment and 58.3 to 68.8 mM in B_{25} treatment. I_1 treatment with B_{25} recorded less total betaine content (58.3) and the highest was in I_2 with B_{25} treatment.

Fluorescence ratio (Fv/Fm):

The genotypic differences were not significantly different, while the stress and betaine level differences were highly significant ($P<0.01$) for Fv/Fm and as shown by

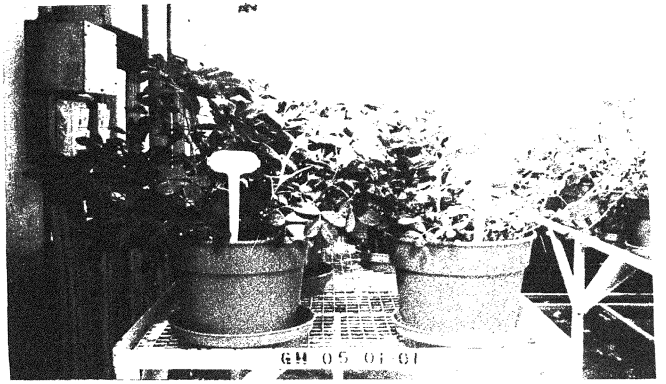


Plate 11 : Effect of glycine betaine on groundnut plants at 30 DAS (TAG 24) in glass house during the study of glycine betaine effects on water stress imposed from 30-60 DAS.

Plate 12 : Effect of glycine betaine on groundnut seedlings of TAG 24 at 30 DAS, during the study of glycine betaine effects on water stress imposed from 30-60 DAS.

Table : 4.4.7 Correlation coefficients

	Correlation coefficient
TB : RGR	0.384
TB : SGR	0.266
TB : CGR	0.439
TB :NAR	0.208
TB : WUE	0.471*
TB : T	0.156
TB : RWC	0.048
TB : Fv / Fm	0.567*
TB : ψ p	0.528*
T : WUE	-0.33
T : DM	0.77**
WUE : SLA	-0.51*
T : DM	0.34

significant G x S, G x B, S x B, G x S x B interactions ($P<0.05$) (Table 4.4.6) . The Fv/Fm ratio decreased with imposition of water stress treatment in all the genotypes studied. The mean Fv/Fm ratio was 0.915 in I_1 and 0.743 in I_2 treatments. Betaine treatments increased Fv/Fm ratio from 0.75 in B_0 to 0.9 in B_{25} treatments. TAG 24 had the highest Fv/Fm ratio (0.951) in B_{25} treatment and the least in (0.562) in CSMG 84-1 genotype with B_0 treatment.

4. 4. 7 Correlations

Correlations of total betaine content with all the parameters were observed, there was no significant positive correlation of total betaine content with WUE (0.471), Fv/Fm ratios (0.567) with total dry matter (0.77)all the parameters.

4. 5 Glycine betaine and high temperature stress

As explained in materials and methods section (3. 5), the seeds of 2 genotypes (ICG 476 and TAG24) are either treated with distilled water (B_0) or 25mM of glycine betaine (B_{25}). The seeds after priming with tretament solutions were planted in pots of size 101mm diameter filled with sand, soil and vermiculite in the ratio of 2:1:1, the plants were grown in glass house at 30^0C upto 15DAS and then 1 set was shifted to a growth chamber (0.75 x 1.82 x 1.4m) at temperatures of 30^0C and 60 - 70% relative humidity, this serves as a control (HT1), and the second set was shifted to another growth chamber of same dimensions and this programmed so as to simulate the naturally occuring diurnal rhytm of temperatures (HT2) as explained in the Fig : 3. 5. The experiment was terminated at 45DAS. Observations on root, shoot growths, specific leaf area water relations were made as described in the materials and methods section 3. 5.

Table 4. 5. 1 : Effect of seed treatment with betaine on root length (rl),shoot length (sl)at 15DAS

	rl(cm)		sl (cm)	
	B0	**B25**	**B0**	**B25**
ICG 476	9.9	10.2	10.8	10.9
TAG24	10.1	10.5	11.3	11.5
G mean	**10.0**	**10.3**	**11.1**	**11.2**
Se M	**± 0.151**		**± 0.017**	
CV %	**2.3**		**1.8**	

	Analysis of variance		
Source of variation	**df**	**rl**	**sl**
MT (genotypes)(G)	**1**	**NS**	**
ST(betaine levels)(B)	**1**	*	**
G X B	**1**	**NS**	*

* Significant at P= 0.05;
** Significant at P=001;
NS Non Significant

4. 5. 1 Root and main stem lengths

At 15 DAS, before the start of high temperature regimes, root length in B_0 treatment varied from 9.9 (ICG 476) to 10.1(TAG 24), while with B_{25} treatment the root lengths were 10% higher than that of the B_0 treatment (Table 4. 5. 1). However there was no genotypic difference and no significant G x B interaction at this stage.

The min stem lengths under B_0 treatment ranged from 10.83(ICG 476) and 11.33 (TAG 24), whereas with B_{25} treatment the main stem lengths increased and ICG 476 had a stem length of 10.9 cm and TAG 24 with 11.53 showing a significant response to betaines. The genotypic variation is observed in main stem lengths, where in which on an average the ICG 476 recorded 10.86 cm, where as TAG24 recorded a stem length of 11.43 cm (Table 4. 5. 1).

4. 5. 2 Root and main stem development

High temperature stress imposed from 15 to 30 DAS resulted in a signifcant reduction in the rate of root and shoot development in both the genotypes (Table 4. 5. 2). HT2 on an average could decrease the root development by 12%. Genotype ICG 476 had more root development (0.263 cm day^{-1}) than that of the TAG24 (0.287 cm day^{-1}). G x S interaction was also found to be significant, in ICG 476 the reduction in the root development due to high temperature stress was only 4%, whereas in TAG 24 the reduction was about 20%. Betaine treatment had a significant positive response showing on an average 22% increase in B_{25} treatment compared to B_0 treatment. S x B interaction was found to be significant, the rate of root development reduced by 18% under B_0 treatment, and it reduced by only 6% in B_{25} treatment. G x B interaction was

Table 4. 5. 2 :Effect of betaine treatment on root dry weight (Rwt), shoot dry weight (Swt), leaf area (LA), total dry matter (TDM) at 15DAS.

	Rwt(g/plant)		Swt(g/plant)		RS(g/g)		LA (cm2)		TDM (g/plant)	
	B0	B25	B0	B25	B0	B25	B0	B25	B0	B25
ICG 476	0.46	0.52	0.68	0.78	0.68	0.67	158	193	1.13	1.30
TAG24	0.41	0.60	0.67	0.79	0.61	0.76	180	200	1.08	1.39
G mean	**0.43**	**0.56**	**0.67**	**0.78**	**0.64**	**0.72**	**169**	**197**	**1.11**	**1.35**
Se M	± 0.027		± 0.012		± 0.120		± 3.945		0.018	
CV %	9.6		9.8		10.7		7.6		8.6	

Analysis of variance						
Source of variation	df	rwt	Swt	RS	LA	TDM
MT (genotypes)(G)	1	NS	NS	NS	NS	NS
ST(betaine levels)(B)	1	*	**	*	**	**
G X B	1	NS	NS	NS	NS	**

* Significant at P= 0.05;
** Significant at P=001;
NS Non Significant

also significant, in ICG 476 the root development increased by 43% with B_{25} treatment and in TAG 24 the B_{25} treatment could increase the root development by only 5%. G x S x B interaction was not significant (Table 4. 5. 2).

4. 5. 3 Seedling growth and development.

At 15 DAS, the root weights had no genoypic variation, but a significant ($P < 0.05$) positive response due to betaine treatment was observed (Table 4. 5. 3). The B_{25} treatment could increase the root weights by 30% on an average, and the root weights ranged from 0.46 to 0.41 in B_0 treatment and 0.52 to 0.61 g plant^{-1} in B_{25} treatment.

The average shoot weight with B_0 treatment was 0.67 g plant^{-1} and 0.78 g/plant in B_{25} treatment. There was no genotypic difference at this stage, whereas with betaine treatment the differences were significant, and the B_{25} treatment could increase the shoot weights by 17%. G x B interaction was not significant(Table 4. 5. 3).

Root shoot ratios were found to have no genotypic differences, and there was a significant difference in RS ratio with betaine treatments ($P < 0.05$), the B_{25} treatment on an average could increase the RS ratio by 11%. G x B interation was not significant a this stage (Table 4. 5. 3).

There were no significant differences between genotypes when observed for leaf areas, there was a significant ($P < 0.01$) positive response due to B_{25} treatment. The B_{25} treatment could increase the leaf areas by 16%. The leaf areas ranged from 158 to 180 cm^2 plant^{-1} in B_0 treatment, whereas with B_{25} treatment the range is 193 to 200 cm^2 plant^{-1}. GxB interaction was not significant (Table 4. 5. 3).

Table : 4. 5. 3 Influence of high temperature stress and betaine treatments on root (RD) and main stem (MsD) development of three groundnut genotypes.

		RD (cm/day)		MsD (cm/day)	
		B0	B25	B0	B25
ICG 476	HT1	0.233	0.301	0.391	0.431
	HT2	0.201	0.318	0.212	0.137
	Mean	0.217	0.310	0.302	0.284
TAG 24	HT1	0.318	0.317	0.347	0.345
	HT2	0.247	0.266	0.222	0.138
	Mean	0.283	0.292	0.285	0.242
	G mean	0.25	0.30	0.29	0.26
	Se M	± 0.0049		± 0.0085	
	CV %	7.8		6.6	

Analysis of variance

source of variation	df	RD	SD
Main trts(stress)(S)	1	**	**
sub trts	1	**	**
geno(G)			
G X S	1	**	**
SST (betaine levels)	1	**	**
S X B	1	*	*
G X B	1	**	NS
G X S X B	1	NS	NS

* Significant at P= 0.05;
** Significant at P=001;
NS Non Significant

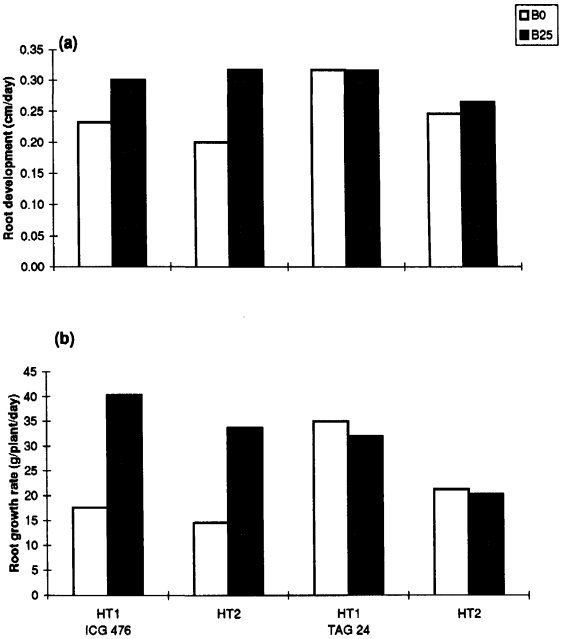


Fig :4. 5.3 Effect of seed treatment of betaine on root development (a) and root growth rate (b) in three groundnut genotypes under two high temperature stress treatments.

The positive response of seed treatment with betaine resulted in a increase in total dry matter by 23% (Table 4. 5. 3). The differences due to betaine treatment were significant ($P < 0.01$) and the genotypic variation was not observed. G x B interaction was significant ($P < 0.01$).

4. 5. 4 Growth components

Root growth rate (RGR) (mg plant^{-1} day^{-1})

Genotypes did not differ significantly for root growth rates, where as G x S interaction was found to be significant (Table 4. 5. 4). The HT2 treatment could decrease the RGR by 17% in ICG 476 and 38% in TAG 24. Betaine levels differed significantly, The B_{25} treatment could increase the RGR by 42% over all. S x B, G x B, G x S x B interactions were not significant.

Shoot growth rate (SGR) (mg plant^{-1} day^{-1})

Shoot growth rates were significantly different ($P < 0.01$) among genotypes and stress levels (Table 4. 5. 4). HT2 could decrease the SGR on an average by 22%. The genotypes varied significantly for SGR ($P < 0.01$), highest SGR was in TAG 24 genotype (74.7mg plant^{-1} day^{-1}) and ICG 476 had SGR of only 56.1mg plant^{-1} day^{-1}. G x S interaction was significant ($P < 0.05$) wherein which in ICG 476 HT2 could decrease the SGR by 5%, whereas in TAG 24 the reduction due to HT2 treatment was 33%. Betaine levels were also significant ($P < 0.01$), and the B_{25} treatment could decrease the SGR by

14%. S x B interaction was significant ($P < 0.05$), in HT1 treatment the decrease in SGR by B_{25} treatment was not significant and it was marginal whereas in HT2 treatment the decrease due to SGR was 27%. The G x B was also significant, in ICG 476 the B_{25} treatment could decrease the SGR by 20%, whereas in TAG 24 the decrease was only 10%. G x S x B interaction was not significant (Table 4. 5. 4).

Rate of expansion of leaf area (cm^2 day^{-1} $plant^{-1}$)

Leaf area expansion rate ranged from 1.8 to 4.7 cm^2 day^{-1} $plant^{-1}$ in B_0 treatment whereas in B_{25} treatment it ranged from 0.7 to 3.1 cm^2 day^{-1} $plant^{-1}$, showing a significant negative response ($P < 0.01$) of B_{25} treatment decreasing the LAER by 48%. Genotypic differences were also significant ($P < 0.05$), TAG 24 had LAER of 2.1 cm^2 day^{-1} $plant^{-1}$ whereas it was 2.9 cm^2 day^{-1} $plant^{-1}$ in ICG 476. Stress levels were also significant ($P < 0.01$), on an average HT2 treatment decreased the LAER by 56%. G x S, S x B, G x B, G x S x B interactions were not significant (Table 4. 5. 4).

Crop growth rate (CGR) (mg plant $^{-1}$ day $^{-1}$)

Genotypes differed significantly in CGR ($P < 0.05$), in ICG 476 the CGR was only 83.4 mg plant-1 day-1 whereas in TAG 24 the CGR was found to be 102 mg $plant^{-1}$ day^{-1} (Table4.5.4) Stress level s were significantly different ($P < 0.05$), HT2 treatment could decrease the CGR by 24%. G x S interaction was significant , in ICG 476 the HT2 treatment could decrease the CGR by only 8%, whereas in TAG 24 the decrease due to HT2 treatment was 35%. There was no significant difference in betaine levels for CGR. S

Table : 4. 5. 4 Effect of seet treatment with betaine on root growth rate (RGR), shoot growth rate (SGR), leaf area expansion rate (LA),crop growth rate (CGR) during water stress imposed from 30 - 60 DAS.

		RGR (mg/plant/day)		SGR (mg/plant/day)		LAER (cm2/day/plant)		CGR (mg/plant/day)	
		B0	B25	B0	B25	B0	B25	B0	B25
ICG 476	HT1	17.7	40.3	58.0	55.3	4.7	3.1	77.0	97.0
	HT2	14.7	33.7	66.3	44.6	2.6	1.1	81.3	78.3
	Mean	16.2	37.0	62.2	50.0	3.7	2.1	79.2	87.7
TAG 24	HT1	35.0	32.0	90.7	88.3	4.0	2.0	126.0	120.7
	HT2	21.3	20.3	66.7	53.0	1.8	0.7	87.3	73.0
	Mean	28.2	26.2	78.7	70.7	2.9	1.3	106.7	96.8
	G mean	22.2	31.6	70.4	60.3	3.3	1.7	92.9	92.2
	Se M	± 2.17		± 3.25		± 0.320		± 4.48	
	CV %	11.3		10.1		23.8		7.8	

		ANALYSIS OF VARIANCE			
source of variation	df	RGR	SGR	LAER	CGR
MT(stress)(S)	1	NS	**	**	*
ST	1	NS	**	*	*
geno(G)					
G X S	1	**	*	NS	*
SST (betaine levels)	1	**	**	**	NS
S X B	1	NS	*	NS	NS
G X B	1	NS	**	NS	**
G X S X B	1	Ns	NS	NS	**

* Significant at P= 0.05;
** Significant at P=001;
NS Non Significant

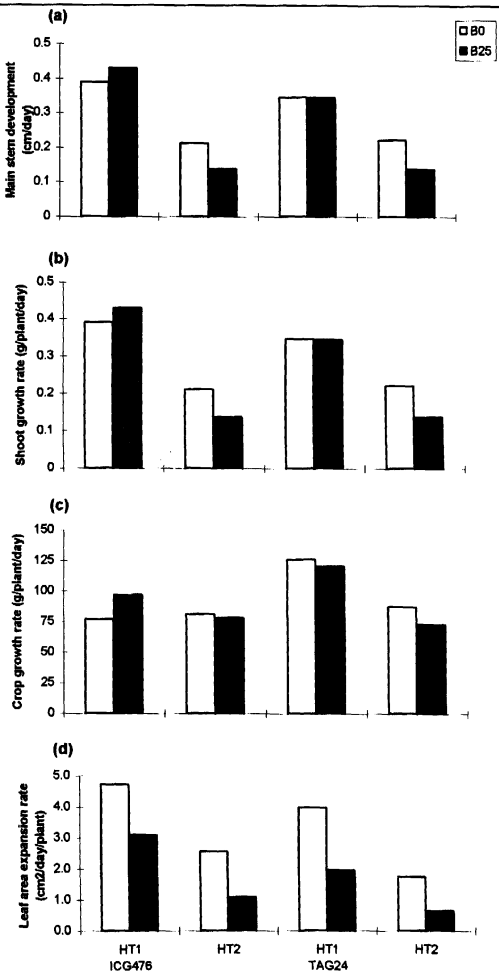


Fig 4. 5. 4 : Effect of seed treatment of betaine on shoot development(a), shoot growth rate(b), crop growth rate (c) and leaf area expansion rate (d) during water stress at 30 - 60 DAS.

x B interaction was also not significant. G x B and G x S x B interactions were significant. In ICG 476 the CGR increased due to B_{25} treatment by 11%, whereas in TAG 24 the differences were marginal.

4. 5. 5 Relative water content (RWC), Leaf water potential (ΨW), Total betaine content (TB), and Fluorescence ratio (Fv/Fm).

Relative water content (RWC) (%)

High temperature stress decreased the RWC significantly ($P < 0.05$), on an average the HT2 treatment could decrease the RWC by 10%. Genotypic variation and G x S interaction were not significant (Table 4. 5. 5). Betaine treatment were found to be significant ($P < 0.05$), on an average the betaine treatment is found to increase the RWC by 6%. S x B interaction was significant ($P < 0.05$), in B_0 treatment the decrease due to HT2 treatment was 10%, whereas in B_{25} treatment the decrease was only 5%. G x B, G x S x B interactions were not significant.

Leaf water potential (ψW) (Mpa)

High temperature stress decreased the ΨW by 44% showing a significant difference due to stress treatment ($P < 0.01$) (Table 4. 5. 5). Genotypes were also significantly different with ICG 476 having an average ΨW of -4.6 Mpa and TAG 24 with a ΨW of -5.7 Mpa. S x B interaction was significant ($P < 0.05$), in B_0 treatment the

decrease in ΨW is 35%, whereas in B_{25} treatment the decrease in ΨW is 24%. G x B and G x S x B interactions were found to be not significant.

Total betaine content (TB) (mM)

Total betaine content did not differ significantly for genotypes and stress levels, the Gx S interaction was also found to be not significant (Table 4. 5. 5), whereas total betaine content differed significantly ($P < 0.01$) with betaine treatments. B_{25} treatment could increase the level of betaine content in the leaf by 356%. S x B and G x S x B interactions were not significant. G x B interaction was significant ($P < 0.05$), In ICG 476 the B_{25} treatment could increase the betaine content by 340% whereas it was 380% in TAG24.

Fluorescence ratio (Fv/Fm)

Stress levels were significantly different for Fv/Fm ratio showing a decrease of 5%. Genotypic variation and G x S interaction were not significant (Table 4. 5. 5). B_{25} treatment could increase the Fv/Fm ratio by 8%. S x B and G x S x B interactions were not significant, whereas the G x B interaction was found to be significantly different. ICG 476 had an increase in Fv/Fm ratio by 14% whereas the difference was marginal in TAG 24.

Table 4. 5. 5 : Effect of betaine treatment on relative water content (RWC) ,leaf Water potential( ),total betaine(TB) and Fv/Fm ratio

		RWC (%)		(bars)		TB(mM)		Fv/ Fm	
		B0	B25	B0	B25	B0	B25	B0	B25
ICG476	HT 1	90.7	92.5	-3.9	-4.8	12.6	65.9	0.836	0.859
vc	HT 2	82.7	86.6	-6.7	-5.8	15.9	59.9	0.764	0.920
	Mean	**86.7**	**89.5**	**-3.9**	**-5.3**	**14.2**	**62.9**	**0.8**	**0.9**
TAG24	HT 1	91.3	93.2	-4.6	-4.1	14.6	61.5	0.894	0.897
	HT 2	80.7	88.9	-6.5	-5.8	12.5	68.6	0.722	0.926
	Mean	**86.0**	**91.1**	**-6.5**	**-5.0**	**13.5**	**65.0**	**0.9**	**0.9**
	G mean	**86.4**	**90.3**	**-5.2**	**-5.1**	**13.9**	**64.0**	**0.847**	**0.900**
	Se M	± 3.36		± 0.220		± 1.87		± 0.254	
	CV %	**17.7**		**12.9**		**20.1**		**12.5**	

Analysis of variance

source of variation	df	RWC	LWP	TB	Fv/FM
Main trts(stress)(S)	1	*	**	NS	*
sub trts geno(G)	1	NS	**	NS	NS
G X S	1	NS	**	NS	NS
sub sub betaine(B) trts	1	*	*	**	*
S X B	1	*	*	NS	NS
G X B	1	NS	NS	*	*
G X S X B	1	NS	NS	NS	NS

* Significant at P= 0.05;
** Significant at P=001;
NS Non Significant

4. 5. 7 Correlation coefficients

	Correlation coefficient
TB : RWC	**0.462***
TB : LWP	**0.125^{NS}**
TB : Fv/Fm	**0.691****
TB : RGR	**0.504***
TB : SGR	**-0.332^{NS}**
TB : CGR	**-0.033^{NS}**
TB : LAER	**-0.595**

In view of the effects of glycine betaine observed in the laboratory and glass house experiments, field experiments were undertaken to study the effect of glycine betaine in the alleviation of drought imposed during mid of the season and end of the season.

4. 5. 6 Correlations

Correlation of total betaine content with all the growth parameters and observations were studied, there was a significant positive correlation (0.6904) between total betaine content Fv/Fm ratio between total betaine content and shoot growth, total betaine content and relative water content (0.461), a significant -ve betaine content and leaf area expansion rate (0.595). (Table 4.4.6).

4. 6 Effect of glycine betaine on sensitivity of groundnut genotypes to mid season drought.

As explained in Materials & Methods section 3.6.1, field experiments were undertaken during the 1996 rainy and 1996/97 post rainy seasons, to examine the effect of mid season and terminal drought stress on groundnut and the role of betaines in alleviating the drought stress. In both the seasons, watering regimes i.e., irrigated and mid season drought were treated as main treatments, genotypes (CSMG 84-1, ICG 476, ICGV 86031, TAG 24, TMV2NLM) as sub treatments & betaine levels (0,3,6,9 kg/ha) (B1, B2, B3, B4) as sub sub treatments.

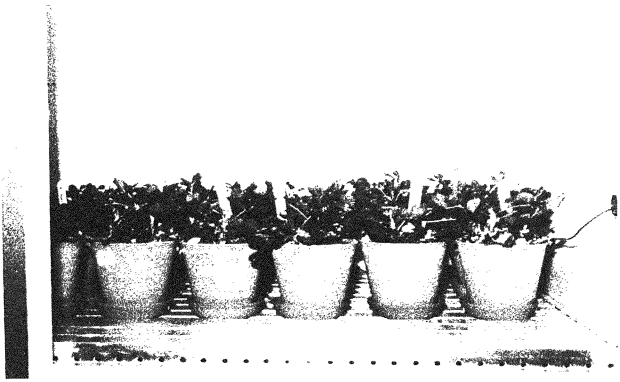


Plate 13 : **Response of treatment with glycine betaine on groundnut seedlings (TAG 24) grown under control conditions in growth chamber - 1.**

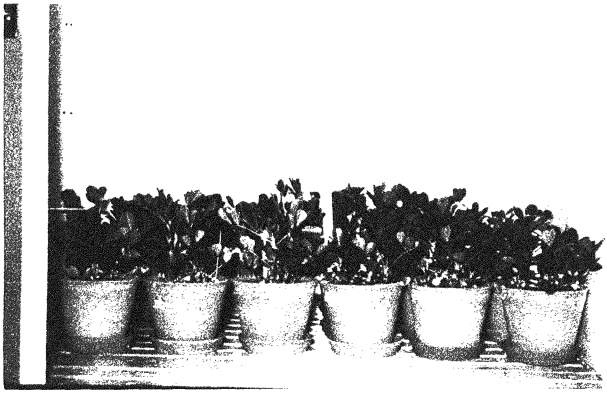


Plate 14 : **Response of treatment with glycine betaine on groundnut seedlings (TAG 24) grown under high temperature stress conditions in the growth chamber - 2**

1996 Rainy season:

During the rainy season the mid season drought spanning from 40-80 DAS was imposed by using portable rain out shelter, while, irrigated treatment received adequate irrigation either by rainfall or supplementary irrigation. The results of the rainy season experiment are given below:

4. 6. 1 Vegetative weight (g m^{-2}), Pod dry weight (g m^{-2}) and harvest index.

Vegetative weight (g m^{-2})

At 100 DAS, effect of drought on shoot growth was significant ($P<0.05$) with the shoot dry weights being 649.6 g m^{-2} in irrigated and 369.9 g m^{-2} under mid season drought conditions (Table 4.6.1). Genotypes also differed significantly ($P<0.05$) for shoot dry weights with TMV2NLM having greatest shoot dry weight (652.8 g m^{-2}) and CSMG84-1 having the least shoot dry weight (233.1 g m^{-2}). S x G interaction was significant ($P<0.05$). The genotypes differed significantly in their reduction of shoot growth due to drought, i.e., in 56% CSMG 84-1, 37% in ICG 476, 43% in TAG 24, 53% in ICGV 86031 and 30% in TMV2NLM. Effect of betaine were found to be not significant neither S x B interaction was significant. However, G x B interaction was found to be significant ($P<0.05$) with 20% increase of shoot dry weight only in case of ICG 476 but the effect of betaine in other genotypes was marginal and not significant. (Table 4.6.1).

Pod Weights (g m $^{-2}$).

On an average there was about 30% reduction in pod weights with an imposition of mid season drought. However genotypic variation was found to be significant ($P<0.01$) (Table 4.6.1). TMV2NLM recorded greatest pod weight (362 g m $^{-2}$). CSMG 84-1 recorded the least 47 g m $^{-2}$. Genotypes differed significantly in their reaction to drought. In ICG 476, there was a 60% reduction, 40% in CSMG 84-1, TMV2NLM, TAG 24 and 15% in ICGV 86031, due to droughts. Betaine effects were not significant and S x B, G x B and G x S x B interactions were also not significant ($P<0.05$) (Table 4.6.1).

Harvest Index

Harvest Index was significantly influenced by drought. There was marginal reduction in HI under irrigated conditions (0.308), compared to that under mid season drought (0.363) conditions. Genotypes also differed significantly for HI where with TMV2NLM having greatest HI (0.558) and TAG 24 having the least (0.152). S x G interaction was significant. ICG 476 recorded 43% reduction in HI under mid season drought. While the differences were marginal in the other genotypes. However, betaine treatments, S x B, G x B and G x S x B interaction were not significant. (Table 4.6.1).

Table : 4. 6.1 Vegetative weights (Veg wt), pod weights (pod wt), and harvest index at 120 DAS as influenced by betaine treatments under irrigated and mid season drought conditions during kharif '96

		Veg wt (g/m2)				Pod wt (g/m2)				HI			
		B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
CSMG 84-1	IRR	565	462	458	661	80	70	72	88	0.14	0.15	0.16	0.13
	MSD	250	234	222	224	51	44	42	49	0.21	0.19	0.19	0.22
	mean	**250**	**234**	**222**	**224**	**51**	**44**	**42**	**49**	**0.21**	**0.19**	**0.19**	**0.22**
ICG 476	IRR	659	785	833	683	140	210	167	167	0.21	0.27	0.20	0.24
	MSD	465	460	470	454	54	59	59	58	0.12	0.13	0.13	0.13
	mean	**562**	**622**	**651**	**568**	**97**	**134**	**113**	**113**	**0.17**	**0.22**	**0.17**	**0.20**
TAG 24	IRR	543	533	516	798	108	141	134	93	0.20	0.27	0.26	0.12
	MSD	399	366	296	296	78	79	61	61	0.20	0.22	0.21	0.21
	mean	**471**	**450**	**406**	**547**	**78**	**79**	**61**	**61**	**0.17**	**0.18**	**0.15**	**0.11**
ICGV 86031	IRR	654	528	571	646	237	234	222	231	0.36	0.44	0.39	0.36
	MSD	234	239	358	290	189	220	189	190	0.81	0.92	0.53	0.66
	mean	**444**	**384**	**464**	**468**	**213**	**227**	**205**	**211**	**0.48**	**0.59**	**0.44**	**0.45**
TMV 2 NLM	IRR	858	758	710	761	345	443	442	487	0.40	0.58	0.62	0.64
	MSD	546	544	512	530	234	331	309	302	0.43	0.61	0.60	0.57
	mean	**702**	**651**	**611**	**645**	**289**	**387**	**375**	**394**	**0.41**	**0.59**	**0.61**	**0.61**
	Gmean	**486**	**468**	**471**	**490**	**146**	**174**	**159**	**166**	**0.30**	**0.37**	**0.34**	**0.34**
	SeM		**± 50.8**				**± 32.5**				**± 0.05**		
	CV%		**28.0**				**20.5**				**16.9**		

Analysis of variance

Source of variation	df	Vegwt	podwt	HI
MT (stress levels (S)	1	**	**	*
ST (genotypes (G)	4	**	**	*
S x G	4	*	**	NS
SST (betaine levels) (B)	3	NS	NS	NS
S x B	3	NS	NS	NS
G x B	12	*	NS	NS
G x S x B	12	NS	NS	NS

4.6.2. Reproductive development

Aerial pegs (AP) (pegs m^{-2})

AP decreased significantly ($P<0.01$) under mid season drought. Under irrigated conditions, mean AP were 511 pegs m^{-2} while under drought AP were reduced to 332 pegs m^{-2}. There was significant genotypic reduction in AP amongst genotypes with TAG 24 recording the greatest number of pegs m^{-2} (693 m^{-2}) and ICG 476 the least (280 m^{-2}) (Table 4.6.2). S x G interaction was also significant ($P<0.05$). The mid season drought reduced the AP by 40% in CSMG 84-1, ICG 476 and Tag 24 and while the reduction in AP was only 22% in ICGV 86031. The effect of betaine was not however, significant and S x B and G x S x B interactions were also not significant. G x B interaction was however significant ($P<0.05$) with CSMG 84-1 sharing no response to betaine treatment (B1 and B4 recorded 358 pegs m^{-2}) whereas in TAG 24, there was a 10% increase in AP with B4 treatment (Table 4.6.2).

Subterranean Pegs (SP) (pegs m^{-2})

Subterranean peg development was significant ($P<0.01$) affected due to drought. For example, while there were 1415 SP no. under irrigated control whereas in drought treatment there were only 510 pegs m^{-2}. There was a significant genotypic variatioin ($P<0.01$) with TAG 24 recording 1701 SP no. m^2 and TMV2NLM recorded only 485 pegs m^2. S x G interaction was found to be significant ($P<0.01$). For example, CSMG 84-1 and ICG 476 had a 60% reduction in SP with an imposition of MSD and the

decrease was only 51% in ICGV 86031. Betaine treatment resulted in a significant increase in SP ($P<0.05$). Particularly in B4 treatment, there was a 30% increase in SP. S x B interaction was found to be significant with MSD resulting in reduction of SP by 58% in B1, 69% in B2, 61% in B3, 65% in B4 respectively. G x B and G x S x B interactions were found to be not significant (Table 4.6.2).

Total Pegs (TP) (pegs m^{-2})

Total pegs m^{-2} (TP) differed significantly ($P<0.01$) for stress levels. Where there were 2152 pegs m^{-2} in irrigated conditions while MSD resulted in only 885 pegs m^{-2}. Significant genotypic variation ($P<0.01$) was observed with TAG 24 having the greatest no. of TP (2633) and TMV2NLM having the least (790). S x G interaction was significant ($P<0.01$) with TAG 24 showing a 67% reduction in TP under MSD and the reduction in TP was only 46% in ICGV 86031. Effect of treatments were also differed significantly ($P<0.05$) with B4 treatment producing 30% more TP than that of B1 treatment. S x B interaction was significant with drought treatment resulting in 60% reduction in TP, while in B2 and B3 treatments, the percentage reduction was 53 and 57% respectively. G x B and G x S x B interactions were not significant. (Table 4.6.2).

Table : 4. 6. 2 Aerial pegs (AP), Subterranean pegs (SP), and total pegs (TP) at 100 DAS as influenced by betaine treatments under irrigated and mid season drought conditions during kharif '96

		AP(pegno m^{-2})				SP(pegno m^{-2})				TP(pegno m^{-2})			
		B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
CSMG 84-1	IRR	495	312	367	397	691	733	648	1082	1186	1045	1014	1479
	MSD	220	189	208	318	354	263	299	342	574	452	507	660
	mean	358	251	287	358	691	733	648	1082	1048	984	935	1439
ICG 476	IRR	345	312	367	397	1324	1044	1159	1238	1669	1356	1525	1636
	MSD	220	189	208	200	546	453	333	545	766	642	541	745
	mean	283	251	287	299	935	748	746	892	1218	999	1033	1190
TAG 24	IRR	752	1008	752	953	2750	2988	2255	2713	3502	3997	3007	3667
	MSD	568	543	458	507	544	825	888	642	1112	1368	1346	1149
	mean	660	776	605	730	1647	1907	1572	1678	2307	2682	2177	2408
ICGV 86031	IRR	733	587	709	684	458	2224	2004	2041	1192	2811	2713	2726
	MSD	495	550	550	513	733	657	867	998	1228	1207	1417	1511
	mean	614	568	629	599	596	1441	1436	1520	1210	2009	2065	2118
TMV 2 NLM	IRR	281	312	306	165	739	721	715	782	1021	1033	1021	947
	MSD	198	187	132	189	312	171	251	189	510	358	383	378
	mean	281	312	306	165	526	446	483	486	807	758	788	651
	Gmean	439	431	423	430	879	1055	977	1131	1318	1486	1400	1561
	SeM		± 34.3				± 88.6				± 92.4		
	CV%		35				50.1				45.1		

Analysis of variance

Source of variation	df	AP	SP	TP
MT (stress levels (S)	1	**	**	**
ST (genotypes (G)	4	**	**	**
S x G	4	*	**	**
SST (betaine levels) (B)	3	NS	*	*
S x B	3	NS	*	*
G x B	12	*	*	NS
G x S x B	12	NS	*	NS

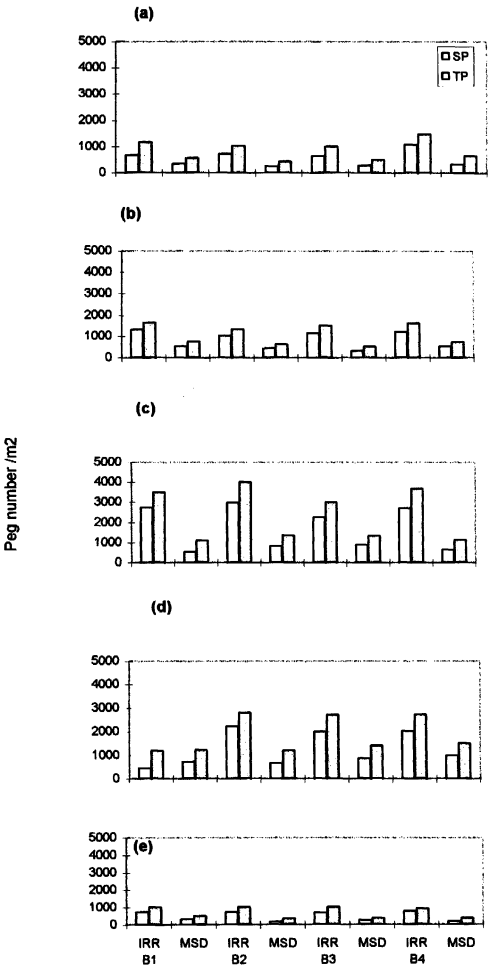


Fig : 4.6.2 Subterranean pegs(SP), total pegs (TP) as influenced by betaine treatment s under irrigated and mid season drought conditions in 5 groundnut genotypes, CSMG 84-(a), ICG 476(b), TAG24 (c), TMV 2 NLM (e) during kharif'96.

4.6.3 Crop Growth Rate (CGR) (g m^{-2} day^{-1}), Pod Growth Rate (PGR) (g m^{-2} day^{-1}), and Partitioning (Part %)

Crop Growth Rate (CGR) (g m^{-2} day^{-1})

CGR was significantly affected due to drought ($P<0.05$). In irrigated conditions the mean CGR was 16.9 g m^{-2} day^{-1} while in MSD conditions it was 7.3 g m^{-2} day^{-1} (Table 4.6.3). Genotypes differed significantly for CGR ($P<0.05$). CSMG 84-1 recorded greatest CGR (17.7) and ICGV 86031 recorded the least (6.6). S x G interaction was significantly different ($P<0.05$) with CSMG 84-1 showing 73% reduction in CGR under drought, while the % decrease was 54, 51, 62 and 25% in ICG 476, TAG 24, ICGV 86031 and TMV2NLM respectively. Betaine effects and other interactions were also not significant.

Pod Growth Rate (PGR) (g m^{-2} d^{-1})

PGR was significantly ($P<0.01$) reduced under MSD. However, genotypes differed significantly for PGR ($p<0.05$) with TAG 24 having the greatest PGR (6.2 g m^{-2} d^{-1}) and TMV2NLM having the least (1.8) S x G type interaction was significant ($P<0.05$). In TMV2NLM, the reduction in PGR due to MSD was 93% while in CSMG 84-1 and ICGV 86031, the PGR reduced by 80%. However, in case of TAG 24, PGR reduced by only 40% due to drought. Betaine treatments did not show significant differences. (Table 4.6.3).

Table : 4. 6. 3 Crop growth rate (CGR), pod growth rate (PGR) and partitioning percentage (part %) as influenced by betaine treatments under irrigated and mid season drought conditions during kharif '96

		CGR ($g\ m^{-2}\ day^{-1}$)				PGR ($g\ m^{-2}\ day^{-1}$)				PART%			
		B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
CSMG 84-1	IRR	27.8	27.1	27.8	28.9	6.8	7.3	7.0	7.6	0.24	0.27	0.25	0.26
	MSD	5.4	7.3	9.9	7.3	0.8	1.0	0.7	1.2	0.15	0.14	0.07	0.17
	mean	**16.6**	**17.2**	**18.9**	**18.1**	**3.8**	**4.2**	**3.8**	**4.4**	**0.23**	**0.24**	**0.20**	**0.24**
ICG 476	IRR	12.9	13.9	11.6	11.9	7.2	5.5	4.6	5.1	0.56	0.40	0.40	0.43
	MSD	7.9	4.0	6.6	4.4	1.4	2.1	1.9	1.0	0.18	0.53	0.29	0.22
	mean	**10.4**	**8.9**	**9.1**	**8.1**	**4.3**	**3.8**	**3.3**	**3.0**	**0.42**	**0.43**	**0.36**	**0.37**
TAG 24	IRR	23.2	21.1	24.5	24.6	6.5	9.6	9.6	6.7	0.28	0.46	0.39	0.27
	MSD	10.9	10.2	11.3	12.8	3.3	5.0	3.8	4.7	0.30	0.49	0.33	0.37
	mean	**17.1**	**15.7**	**17.9**	**18.7**	**4.9**	**7.3**	**6.7**	**5.7**	**0.29**	**0.47**	**0.38**	**0.31**
ICGV 86031	IRR	9.9	8.4	10.1	10.4	4.6	4.2	3.9	4.3	0.47	0.50	0.38	0.41
	MSD	3.3	2.5	4.6	4.0	0.6	0.9	0.6	0.5	0.18	0.37	0.13	0.14
	mean	**6.6**	**5.5**	**7.3**	**7.2**	**2.6**	**2.6**	**2.2**	**2.4**	**0.39**	**0.47**	**0.30**	**0.34**
TMV 2 NLM	IRR	12.1	11.5	10.2	11.0	3.6	3.1	3.1	3.6	0.30	0.27	0.31	0.32
	MSD	9.6	8.2	7.7	8.1	0.2	0.0	0.3	0.4	0.02	0.00	0.04	0.05
	mean	**10.9**	**9.9**	**8.9**	**9.6**	**1.9**	**1.6**	**1.7**	**2.0**	**0.17**	**0.16**	**0.19**	**0.21**
	Gmean	**12.3**	**11.4**	**12.4**	**12.3**	**3.5**	**3.9**	**3.5**	**3.5**	**0.29**	**0.34**	**0.29**	**0.28**
	SeM		± 1.3				± 0.099				± 0.008		
	CV%		23.6				19.9				12.6		

Analysis of variance

Source of variation	df	CGR	PGR	PART%
MT (stress levels (S)	1	**	**	NS
ST (genotypes (G)	4	*	*	NS
S x G	4	**	*	*
SST (betaine levels) (B)	3	NS	NS	NS
S x B	3	*	*	*
G x B	12	NS	*	NS
G x S x B	12	NS	NS	NS

Partitioning % (Part %)

Imposition of drought resulted in marginal reduction in part % from 0.51 in irrigated conditions to 0.45 under MSD conditions. There was a significant genotypic variation with TAG 24 having the greatest and TMV2NLM having the least (0.38) partitioning. S x G interaction was found to be significantly different ($P<0.05$). For example, the reduction in partitioning due to drought was 28 in TMV2NLM and 24% in ICGV 86031. However, in ICG 476, the partitioning was unaffected due to drought. However, betaine treatments did not show any significant effects on part. (Table 4.6.3).

4.6.4 Photosynthetc rate (Pn), Relative Water Content (RWC), Osmotic Potential (OP) and Light Interception (LI)

Photosynthetic rate (Pn) (μ mol m^{-2} sec^{-1})

Pn was significantly ($P<0.01$) influenced by drought. The mean Pn in irrigated conditions was about 16.2 while under MSD conditions, the Pn was 12 (μ mol m^{-2} sec^{-1}). Genotypes however, did not differ significantly for Pn. S x G interaction was found to be significant with reduction of Pn in ICG 476 was 35% whereas in other genotypes the reduction was 25%. Effect of betaines and associated interactions were not significant (Table 4.6.4).

Omotic Potential (OP) (milli osmoles)

The osmotic potentials were significantly influenced by the drought. In irrigated treatment, the OP ranged from 282 in TMV2NLM to 376 in ICG 476 resulting in significant differences between genotypes. On average, ICGV 86031 recorded highest OP (324) and the least OP was recorded in TAG 24 (276). S x G interaction was found to be significant ($P<0.05$). Effects of betaines and S x B interaction were not significant. Although there was some trend for an increase in OP under Betaine treatment for some genotypes (CSMG 84-1). The data was not conclusive enough in all the genotypes. The highest OP was recorded in B3 treatment in TMV2NLM genotype and the least was recorded in B1 treatment of TAG 24 (Table 4.6.4.).

Relative Water Content (RWC) (%)

The drought effects were significant for RWC with RWC being 94% under irrigated and 87% under MSD conditions. However, genotypes and betaine treatments did not show significant differences for RWC. For the S x B, G x B, S x G x B interactions were also not significant (Table 4.6.4).

Light Interception (LI) (%)

There was a significant reduction in LI due to drought. In irrigated conditions, the LI% was 80 while in MSD conditions it was only 67%. There were no differences for

Table : 4. 6. 4 Photosynthetic rate (Pn), osmotic potential (OP), relative water content (RWC) and light interception % (LI%) as influenced by betaine treatments under irrigated and mid season drought conditions at 80AS during kharif '96.

		Pn (µmol m^{-2} sec^{-1})				OP(milliosmoles)				RWC(%)				LI (%)			
		B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
CSMG 84-1	IRR	12.7	13.7	14.7	13.2	289	265	356	376	95.4	95.6	94.7	98.3	75	76	82	86
	MSD	12.0	11.2	11.4	11.0	245	312	287	363	87.1	88.0	87.1	86.2	71	69	70	68
	mean	**12.3**	**12.4**	**13.0**	**12.1**	**267**	**289**	**322**	**370**	**91.3**	**91.8**	**90.9**	**92.2**	**73**	**72.5**	**76**	**77**
ICG 476	IRR	16.5	17.8	18.8	19.0	376	226	328	338	92.1	92.0	92.0	93.1	78	73	71	76
	MSD	12.3	12.0	11.4	11.0	389	354	270	392	86.9	87.1	85.3	88.0	63	64	65	65
	mean	**14.4**	**14.9**	**15.1**	**15.0**	**382.5**	**290**	**299**	**365**	**89.5**	**89.6**	**88.6**	**90.6**	**70.5**	**68.5**	**68**	**70.5**
TAG 24	IRR	14.3	17.4	17.7	14.3	341	208	294	249	92.4	91.2	91.7	90.1	84	82	72	87
	MSD	12.0	13.0	12.0	11.0	214	357	235	317	85.6	86.2	86.2	87.3	68	66	68	66
	mean	**13.2**	**15.2**	**14.8**	**12.7**	**277.5**	**283**	**265**	**283**	**89.0**	**88.7**	**89.0**	**88.7**	**76**	**74**	**70**	**76.5**
ICGV 86031	IRR	18.8	13.5	18.2	16.8	321	277	295	358	93.6	97.8	98.2	97.3	73	84	76	89
	MSD	12.1	12.5	13.1	12.0	258	398	382	305	89.4	85.5	88.3	86.0	65	68	69	70
	mean	**15.4**	**13.0**	**15.7**	**14.4**	**289.5**	**338**	**339**	**332**	**91.5**	**91.6**	**93.2**	**91.7**	**69**	**76**	**72.5**	**79.5**
TMV 2 NLM	IRR	14.4	18.9	13.2	19.3	282	365	357	272	90.0	91.0	90.0	90.3	82	85	87	76
	MSD	12.0	13.0	12.2	13.5	322	251	318	255	86.3	86.5	90.6	88.1	67	69	70	69
	mean	**13.2**	**16.0**	**12.7**	**16.4**	**302**	**308**	**338**	**264**	**88.2**	**88.8**	**90.3**	**89.2**	**74.5**	**77**	**78.5**	**72.5**
	Gmean	**13.7**	**14.3**	**14.3**	**14.1**	**303.7**	**301**	**312**	**323**	**89.9**	**90.1**	**90.4**	**90.5**	**72.6**	**73.6**	**73**	**75.2**
	SeM		± 1.0				± 23.76				± 16.0				± 12.6		
	CV%		21.8				19.54				31.0				22		

Analysis of variance

Source of variation	df	Pn	OP	RWC	LI
MT (stress levels (S)	1	**	**	*	*
ST (genotypes (G)	4	NS	*	NS	NS
S x G	4	*	*	NS	NS
SST (betaine levels) (B)	3	NS	NS	NS	NS
S x B	3	NS	NS	NS	NS
G x B	12	NS	*	NS	NS
G x S x B	12	NS	NS	NS	NS

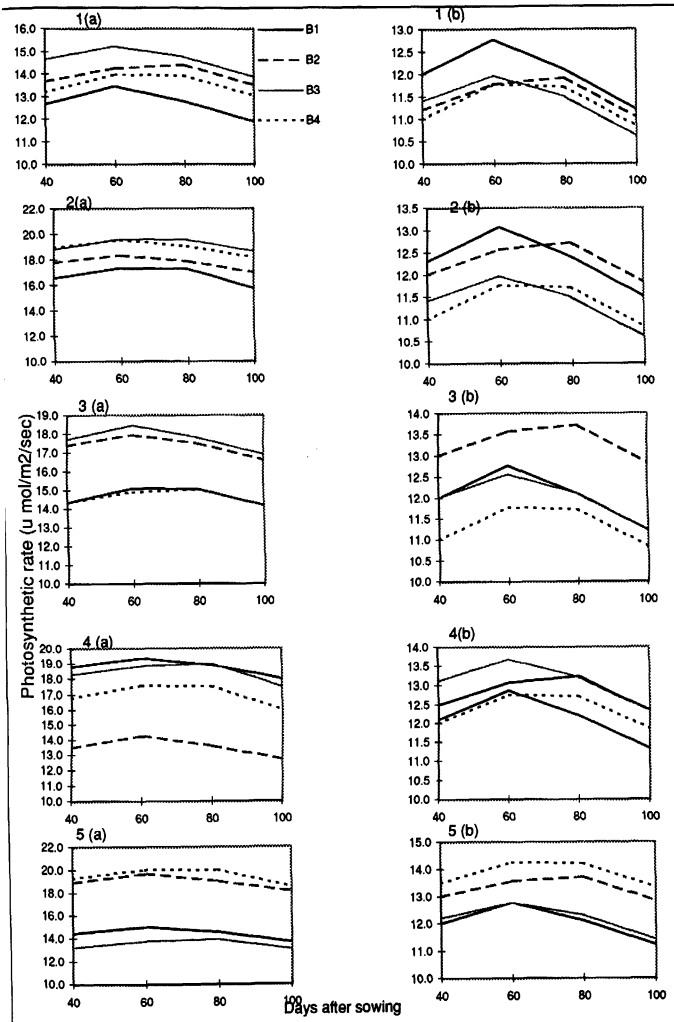


Fig: 4.6.4 - Photosynthetic rate as influenced by betaine treatments under irrigated (a) and mid season drought (b)conditions during kharif '96 in 5 groundnut genotypes, CSMG 84-1(1), ICG 476 (2), TAG 24 (3), ICGV 86031 (4), TMV2NLM (5).

LI%. Betaine level differences were also not observed. S x B, G x B, G x S x B interactions were also not significant (Table 4.64).

4. 7 Effect of glycine betaine in the alleviation of mid season drought during Rabi 96- 97.

Another field experiment was conducted during post rainy season of 96-97 to study the effect of glycine betaine spray (0, 3 and 6 kg ha^{-1}) (B1, B2 and B3) on the alleviation of drought stress (mid season drought)(MSD) in groundnut genotypes (CSMG 84-1, ICG 476, TAG24, ICGV 86031 and TMV 2 NLM). Mid season drought spanning from 40 - 80DAS was imposed by withholding water.

4. 7. 1 Vegetative weights (Veg wt) (g m^{-2}), pod dry weights (pod dry wt) (g m^{-2}) and harvest index

Vegetative weights (Veg wt) (g m^{-2})

Shoot dry wts were found to be significantly different ($P<0.01$) between stress treatments. On an average, IRR treatment produced a shoot dry wt of 524 g m^{-2} and MSD had a shoot dry wt of 229 g m^{-2}(Table 4.7.1) Genotypes also were found to be significantly different ($P<0.05$). ICG 476 recorded more shoot dry wt (558) while the least was observed iin ICGV 86031 (265). SxG interaction was found to be significant ($P<0.05$) where CSMG 84-1 had a 20% decrease in shoot dry wts with imposition of MSD while in other four genotypes it was nearly 60%. Veg weights differed

significantly with betaine treatment ($P<0.05$) with B3 having a shoot dry wt of 443 g m^{-2} while B1 treatment had only 393 g m^{-2} representing a positive significant effect of betaine treatments on shoot dry wts. SxB interaction was found to be not significantly different while GxB interaction was found to be significant. GxSxB interaction was not significant(Table 4.7.1).

Pod dry weight (Pod dry wt) (g m^{-2})

IRR treatment is found to produce 159% more pod dry wt than that of MSD treatment. Genotypic variation was significant where ICG 476 was found to produce more pod dry wt (335) and the least was observed in TAG 24 (213). SxG interaction was found to be significant ($P<0.05$) where in TMV2NLM there is a 79% decrease in pod dry weights in MSD conditions and it is only 37% decrease in CSMG 84-1. Betaine levels, SxB, GxB and GxSxB interactions were not significant(Table 4.7.1).

Harvest Index (HI)

With an imposition of MSD there is a 10% increase in HI. CSMG 84-1 had a 22% decrease in HI with an imposition of MSD ($P<0.05$). Where in other four genotypes the decrease was less than 10%. Betaine levels GxB, SxB and GxSxB interactions were not significant(Table 4.7.1).

Table : 4. 7.1 Vegetative weight (veg wt), pod dry weights(pod dry wt), and harvest index as influenced by betaine tretments under mid season drought during rabi 96-97.

		Veg wt. (g m^{-2})			pod dry wt. (g m^{-2})			HI		
		B1	B2	B3	B1	B2	B3	B1	B2	B3
CSMG 84-1	MSD	330	387	338	198	174	179	0.6	0.45	0.53
	IRR	434	423	444	330	313	240	0.76	0.74	0.54
	mean	**382**	**405**	**391**	**260**	**241**	**209**	**0.68**	**0.595**	**0.535**
ICG 476	MSD	135.2	223.3	156.4	81	125	92	0.6	0.56	0.59
	IRR	510	457	707	321	270	445	0.63	0.59	0.63
	mean	**510**	**457**	**707**	**314**	**263**	**431**	**0.62**	**0.575**	**0.61**
TAG 24	MSD	223.3	224	156.4	134	125	91	0.6	0.56	0.58
	IRR	554	504	572	338	282	297	0.61	0.56	0.52
	mean	**389**	**364**	**364**	**235**	**204**	**200**	**0.61**	**0.56**	**0.55**
ICGV 86031	MSD	235.7	195.2	234	153	109	138	0.65	0.56	0.59
	IRR	405	613	510	288	405	342	0.71	0.66	0.67
	mean	**320**	**404**	**372**	**218**	**247**	**234**	**0.68**	**0.61**	**0.63**
TMV 2 NLM	MSD	135.7	240	221.4	76	115	144	0.56	0.48	0.65
	IRR	595	599	547	345	467	350	0.58	0.78	0.64
	mean	**365**	**420**	**384**	**208**	**264**	**248**	**0.57**	**0.63**	**0.645**
	Gmean	**393**	**410**	**444**	**248**	**243**	**264**	**0.63**	**0.594**	**0.594**
	SeM		**± 26.77**			**± 23.35**			**± 0.01**	
	CV%		**± 12.6**			**± 25.8**			**± 21.9**	

Analysis of variance

Source of variation	df	shoot dry wt	pod dry wt	HI
MT (stress levels (S)	1	**	**	*
ST (genotypes (G)	4	*	*	NS
S x G	4	*	**	*
SST (betaine levels) (B)	2	*	NS	*
S x B	2	NS	NS	NS
G x B	8	*	NS	NS
G x S x B	8	NS	NS	NS

4.7.2 Reproductive development

4. 7. 2 (a) Aerial pegs (AP) (pegs m^{-2}), Subterranean pegs (SP) (pegs m^{-2}) Total pegs (TP) (pegs m^{-2}).

Aerial pegs (AP) (pegs m^{-2})

IRR treatment was found to produce 343 pegs m^{-2} while MSD had only 283 pegs m^{-2}. Genotypes differed significantly for AP ($P<0.05$) (Table 4.7.2(a)). Where ICGV 86031 was found to produce more pegs m^{-2} (426) while TAG 24 had only 260 pegs m^{-2}. SxG interaction was found to be significant where in TMV2NLM, CSMG 84-1, there was a 40% decrease in AP. While in other three genotypes, the differences were marginal. B3 treatment had 410 pegs m^{-2}. Whereas B1 had only 235 pegs m^{-2} showing a significant ($P<0.01$) positive increase in AP with B2 and B3 treatment. SxB interaction was found to be significant where in B1 treatment, the percent decrease due to MSD was 35 while in B2 treatment, it is 20 and in B3 treatment the difference was only 2%. GxB interaction was significant where in CSMG 84-1 there was a 150% increase in AP with B3 treatment while the other four genotypes the increase was 40-60%. GxSxB interaction was not significant (Table 4.7.2(a)).

Subterranean pegs (SP) (pegs m^{-2})

IRR treatment was found to produce 532 pegs m^{-2} while MSD had only 396 pegs m^{-2}. Genotypes differed significantly for SP ($P<0.05$) (Table 4.7.2(a)). Where ICGV-

86031 was found to produce more pegs m^{-2} (581) while TAG 24 had only 387 pegs m^{-2}. SxG interaction was found to be significant ($P<0.05$) where in CSMG 84-1 and TMV2NLM there was a 50% decrease in SP while in other three genotypes, the differences were less than 20%. B2 treatment had 512 pegs m^{-2}. Whereas B1 had only 418 pegs m^{-2} showing a significant ($P<0.01$) positive increase in SP with B3 treatment. SxB interaction was found to be significant where in B1 treatmnet, the percent decrease due to MSD was 34 while in B2 treatment, it is 38 and in B3 treatment the difference was only 25%. GxB interaction was significant where in CSMG 84-1 there was a 33% increase in SP with B3 treatment while the other four genotypes the increase was 20-25%. GxSxB interaction was not significant (Table 4.7.2(a)).

Total pegs (TP) (pegs m^{-2})

IRR treatment was found to produce 883 pegs m^{-2} while MSD had only 612 pegs m^{-2}. Genotypes differed significantly for TP ($P<0.05$). Where ICGV 86031 was found to produce more pegs m^{-2} (1014) while CSMG 84-1 had only 647 pegs m^{-2}. (Table 4.7.2(a)) SxG interaction was found to be significant ($P<0.05$) where in CSMG 84-1 and TMV2NLM there was a 55% decrease in TP while in other three genotypes, the differences were less than 15%. B2 treatment had 788 pegs m^{-2} whereas B1 had only 684 pegs m^{-2} showing a significant ($P<0.01$) positive increase in SP with B2 treatment. SxB interaction was found to be not significant. GxB interaction was significant where in TAG 24, there was a 24% increase in TP with B3 treatment while in the other four genotypes the increase was less than 20%. GxSxB interaction was not significant (Table 4.7.2(a)).

Table :4. 7. 2 (a)Aerial pegs(AP) subterranean pegs(SP), and total pegs(TP) as influenced by betaine treatments under irrigated and mid season drought conditions at 60 DAS during rabi '96-'97.

		AP (peg no. m^{-2})			SP (peg no. m^{-2})			TP (peg no. m^{-2})		
		B1	B2	B3	B1	B2	B3	B1	B2	B3
CSMG 84-1	MSD	110	220	450	187	352	330	407	462	408
	IRR	294	269	554	488	662	597	782	931	893
	mean	**201.9**	**244**	**502**	**338**	**507**	**464**	**595**	**696.3**	**650.3**
ICG 476	MSD	286	319	363	341	287	616	616	701	627
	IRR	243	208	388	441	721	309	683	929	696
	mean	**264.3**	**263**	**375.4**	**391**	**504**	**462**	**650**	**815**	**661.7**
TAG 24	MSD	154	165	440	407	396	297	561	619	737
	IRR	157	297	351	314	459	455	470	756	807
	mean	**155.4**	**231**	**395.7**	**360**	**427**	**376**	**516**	**687.3**	**771.9**
ICGV 86031	MSD	264	506	504	415	616	440	944	715	1122
	IRR	361	376	548	614	667	738	975	1043	1285
	mean	**312.7**	**441**	**525.9**	**514**	**641**	**589**	**960**	**878.9**	**1204**
TMV 2 NLM	MSD	112	100	264	297	308	297	363	561	351
	IRR	366	508	238	678	657	563	1044	1165	800
	mean	239	304	251	488	482	430	704	863	576
	Gmean	**235**	**297**	**410**	**418**	**512**	**464**	**685**	**788**	**773**
	SeM		± 23.6			± 49.9			± 54.0	
	CV%		± 19.7			± 21.5			± 32.8	

Analysis of variance

Source of variation	df	AP	SP	TP
MT (stress levels (S)	1	**	**	**
ST (genotypes (G)	4	*	*	**
S x G	4	*	*	*
SST (betaine levels) (B)	2	**	**	*
S x B	2	*	*	*
G x B	8	*	*	*
G x S x B	8	NS	*	*

4.7.2 (b) Aerial peg, subterranean peg and total peg addition rates

Aerial peg addition rate (peg m^{-2} day^{-1})

Effect of drought on AP addition rates was significantly different ($P < 0.05$) with AP addition rates being 4.65 pegs m^{-2} day^{-1} in IRR conditions while the rate being only 2.22 in MSD conditions. Peg addition rates were significantly different ($P < 0.05$) for genotypes with ICGV 86031 having the greatest peg addition rate (4.75) and the least in CSMG 84-1 (1.76). S x G interaction was also significant with 90% reduction in TMV2NLM with an imposition of drought whereas in ICG 476 and ICGV 86031, reduction was less than 10%. AP addition rates showed significant ($P < 0.05$) differences with betaine levels wherein B3 treatment had an addition rate of 5.63 pegs m^{-2} day^{-1} while it is 2.8 in B2 and 1.88 in B1. S x B interaction was significant ($P < 0.05$) in B3 treatment, the percent reduction due to drought was 63 while the other two genotypes it was 30-40%. G x B, G x S x B interactions were not significant.

Subterranean peg addition rate (pegs m^{-2} day^{-1})

SP addition rates were significantly different for stress levels ($P < 0.05$), with an imposition of MSD, SP addition rates decreased from 5.25 in IRR conditions to 2.82 in MSD conditions. Genotypic variations was also observed with greatest peg addition rate of 5.35 in ICGV 86031 while the least (2.36) in ICG 476. S x G interaction was significant ($P < 0.05$) with TMV2NLM showing 85% reduction with MSD and the percent reduction was only 12 in ICG 476. SP addition rates were significantly different

with betaine levels where B3 treatment recorded 6.22 pegs m^{-2} day^{-1} while it was only 2.48 pegs m^{-2} day^{-1} in B1 treatment. S x B interaction was also significant ($P < 0.05$), in B3 treatment with an imposition of MSD, the SP addition rates decreased by 100%. While in B1 and B2 treatments, the decrease was less than 30%. G x B interaction was also significant ($P < 0.05$) with ICGV 86031 showing the greatest increase in SP addition rates by 279% while it was 68% increase in TMV2NLM with B3 treatment. G x S x B interaction was not significant.

Total peg addition rates (pegs m^{-2} day^{-1})

The total peg addition rate was 9.9 pegs m^{-2} day^{-1} in IRR conditions while it was only 5.04 pegs m^{-2} day^{-1} under MSD conditions showing a significant difference ($P < 0.05$) in TP addition rates between stress levels. Genotypic differences were also significant with ICGV 86031 having greatest TP addition rate of 10.01 pegs m^{-2} day^{-1} while the least rate was in ICG 476. S x G interaction was significant ($P < 0.05$) with TMV2NLM showing 88% reduction in TP addition rate under MSD while it was only 12% in ICGV 86031. Betaine treatment increased the peg addition rates, B3 treatment had a peg addition rate of 11.85 while it was only 4.37 pegs m^{-2} day^{-1} in B1 treatment. S x B interaction was also significant where in B1 treatment there was a 37% decrease in TP addition rate with an imposition of MSD while it was only 27% in B2 treatment. G x B interaction was also significant ($P < 0.05$), betaine treatment could increase the TP addition rates by 344% in TAG 24 while the increase was only 73% in TMV2NLM. G x S x B interaction was not significant.

Table :4. 7. 2 (b)Aerial peg addition rate,subterranean peg addition rate and total peg addition rate as influenced by betaine treatments under irrigated and mid season drought conditions during rabi '96-'97.

		AP addn rate (peg no. m^{-2} day^{-1})			SP addn rate (peg no. m^{-2} day^{-1})			TP addn rate (peg no. m^{-2} day^{-1})		
		B1	B2	B3	B1	B2	B3	B1	B2	B3
CSMG 84-1	IRR	2.13	2.25	4.85	2.73	2.85	5.45	4.86	5.10	10.30
	MSD	0.28	0.19	0.85	0.87	0.79	1.45	1.15	0.98	2.30
	mean	1.20	1.22	2.85	1.80	1.82	3.45	3.00	3.04	6.30
ICG 476	IRR	1.48	2.32	8.00	2.07	2.92	8.60	3.55	5.24	16.60
	MSD	1.09	3.88	1.98	1.68	4.48	2.58	2.77	8.35	4.56
	mean	1.28	3.10	4.99	1.88	3.70	5.59	3.16	6.80	10.58
TAG 24	IRR	1.93	2.75	10.60	2.52	3.35	11.20	4.45	6.10	21.80
	MSD	3.12	3.58	6.54	3.72	4.17	7.14	6.84	7.75	13.68
	mean	2.52	3.16	8.57	3.12	3.76	9.17	5.64	6.92	17.74
ICGV 86031	IRR	0.13	1.20	7.50	0.72	1.80	8.10	0.85	3.00	15.60
	MSD	1.97	3.79	3.77	2.56	4.39	4.37	4.53	8.19	8.14
	mean	1.05	2.50	5.64	1.64	3.10	6.23	2.69	5.59	11.87
TMV 2 NLM	IRR	6.28	7.95	10.40	6.87	8.55	11.00	13.15	16.50	21.40
	MSD	0.47	0.07	1.76	1.07	0.67	2.36	1.53	0.74	4.12
	mean	3.37	4.01	6.08	3.97	4.61	6.68	7.34	8.62	12.76
	Gmean	**1.88**	**2.80**	**5.63**	**2.48**	**3.40**	**6.22**	**4.37**	**6.19**	**11.85**
	SeM		± 0.98			± 1.02			± 1.89	
	CV%		± 21.5			± 25.7			± 33.0	

Analysis of variance

Source of variation	df	AP	SP	TP
MT (stress levels (S)	1	*	*	*
ST (genotypes (G)	4	*	*	*
S x G	4	*	*	*
SST (betaine levels) (B)	2	*	*	*
S x B	2	*	*	*
G x B	8	NS	*	**
G x S x B	8	NS	NS	NS

4. 7. 3 Crop growth rate (CGR) (g m^{-2} day^{-1}), Pod growth rate (PGR) (g m^{-2} day^{-1}) and Partitioning %. (Part %).

Crop growth rate (CGR) (g m^{-2} day^{-1})

CGR differed significantly (P<005) between stress levls. On an average IRR had a CGR of 19.5 (g m^{-2} day^{-1}) while MSD had a CGR of 11.8 (g m^{-2} day^{-1}) (Table 4.7.3). Genotypic variation is also seen where in CSMG 84-1 recorded highest CGR (19.2) while the least was observed in ICG 476 (11.8). S x G interaction (P<0.05) was found to be significant where in ICG 476 had a 68% decreased in CGR with an imposition of MSD, ICGV 86031 had a 53% decrease while in TAG 24 and TMV2NLM the differences were marginal. Betaine treatments were found to increase the CGR by 25% and the differences were significant (P<0.05). S x B, G x B, S x G x B interactions were not significant (Table 4.7.3).

Pod growth rate (PGR) (g m^{-2} day^{-1})

On an average, IRR treatment had a PGR of 13.2 whereas MSD had a PGR of 7.8. Genotypes had a significant difference (P<0.05) for PGR. The highest PGR was observed in CSMG 84-1 (13.3) and the lowest in ICGV 86031 (7.8) (Table 4.7.3). SxG interaction was significant (P<0.05) where CSMG 84-1, ICGV 86031 and TMV2NLM had a 38% decrease in PGR with an imposition of MSD while TAG 24 and ICG 476 had 43% decrease with MSD. Betaine levels SxB, GxB, GxSxB interactions were not significant.

Table :4. 7. 3 Crop growth rate(CGR),pod growth rate (PGR), partitioning (PART%) as influenced by betaine tretments under mid season drought in raabi '96-'97.

		CGR (g m^{-2} day^{-1})			PGR (g m^{-2} day^{-1})			PART%		
		B1	B2	B3	B1	B2	B3	B1	B2	B3
CSMG 84-1	MSD	16.0	16.2	17.7	9.9	9.7	11.0	0.720	0.510	0.620
	IRR	21.0	22.0	22.4	14.4	20.6	14.3	0.687	0.934	0.639
	mean	**18.5**	**19.1**	**20.1**	**12.2**	**15.1**	**12.7**	**0.703**	**0.722**	**0.629**
ICG 476	MSD	4.7	5.4	6.6	5.9	5.5	13.7	0.690	0.560	0.720
	IRR	19.2	16.1	18.7	12.7	11.4	17.3	0.661	0.709	0.925
	mean	**11.9**	**10.7**	**12.7**	**9.3**	**8.5**	**15.5**	**0.676**	**0.634**	**0.823**
TAG 24	MSD	6.6	22.8	13.2	4.2	7.4	9.9	0.634	0.324	0.760
	IRR	21.5	18.9	25.2	13.9	11.0	14.4	0.645	0.583	0.572
	mean	**14.0**	**20.8**	**19.2**	**9.0**	**9.2**	**12.2**	**0.640**	**0.453**	**0.666**
ICGV 86031	MSD	6.7	6.0	14.7	4.7	4.7	7.4	0.686	0.793	0.670
	IRR	12.1	25.6	22.7	10.1	10.0	10.2	0.832	0.539	0.619
	mean	**9.4**	**15.8**	**18.7**	**7.4**	**7.4**	**8.8**	**0.759**	**0.666**	**0.644**
TMV 2 NLM	MSD	11.8	15.1	14.7	8.5	9.9	5.5	0.950	0.457	0.667
	IRR	12.5	18.6	16.5	12.5	14.8	11.3	1.064	0.816	0.770
	mean	**11.8**	**15.1**	**14.7**	**10.5**	**12.3**	**8.4**	**1.007**	**0.637**	**0.718**
	Gmean	**13.1**	**16.3**	**17.1**	**9.66**	**10.5**	**11.5**	**0.7568**	**0.623**	**0.696**
	SeM		**± 2.54**			**± 1.678**			**± 0.098**	
	CV%		**± 12.54**			**± 21.65**			**± 20.98**	

Analysis of variance

Source of variation	df	CGR	PGR	PART%
MT (stress levels (S)	1	*	**	*
ST (genotypes (G)	4	*	*	NS
S x G	4	*	*	NS
SST (betaine levels) (B)	2	*	NS	NS
S x B	2	NS	NS	NS
G x B	8	NS	NS	**
G x S x B	8	NS	NS	NS

Partitioning % (Part %)

Part % decreased significantly with an imposition of MSD ($p<0.05$). On an average IRR had a Part % of 0.73 while MSD had a Part % of 0.65 (Table 4.7.3). Genotypes SxG, B, SxB interactions were not significant whereas GxB interaction was found to be significant. In ICG 476 there was a 20% increase in Part % with B3 treatment. While in other genotypes, the differences were marginal. GxSxB interaction was not significant.

4.7.4 Net assimilation rate (NAR) (g m^{-2} day^{-1}), Leaf area duration (LAD) (days)

Net assimilation rate (NAR) (g m^{-2} day^{-1})

Stress levels were found to be significantly different ($P<0.05$) for NAR. IRR treatment was found to produce 9.7 g m^{-2} day^{-1} while MSD had only an NAR of 5.9 (Table 4.7.4). Genotypic variation is not seen. SxG interaction was significant, CSMG 84-1, ICGV 86031 and TMV2NLM had a 50% decrease in NAR with MSD treatment while in the other two genotypes, the decrease was less than 20%. Betaine levels were found to be significantly different ($P<0.01$), B2 treatment had an NAR of 8.5 and B1 had only 6.5 g m^{-2} day^{-1}. SxB interaction was found to be significantly different, the decrease in NAR due to MSD was 48% in B1 treatment whereas it was only 32 to 39% in B2 and B3 treatments respectively. G x B interaction was found to be significantly different, ICGV 86031 and TMV2NLM had a 60% increase in NAR due to B3 treatment

Table : 4.7.4 Net assimilation rate(NAR), Leaf area duration(LAD) as influenced by betaine treatments under Mid season drought during rabi '96-'97.

		NAR (g m^{-2} day^{-1})			LAD (days)		
		B1	B2	B3	B1	B2	B3
CSMG 84-1	MSD	3.7	9.0	5.0	78.9	113.6	55.7
	IRR	10.2	8.6	10.6	95.0	152.0	112.0
	mean	**7.0**	**8.8**	**7.8**	**87.0**	**132.8**	**83.9**
ICG 476	MSD	4.8	6.1	7.1	148.4	150.9	114.3
	IRR	7.1	8.1	6.1	101.0	107.0	87.0
	mean	**6.0**	**7.1**	**6.6**	**124.7**	**128.9**	**100.7**
TAG 24	MSD	5.9	6.7	7.3	82.8	154.8	112.9
	IRR	7.7	5.0	11.2	78.0	157.0	134.0
	mean	**6.8**	**5.9**	**9.2**	**80.4**	**155.9**	**123.5**
ICGV 86031	MSD	4.5	6.7	6.8	56.8	79.4	94.9
	IRR	9.0	14.6	12.0	102.0	73.0	85.0
	mean	**6.7**	**10.6**	**9.4**	**79.4**	**76.2**	**90.0**
TMV 2 NLM	MSD	3.9	6.3	5.2	127.2	147.2	147.6
	IRR	9.0	14.6	12.0	102.0	73.0	85.0
	mean	**6.4**	**10.4**	**8.6**	**114.6**	**110.1**	**116.3**
	Gmean	**6.6**	**8.6**	**8.3**	**97.2**	**120.8**	**102.9**
	SeM		± **1.9**			± **11.0**	
	CV%		± **19.8**			± **21.8**	

Analysis of variance			
Source of variation	**df**	**NAR**	**LAD**
MT (stress levels (S)	**1**	*	*
ST (genotypes (G)	**4**	**NS**	*
S x G	**4**	*	*
SST (betaine levels) (B)	**2**	**NS**	*
S x B	**2**	**	**NS**
G x B	**8**	*	**NS**
G x S x B	**8**	**NS**	**NS**

while in the other three genotypes, the increase was 25%. G x S x B interaction was not significant (Table 4.7.4).

Leaf area duration (LAD) (days)

LAD was found to be not significant with stress levels, while genotypes had a significant difference ($P<0.05$) with ICG 476 having LAD of 118 days while in ICGV 86031 the LAD was 81. S x G interaction was found to be significant where in CSMG 84-1 had a 31% decrease in NAR due to MSD while in the other four genotypes, the differences were marginal. Betaine treatments were found to be significantly different ($P<0.01$). B2 treatment had an LAD of 120 while B1 had an LAD of only 97. S x B, G x B, G x S x B interactions were not significant (Table 4.7.4).

4.7.5 Photosynthetic rates (Pn) (μ mol m^{-2} sec^{-1}), Relative water content (RWC) (%), Osmotic Potential ($\psi\pi$) (milliosmoles), Light Interception (LI) (%)

Photosynthetic rates (Pn) (μ mol m^{-2} sec^{-1})

Photosynthetic rates differed significantly for stress levels($P<0.05$) IRR treatment had on an average 19.5 μ mol m^{-2} sec^{-1} while MSD had a Pn rate of 16.3 μ mol m^{-2} sec^{-1}. Genotypes, S x G interaction were not significant. Betaine levels were found to be significant ($P<0.05$). On an average B1 treatment had a Pn rate of 16.8 μ mol m^{-2} sec^{-1} while B2 and B3 treatments had 18.6 and 19.8 μ mol m^{-2} sec^{-1} respectively. S x B, G x B, G x S x B interactions were not significant (Table 4.7.5).

Relative water content (RWC) (%)

There were no significant differences in RWC for stress, genotypes, betaine S x G, G x B, S x B, G x S x B interactions. The RWC ranged from 77 - 90% in MSD treatment and 90 - 96% under IRR conditions (Table 4.7.5).

Osmotic Potential (OP)

OP differed significantly for stress levels($P<0.05$). IRR treatment had a OP of 318 milli osmoles whereas MSD had 367 milli osmoles. Genotypes had no significant difference S x G interaction was not significant. Betaine levels differed significantly ($P<0.01$). On an average B1 treatment had an OP of 414 while B2 had an OP of 311 and B3 had an OP of 303 milli osmoles. S x B interaction found to be significant where in B1 treatment, the decrease due to MSD was 13% whereas in B3 treatment the decrease was 10%.

G x S x B interactions were not significant (Table 4.7.5).

Light interception (LI) (%)

LI % differed significantly with stress levels($P<0.05$). IRR had an LI of 78% whereas MSD had only 68% LI. Genotypes, betaine levels did not differ significantly for LI.

S x G, G x B, S x B, G x S x B interactions were not significant (Table 4.7.5).

Table :4.7.5 **Relative water content (RWC), photosynthetic rates (Pn) osmotic potentials (OP),and light interception(LI%) as influenced by betaine treaments under mid season drought at 100DAS during rabi '96-'97.**

		RWC (%)			Pn (μ mol m^{-2} sec^{-1})			OP (milli osmoles)			LI(%)		
		B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
CSMG 84-1	MSD	88	90	90	15.2	18.7	18.7	345	300	312	65	64	68
	IRR	90	90	96	19.9	21.5	22.8	327	256	245	72	74	77
	mean	**89**	**90**	**93**	**19.9**	**21.5**	**22.8**	**336**	**278**	**278.5**	**68.5**	**69**	**72.5**
ICG 476	MSD	77	79	88	19.9	20.5	23.7	399	287	259	54	67	65
	IRR	87	90	96	20.7	21.0	19.9	327	256	245	75	78	80
	mean	**82**	**85**	**92**	**20.3**	**20.8**	**21.8**	**363**	**271.5**	**252**	**64.5**	**72.5**	**72.5**
TAG 24	MSD	77	79	83	11.2	11.3	13.3	354	259	254	65	67	64
	IRR	87	94	93	17.4	19.9	19.8	399	245	286	77	79	72
	mean	**82**	**87**	**88**	**14.3**	**15.6**	**16.5**	**377**	**252**	**270**	**71**	**73**	**68**
ICGV 86031	MSD	87	87	80	13.9	19.9	20.1	398	284	206	69	70	72
	IRR	88	97	90	17.0	17.4	22.8	352	214	263	74	83	88
	mean	**87**	**92**	**85**	**15.4**	**18.6**	**21.4**	**375**	**249**	**234.5**	**71.5**	**76.5**	**80**
TMV 2 NLM	MSD	89	87	88	9.9	14.5	14.5	716	589	548	76	78	82
	IRR	90	93	95	15.7	19.3	19.1	523	421	412	79	88	87
	mean	**90**	**90**	**92**	**12.8**	**16.9**	**16.8**	**620**	**505**	**480**	**77.5**	**83**	**84.5**
	Gmean	**86**	**89**	**90**	**16.5**	**18.7**	**19.9**	**414**	**311.1**	**303**	**70.6**	**74.8**	**75.5**
	SeM		**± 19.7**			**± 2.9**			**± 34.7**			**± 13.9**	
	CV%		**± 30.2**			**± 19.8**			**± 20.0**			**± 25.0**	

Analysis of variance

Source of variation	df	RWC	Pn	OP	LI
MT (stress levels (S)	1	NS	*	**	NS
ST (genotypes (G)	4	NS	NS	NS	NS
S x G	4	NS	NS	NS	NS
SST (betaine levels) (B)	2	NS	*	**	NS
S x B	2	NS	NS	**	NS
G x B	8	NS	NS	**	NS
G x S x B	8	NS	NS	NS	NS

Table : 4. 7. 6 Total betaine content (TB) as influenced by betaine tretments under mid season drought during rabi '96-'97.

		TB (mM)		
		B1	B2	B3
CSMG 84-1	MSD	12.2	74.5	77.8
	IRR	15.3	80.9	93.5
	mean	**13.7**	**77.7**	**85.6**
ICG 476	MSD	11.0	71.4	82.3
	IRR	9.9	69.4	83.0
	mean	**10.5**	**70.4**	**82.6**
TAG 24	MSD	10.5	89.2	86.9
	IRR	9.5	55.8	70.5
	mean	**10.0**	**72.5**	**78.7**
ICGV 86031	MSD	13.5	77.7	75.0
	IRR	10.3	60.0	92.2
	mean	**11.9**	**68.8**	**83.6**
TMV 2 NLM	MSD	9.1	51.3	92.1
	IRR	5.4	48.1	91.2
	mean	**7.2**	**49.7**	**91.7**
	Gmean	**10.7**	**67.8**	**84.4**
	SeM		± 2.0	
	CV%		± 23.7	

Analysis of variance		
Source of variation	df	TB
MT (stress levels (S)	1	NS
ST (genotypes (G)	4	NS
S x G	4	NS
SST (betaine levels) (B)	2	**
S x B	2	NS
G x B	8	NS
G x S x B	8	NS

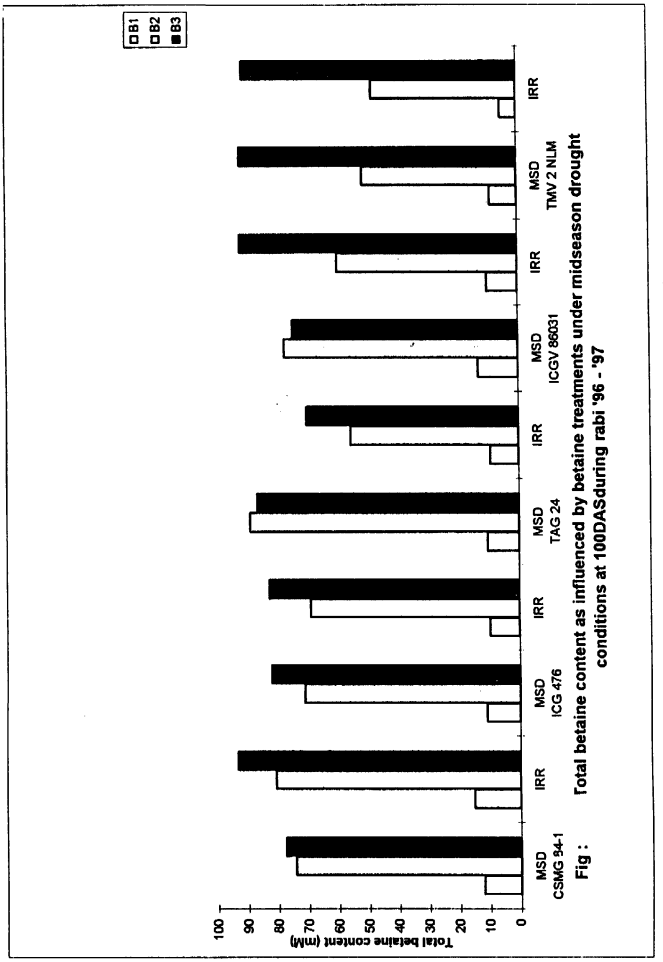


Fig : Total betaine content as influenced by betaine treatments under midseason drought conditions at 100DASduring rabi '96 - '97

4.7.6 Total betaine content (TB) (mM)

Stress levels and genotypes did not differ significantly for TB. S x G interaction was also not significant whereas betaine levels differed significantly ($P<0.01$). With a spray of betaine, the TB increased from 10 mM in B1 to 84 mM in B3, showing a 740% increase in TB with B3 treatment. S x B, G x B, G x S x B interactions were not significant (Table 4.7.6).

4.7.7 Correlations

Total betaine content had a significant positive correlation (with CGR (0.464*), PGR (0.461*), NAR (0.702**) and OP (0.582**) under irrigated conditions whereas with MSD, betaine content had no significant correlation with CGR, PGR and NAR whereas there was significant positive correlation between total betaine content and OP (0.657**) and RWC (0.661**)

4. 8 Effect of glycine betaine in the alleviation of end season drought (ESD) (80 - 100DAS) during rabi '96-'97.

A field experiment was conducted during rabi '96 - '97, with stress levels as main treatments, irrigated (IRR) and end season drought (ESD), genotypes as sub treatments (CSMG 84-1, ICG 476, TAG24, ICGV 86031 and TMV 2 NLM) and betaine spray as sub sub treatments (0, 3 and 6 kg ha^{-1}). The end season drought was imposed from

80 DAS to Final harvest by line source sprinkler irrigation system which develops a systematic gradient of soil moisture(different drought intensities).

4. 8. 1 Shoot dry weights (shoot dry wt) (g m^{-2}), pod dry weights (pod dry wt) (g m^{-2}) and harvest index

Shoot dry weights (Shoot dry wt) (g m^{-2})

Shoot dry wts were found to be significantly different ($P<0.01$) between stress treatments. On an average, 67.54% water deficit recorded a shoot dry wt of 428 g m^{-2} and 8.6% water deficit had a shoot dry wt of 931 g m^{-2} (Table 4.8.1). Genotypes also were found to be significantly different ($P<0.05$). ICGV 86031 recorded more shoot dry wt (806) while the least was observed in CSMG 84-1 (471). S x G interaction was found to be significant ($P<0.05$) where ICG 476 had a 45% decrease in shoot dry wt with imposition of 67.5% WD while in other four genotypes it was nearly 58%. Betaine levels differed significantly ($P<0.01$) with B3 having a shoot dry wt of 717 g m^{-2} while B1 treatment had only 593 g m^{-2} representing a positive significant effect of betaine treatments on shoot dry wts. S x B interaction was found to be significant, shoot dry weights decreased by 63% in B1 while in B2 and B3 the decrease was 50%. G x B interaction was found to be significant, CSMG 84-1 had a 49% increase in shoot dry weights with B3 treatment while TAG 24 had a 36% increase due to B2 treatment. G x S x B interaction was not significant (Table 4.8.1).

Pod dry weight (Pod dry wt) (g m^{-2})

8.6% WD treatment is found to produce 93% more pod dry wt than that of 67.5% WD treatment. Genotypic variation was significant where TAG 24 was found to produce more pod dry wt (449) and the least was observed in CSMG 84-1 (381). S x G interaction was found to be significant ($P<0.05$) where in TMV2NLM there is a 53% decrease in pod dry weights in 67.5% WD conditions and it is 50% decrease in CSMG 84-1. Betaine levels were found to be significantly different ($P<0.05$) with B1 producing 383 g m^{-2} while B3 treatment produced 442 g m^{-2}. SxB interaction was found to be significant. In B1 treatment, the decrease due to 67.5% WD was 61% while in B2 treatment, the decrease was only 33%. GxB and GxSxB interactions were not significant (Table 4.8.1).

Harvest Index (HI)

With an imposition of 67.5% WD there is a 15% increase in HI. Genotypic variation was observed, CSMG 84-1 recorded a HI of 0.83 while in ICG 476 it was only 0.57. S x G interaction was significant ($P<0.05$), CSMG 84-1 had a 30% decrease in HI with 67.5 % WD. Whereas it was less than 20% in all the other four genotypes. Betaine treatments were found to be not significantly different, GxB, SxB and GxSxB interactions were not significant (Table 4.8.1).

Table:4.8.1 Vegetative weights (Veg wt), pod dry weights (pod dry wt), and harvest index (HI) as influenced by betaine treatments under end season drought at 120DAS during rabi '96-'97.

		Veg wt(g m^{-2})			pod dry w (g m^{-2})			HI		
	WD%	B1	B2	B3	B1	B2	B3	B1	B2	B3
CSMG 84-1	8.65	912	829	1107	560	445	581	0.61	0.54	0.52
	39.9	519	583	543	396	283	297	0.76	0.49	0.55
	67.5	251	561	367	176	443	250	0.70	0.79	0.68
	mean	385	572	455	377	390	376	0.98	0.68	0.83
ICG476	8.65	865	867	1103	510	457	707	0.59	0.53	0.64
	39.9	654	678	1019	497	499	435	0.76	0.74	0.43
	67.5	299	704	549	211	312	332	0.71	0.44	0.60
	mean	606	750	1061	406	423	491	0.67	0.56	0.46
ICGV 86031	8.65	1078	877	992	554	504	572	0.51	0.57	0.58
	39.9	779	888	866	475	345	324	0.61	0.39	0.37
	67.5	454	444	458	196	302	311	0.43	0.68	0.68
	mean	770	736	772	408	384	402	0.53	0.52	0.52
TAG24	8.65	580	847	705	405	613	510	0.70	0.72	0.72
	39.9	452	556	657	413	432	398	0.91	0.78	0.61
	67.5	207	473	311	140	389	342	0.68	0.82	1.10
	mean	516	702	681	409	523	417	0.79	0.74	0.61
TMV2NLM	8.65	927	1292	997	595	599	787	0.64	0.46	0.79
	39.9	546	456	549	324	432	453	0.59	0.95	0.83
	67.5	384	421	543	298	291	342	0.78	0.69	0.63
	mean	619	723	696	406	441	527	0.66	0.61	0.76
	Gmean	579	696	733	401	432	443	0.73	0.62	0.64
	SeM		± 59.8			± 28.9			± 0.010	
	CV%		± 22.9			± 23.4			± 19.8	

Analysis of variance

Source of variation	df	pod dry wt	shoot dry wt	HI
MT (stress levels (S)	2	**	*	*
ST (genotypes (G)	4	**	**	*
S x G	8	*	*	*
SST (betaine levels) (B)	2	**	*	*
S x B	4	*	NS	*
G x B	8	*	*	*
G x S x B	16	NS	NS	NS

4. 8. 2 (a) Aerial pegs (AP) (pegs m^{-2}), Subterranean pegs (SP) (pegs m^{-2}) Total pegs (TP) (pegs m^{-2}) at 80DAS.

Aerial pegs (AP) (pegs m^{-2})

Genotypes differed significantly for AP at 80 DAS, ICGV 86031 had 268 pegs m^{-2} whereas in TAG 24 there are 428 pegs m^{-2}. Betaine treatments also differed significantly ($P<0.01$), B3 treatment is able to produce 364 pegs m^{-2} whereas B1 treatment had only273 pegs m^{-2}. G x B interaction was found to be significant(Table 4.8.2(a)).

Subterranean pegs (SP) (pegs m^{-2})

Genotypes differed significantly for SP at 80 DAS, ICGV 86031 had 409 pegs m^{-2} whereas in TAG 24 there are 672 pegs m^{-2}. (Table 4.8.2(a)). Betaine treatments also differed significantly ($P<0.01$), B2 treatment is able to produce 633 pegs m^{-2} whereas B1 treatment had only 507 pegs m^{-2}. G x B interaction was found to be significant.

Total pegs (TP) (pegs m^{-2})

Genotypes differed significantly for TP at 80 DAS, ICGV 86031 had 677 pegs m^{-2} whereas in TAG 24 there are 1101 pegs m^{-2}(Table 4.8.2(a)). Betaine treatments also differed significantly ($P<0.01$), B2 treatment is able to produce 965 pegs m^{-2} whereas B1 treatment had only 791 pegs m^{-2}. G x B interaction was found to be significant.

Table:4. 8. 2 (a)Aerial pegs (AP), subterranean pegs(SP), and total pegs (TP)as influenced by betaine treatments at 80DAS under end season drought during rabi '96-'97.

	AP (peg no m^{-2})			SP (peg no m^{-2})			TP(peg no m^{-2})		
Geno	**B1**	**B2**	**B3**	**B1**	**B2**	**B3**	**B1**	**B2**	**B3**
CSMG 84-1	294	269	295	488	662	597	782	931	893
ICG 476	243	208	388	441	721	309	683	929	696
ICGV 86031	157	297	351	314	459	455	470	756	807
TAG24	361	376	548	614	667	738	975	1043	1285
TMV2NLM	311	508	238	678	657	563	1044	1165	800
Gmean	**273**	**332**	**364**	**507**	**633**	**532**	**791**	**965**	**896**
SeM		**± 54.8**			**± 50.4**			**± 85.3**	
Cv%		**± 28.6**			**± 15.0**			**± 27.4**	

Analysis of variance

Source of variation	**df**	**AP**	**SP**	**TP**
MT (genotypes (G)	**4**	**	*	**
SST (betaine levels) (B)	**2**	*	**	**
G x B	**8**	*	*	*

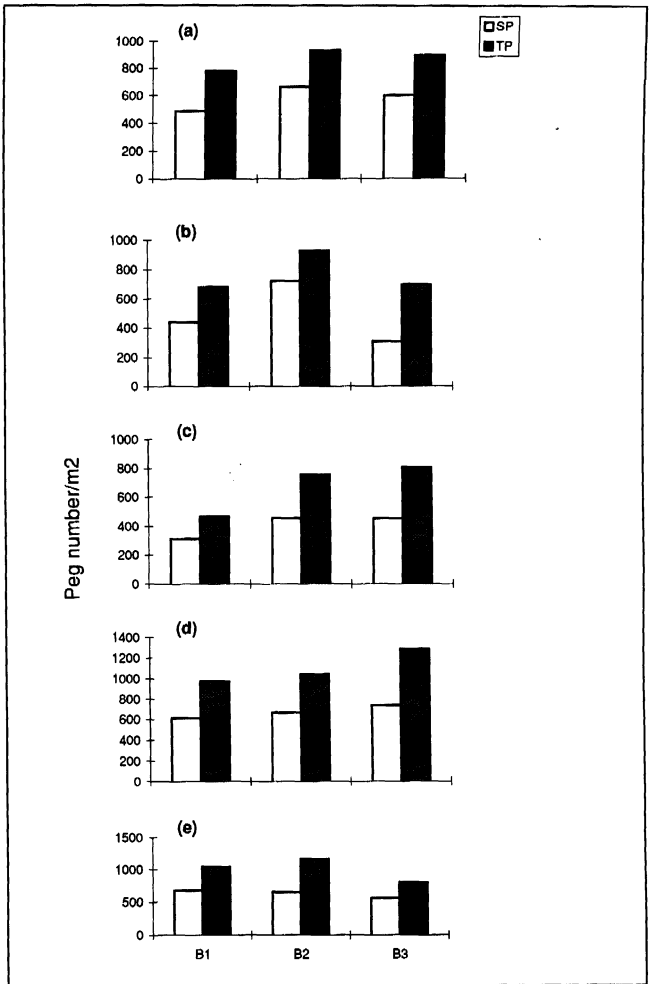


Fig : 4.8.2 Subterranean pegs and Total pegs as influenced by betaine treatments at 80DAS before the imposition of end season drought in 5 groundnut genotypes, CSMG 84-1(a), ICG476(b),ICGV 86031(c), TAG24(d), TMV2NLM(e).

4. 8. 2 (b) Aerial peg, (pegs m^{-2} day^{-1}), Subterranean peg (pegs m^{-2} day^{-1}) Total peg (pegs m^{-2} day^{-1}) addition rates.

Aerial peg addition rate (pegs m^{-2} day^{-1})

AP addition rates were significantly different with stress levels where 67.5% WD level could record an AP addition rate of 2.92 pegs m^{-2} day^{-1} while 8.65% WD level had 5.35 pegs m^{-2} day^{-1}. Genotypic variation was also significant where in ICGV 86031, the AP addition rate was 5.45 while the least was observed in CSMG 84-1 (2.45 pegs m^{-2} day^{-1}). S x G interaction is found to be significant. TMV2NLM had a 84% reduction due to end season drought, the reduction was only 12% in ICG 476. AP addition rates for betaine levels were significant with B3 treatment, the AP addition rates increased from 2.88 (B1) to 6.62. S x B interaction was also significant. The percent reduction due to ESD was 57 in B3 treatment, while it was 29% in B1 and B2 treatments. G x B interaction was significant, with betaine treatment the AP addition rates increased by 224% in TAG 24 while it was 62% in TMV2NLM. G x S x B interaction was not significant.

Subterranean peg addition rate (pegs m^{-2} day^{-1})

SP addition rates were significantly different with stress levels where 67.5% WD level could record an SP addition rate of 3.53 pegs m^{-2} day^{-1} while 8.65% WD level had 5.89 pegs m^{-2} day^{-1}. Genotypic variation was also significant where in ICG 476, the SP addition rate was 6.06 while the least was observed in CSMG 84-1 (3.06 pegs m^{-2} day^{-1}).

S x G interaction is found to be significant. TMV2NLM had a 78% reduction due to end season drought, the reduction was only 10% in ICG 476. SP addition rates for betaine levels were significant with B3 treatment, the SP addition rates increased from 2.58 (B1) to 7.13 pegs m^{-2} day^{-1}. S x B interaction was also significant. The percent reduction due to ESD was 52 in B3 treatment, while it was less than 30% in B1 and B2 treatments. G x B interaction was significant, with betaine treatment the SP addition rates increased by 315% in TAG 24 while it was 89% in TMV2NLM. G x S x B interaction was not significant.

Total peg addition rate (pegs m^{-2} day^{-1})

TP addition rates were significantly different with stress levels where 67.5% WD level could record an TP addition rate of 5.74 pegs m^{-2} day^{-1} while 8.65% WD level had 10.59 pegs m^{-2} day^{-1}. Genotypic variation was also significant where in ICGV 86031, the TP addition rate was 10.8 while the least was observed in CSMG 84-1 (4.81 pegs m^{-2} day^{-1}). S x G interaction is found to be significant. TMV2NLM had a 84% reduction due to end season drought, the reduction was only 11% in ICG 476. TP addition rates for betaine levels were significant with B3 treatment, the TP addition rates increased from 5.36 (B1) to 12.84 pegs m^{-2} day^{-1}. S x B interaction was also significant. The percent reduction due to ESD was 58 in B3 treatment, while it was less than 30% in B1 and B2 treatments. G x B interaction was significant, with betaine treatment the TP addition rates increased by 315% in TAG 24 while it was 89% in TMV2NLM. G x S x B interaction was not significant.

Table :4. 8. 2 (b)Aerial peg addition rate,subterranean peg addition rate and total peg addition rate as influenced by betaine treatments under irrigated and end season drought conditions during rabi '96-'97.

		AP addn rate			SP addn rate			TP addn rate		
	WD%	B1	B2	B3	B1	B2	B3	B1	B2	B3
CSMG 84-1	8.65	3.13	2.35	5.84	2.83	3.86	5.43	5.85	5.20	11.29
	39.9	1.27	0.29	1.84	0.97	1.80	2.46	2.14	1.08	3.29
	67.5	1.18	0.19	1.75	0.87	1.70	2.36	2.04	0.98	3.19
	mean	**1.86**	**0.94**	**3.14**	**1.56**	**2.45**	**3.42**	**3.35**	**2.42**	**5.93**
ICG 476	8.65	2.47	2.42	8.99	2.17	3.93	9.61	4.54	5.34	17.59
	39.9	2.08	3.98	2.97	1.78	5.49	3.59	3.76	8.45	5.55
	67.5	1.99	3.88	2.88	1.69	5.39	3.49	3.67	8.35	5.45
	mean	**2.18**	**3.42**	**4.95**	**1.88**	**4.93**	**5.56**	**3.99**	**7.38**	**9.53**
TAG 24	8.65	2.92	2.85	11.59	2.62	4.36	12.21	5.44	6.20	22.79
	39.9	4.12	3.67	7.54	3.82	5.18	8.15	7.83	7.85	14.68
	67.5	4.02	3.58	7.44	3.72	5.09	8.05	7.73	7.75	14.58
	mean	**3.69**	**3.37**	**8.86**	**3.39**	**4.88**	**9.47**	**7.00**	**7.26**	**17.35**
ICGV 86031	8.65	1.12	1.30	8.49	0.82	2.81	9.11	1.84	3.10	16.59
	39.9	2.96	3.89	4.76	2.66	5.40	5.38	5.52	8.29	9.13
	67.5	2.87	3.80	4.67	2.57	5.30	5.28	5.43	8.19	9.03
	mean	**2.32**	**3.00**	**5.97**	**2.02**	**4.51**	**6.59**	**4.26**	**6.52**	**11.59**
TMV 2 NLM	8.65	7.27	8.05	11.39	6.97	9.56	12.01	14.14	16.60	22.39
	39.9	1.47	0.17	2.75	1.16	1.68	3.37	2.53	0.84	5.11
	67.5	1.37	0.07	2.65	1.07	1.58	3.27	2.43	0.74	5.01
	mean	**3.37**	**2.76**	**5.60**	**3.07**	**4.27**	**6.22**	**6.37**	**6.06**	**10.84**
	Gmean	**2.68**	**2.70**	**5.70**	**2.38**	**4.21**	**6.25**	**4.99**	**5.93**	**11.05**
	SeM		**± 1.01**			**± 1.26**			**± 1.99**	
	CV%		**± 20.2**			**± 17.3**			**± 23.2**	

Analysis of variance

Source of variation	df	AP	SP	TP
MT (stress levels (S)	1	*	*	*
ST (genotypes (G)	4	*	*	*
S x G	4	*	*	*
SST (betaine levels) (B)	2	*	*	*
S x B	2	*	*	*
G x B	8	*	*	**
G x S x B	8	NS	NS	NS

4. 8. 3 Crop growth rate (CGR) (g m^{-2} day^{-1}), Pod growth rate (PGR) (g m^{-2} day^{-1}) and Partitioning %. (Part %).

Crop growth rate (CGR) (g m^{-2} day^{-1})

Stress levels differed significantly (P<005) for CGR. On an average 8.6% WD had a CGR of 20 (g m^{-2} day^{-1}) while 67.5% WD had a CGR of 11 (g m^{-2} day^{-1}) (Table 4.8.3). Genotypic variation is not seen. SxG interaction (P<0.05) was found to be significant where in CSMG 84-1 had a 67% decrease in CGR with an imposition of 67.5% WD, TMV2NLM had only 14% decrease. Betaine treatments were found to increase the CGR by 20% and the differences were significant (P<0.05). SxB interaction was significant, in B1 treatment, the decrease due to water deficit was 40% while in B2 treatment, the decrease was 36%. GxB interaction was significant, CSMG 84-1 had a 50% increase in CGR due to B3 treatment whereas it was only 18% in TAG 24. GxSxB interactions were not significant (Table 4.8.3).

Pod growth rate (PGR) (g m^{-2} day^{-1})

On an average, 8.6% WD treatment had a PGR of 12 whereas 67.5% WD had a PGR of 8. Genotypes had no significant difference (Table 4.8.3). SxG interaction was not significant. Betaine levels were found to be significantly different (P<0.05), B3 had a PGR of 12 whereas it was only 9 in B1 treatment. SxB was significant, in B1 treatment, the decrease due to water deficit was 49% while in B2 and B3 treatments it was less than 25%. GxB, GxSxB interactions were not significant (Table 4.8.3).

Table:4.8.3 Crop growth rate (CGR), pod growth rate (PGR) and partitioning % (part%) as influenced by betaine treatments under end season drought during rabi '96-'97.

		CGR ($g\ m^{-2}\ day^{-1}$)			PGR ($g\ m^{-2}\ day^{-1}$)			part %		
	WD%	**B1**	**B2**	**B3**	**B1**	**B2**	**B3**	**B1**	**B2**	**B3**
CSMG 84-1	**8.65**	21.0	29.7	22.4	14.4	20.6	14.3	0.69	0.69	0.64
	39.9	18.1	24.4	15.2	10.3	15.1	12.1	0.57	0.62	0.80
	67.5	4.9	12.2	6.7	4.3	10.3	6.2	0.86	0.84	0.92
	mean	**14.7**	**22.1**	**14.8**	**9.7**	**15.3**	**10.9**	**0.70**	**0.72**	**0.78**
ICG476	**8.65**	19.2	16.1	25.3	12.7	11.4	17.3	0.66	0.71	0.69
	39.9	15.3	16.7	21.6	9.6	14.5	19.6	0.63	0.87	0.91
	67.5	3.5	18.6	6.4	3.4	13.2	5.2	0.97	0.71	0.81
	mean	**12.7**	**17.1**	**17.8**	**8.6**	**13.0**	**14.0**	**0.76**	**0.76**	**0.80**
ICGV 86031	**8.65**	21.5	18.9	25.2	13.9	11.0	14.4	0.65	0.58	0.57
	39.9	20.5	22.8	24.6	11.8	15.0	16.3	0.65	0.66	0.66
	67.5	18.1	19.3	11.4	12.4	12.4	10.5	0.60	0.64	0.92
	mean	**21.0**	**20.3**	**20.4**	**12.7**	**12.8**	**13.7**	**0.63**	**0.63**	**0.72**
TAG24	**8.65**	12.1	25.6	22.7	10.1	10.0	4.7	0.83	0.82	0.88
	39.9	19.8	12.2	11.1	9.8	7.3	11.3	0.49	0.60	1.01
	67.5	11.7	12.2	5.4	6.7	6.4	14.0	0.34	0.25	0.62
	mean	**16.0**	**18.9**	**16.9**	**8.8**	**7.9**	**10.0**	**0.55**	**0.56**	**0.84**
TMV2NLM	**8.65**	11.8	19.8	14.6	12.5	14.8	11.3	1.06	0.24	0.78
	39.9	15.9	21.3	23.2	10.6	4.8	15.1	0.33	0.69	0.65
	67.5	12.5	7.9	19.2	5.2	6.8	14.0	0.85	0.86	0.73
	mean	**13.4**	**16.3**	**19.0**	**11.5**	**8.8**	**13.5**	**0.75**	**0.60**	**0.72**
	Gmean	15.5	18.9	17.8	10.3	11.6	12.4	0.68	0.65	0.77
	SeM		± 2.1			± 1.4			± 0.0657	
	CV%		± 19.7			± 20.4			± 22.2	

Analysis of variance

Source of variation	**df**	**CGR**	**PGR**	**part%**
MT (stress levels (S)	**2**	*	*	*
ST (genotypes (G)	**4**	**NS**	**NS**	*
S x G	**8**	*	**NS**	*
SST (betaine levels) (B)	**2**	**	*	**
S x B	**4**	**	**NS**	*
G x B	**8**	*	*	*
G x S x B	**16**	**NS**	**NS**	**NS**

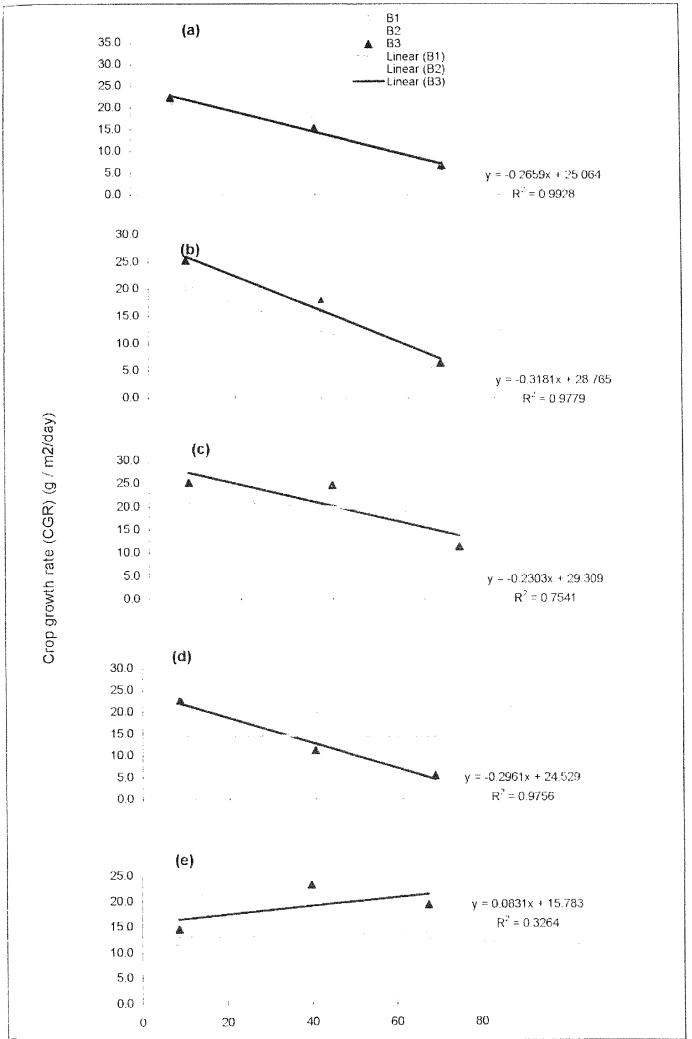


Fig : 4.8.3(a) Crop growth rate (CGR) as influenced by betaine treatments under end season drought during rabi '96-'97 in 5 groundnut genotypes, CSMG 84-1(a), ICG476(b), ICGV86031(c), TAG24(d)and TMV2NLM(e).

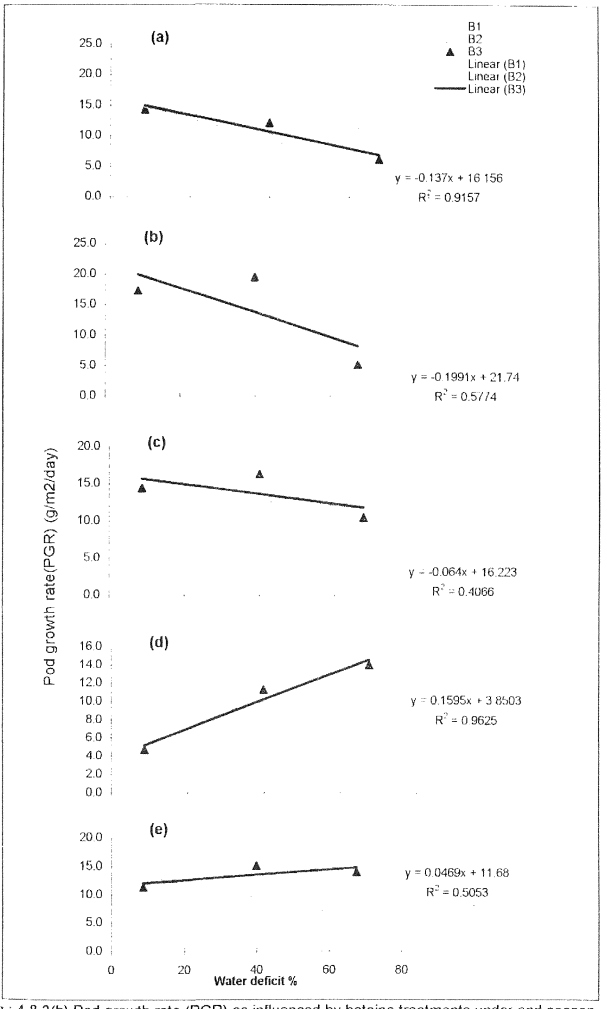


Fig : 4.8.3(b) Pod growth rate (PGR) as influenced by betaine treatments under end season drought during rabi '96-'97 in 5 groundnut genotypes, CSMG 84-1(a), ICG476(b), ICGV86031(c), TAG24(d)and TMV2NLM(e).

Partitioning % (Part %)

Part % decreased significantly with an imposition of 67.5% WD ($p<0.05$). On an average 8.6% WD had a Part % of 0.69 while 67.5% WD had a Part % of 0.67. Genotypes differed significantly for Part % with ICG 476 recording the highest Part % (0.77) whereas the lowest was in TAG 24 (0.65). SxG interaction was also found to be significantly different, in TAG 24 the decrease due to water deficit was 52% while in the other four genotypes, the decrease was less than 30%. Betaine levels were also found to be significantly different ($P<0.01$), B1 had a Part % of 0.67 whereas it was 0.77 in B3 treatment. SxB, G x B, G x S x B interactions were not significant (Table 4.8.3).

4.8.4 Net assimilation rate (NAR) (g m^{-2} day^{-1}), Leaf area duration (LAD) (days)

Net assimilation rate (NAR) (g m^{-2} day^{-1})

Stress levels were found to be significantly different ($P<0.05$). 8.6% WD treatment was found to produce 9.4 g m^{-2} day^{-1} while 67.5% WD had only an NAR of 5.4 (Table 4.8.4). Genotypic variation is not seen. SxG interaction was not significant. Betaine levels were found to be significantly different ($P<0.01$), B2 treatment had an NAR of 9.1 and B1 had only 7.1 g m^{-2} day^{-1}. SxB interaction was found to be significantly different, the decrease in NAR due to 67.5% WD was 52% in B1 treatment whereas it was only 29 to 45% in B2 and B3 treatments respectively. G x B interaction was found to be significantly different, ICG 476 had a 88% increase in NAR due to B3

Table:4. 8. 4 Net assimilation rate (NAR) and leaf area duraion (LAD) as influenced by betaine treatments under end season drought during rabi '96-'97.

		NAR (g m^{-2} day^{-1})			LAD (days)		
	WD%	B1	B2	B3	B1	B2	B3
CSMG 84-1	8.65	10.2	8.6	10.6	95	152	112
	39.9	7.4	7.3	7.2	73	121	90
	67.5	4.8	4.4	4.8	61	109	78
	mean	6.1	7.9	6.0	76	127	93
ICG476	8.65	7.1	8.1	6.1	101	107	87
	39.9	6.3	12.8	18.8	98	67	82
	67.5	2.4	8.8	5.2	89	76	65
	mean	5.3	9.9	10.0	96	83	74
ICGV 86031	8.65	7.7	5.0	11.2	78	157	134
	39.9	7.8	12.1	17.0	108	98	107
	67.5	5.5	7.0	4.1	109	97	102
	mean	7.0	8.0	10.8	98	117	105
TAG24	8.65	9.0	14.6	12.0	102	98	97
	39.9	8.3	10.1	13.5	101	87	90
	67.5	2.8	8.0	6.0	91	71	73
	mean	6.7	10.9	10.5	98	85	87
TMV2NLM	8.65	10.9	12.6	8.4	80	115	145
	39.9	11.7	12.1	7.9	69	71	116
	67.5	6.1	6.3	6.3	53	67	98
	mean	9.6	10.3	7.5	67	84	107
	Gmean	6.9	9.4	9.0	87	100	93
	SeM		± 1.31			± 15.9	
	CV%		± 22.8			± 20.6	

Analysis of variance

Source of variation	df	NAR	LAD
MT (stress levels (S)	2	*	*
ST (genotypes (G)	4	NS	NS
S x G	8	N	*
SST (betaine levels) (B)	2	*	*
S x B	4	*	*
G x B	8	*	*
G x S x B	16	*	NS

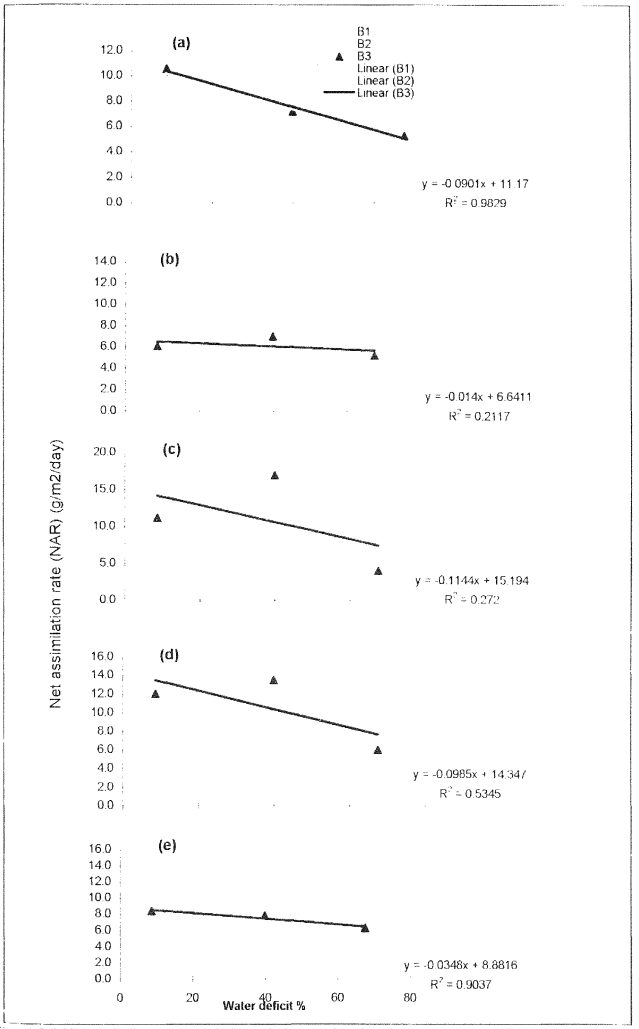


Fig : 4.8.4 Net assimilation rate (NAR) as influenced by betaine treatments under end season drought during rabi '96-'97 in 5 groundnut genotypes, CSMG 84-1(a), ICG476(b), ICGV86031(c), TAG24(d)and TMV2NLM(e).

treatment while in the other three genotypes, the increase was 30 - 60%. G x S x B interaction was not significant.

Leaf area duration (LAD) (days)

Stress levels were found to be significant, with an increase in water deficit there was a 25% decrease in LAD. Genotypes had no significant difference (Table 4.8.4). S x G interaction was found to be significant where in TMV2NLM had a 36% decrease in NAR due to 67.5% WD while in the other four genotypes, the decresae was 20- 25%. Betaine treatments were found to be significantly different ($P<0.01$). B2 treatment had an LAD of 100 while B1 had an LAD of only 87. S x B interaction was significant, G x B interaction was also significant, CSMG 84-1 had a 67% increase in LAD due to B3 treatment whereas in the other 4 genotypes the % increase due to B3 treatment was 25%. G x S x B interactions were not significant.

4.8.5 Photosynthetic rates (Pn) (μ mol m^{-2} sec^{-1}), Relative water content (RWC) (%), Osmotic Potential ($\psi\pi$) (milliosmoles), Light Interception (LI) (%)

Photosynthetic rates (Pn) (μ mol m^{-2} sec^{-1})

Stress levels differed significantly for photosynthetic rates ($P<0.05$) 8.6% WD treatment had on an average 18.6 μ mol m^{-2} sec^{-1} while 67.5% WD had a Pn rate of 14.7 μ mol m^{-2} sec^{-1}. Genotypes, S x G interaction were not significant (Table 4.8.5). Betaine levels were found to be significant ($P<0.05$). On an average B1 treatment had a

Pn rate of 15.9 μ mol m^{-2} sec^{-1} while B2 and B3 treatments had 17.5 and 18.4 μ mol m^{-2} sec^{-1} respectively. S x B, G x B, G x S x B interactions were not significant.

Relative water content (RWC) (%)

There were significant differences in RWC for stress levels, the RWC decreased from 92% to 67% with an increase in the water deficit (Table 4.8.5) Genotypes, S x G interaction were not significant. Betaine levels were found to be significantly different ($P<0.05$), on an average the B1 treatment had an RWC of 75%, while it was 86 and 84% in B2 and b3 respectiely. G x B interaction was significant, whereas G x S x B interaction was not significant.

Osmotic Potential ($\psi\pi$) (milli osmoles)

Stress levels did not differ significantly for OP (Table 4.8.5). Genotypes had significant difference, ICGV 86031 recorded a OP of 290 milliosmoles whereas OP was 358 in ICG 476. S x G interaction was significant. Betaine levels differed significantly ($P<0.01$). On an average B1 treatment had an OP of 370 while B2 had an OP of 274 and B3 had an OP of 287 milli osmoles. S x B interaction found to be significant where in B1 treatment, the decrease due to 67.5% WD was 5% whereas in B3 treatment the decrease was 0%. G x S x B interactions were not significant.

Table: 4. 8. 5 Total Betaine content(TB) as influenced by Betaine Treatments under end season drought conditions at 100 DAS during rabi '96-'97.

		TB (mM)		
WD%		B1	B2	B3
CSMG 84-1	8.65	15.3	80.9	93.5
	39.9	33.2	79.1	86.6
	67.5	23.1	72.2	90.1
	mean	23.9	77.4	90.1
ICG476	8.65	9.9	69.4	83.0
	39.9	13.6	74.0	87.9
	67.5	13.9	76.9	90.0
	mean	12.5	73.4	86.9
ICGV 86031	8.65	9.5	55.8	70.5
	39.9	13.5	54.2	98.5
	67.5	11.2	65.1	91.6
	mean	11.4	58.4	86.9
TAG24	8.65	10.3	60.0	92.2
	39.9	16.1	65.8	94.8
	67.5	15.5	65.7	96.3
	mean	14.0	63.8	94.4
TMV2NLM	8.65	5.4	48.1	91.2
	39.9	12.9	57.7	93.3
	67.5	8.9	48.1	91.8
	mean	9.1	51.3	92.1
	Gmean	14.2	64.9	90.1
	SeM		± 2.44	
	CV%		±14.60	

Analysis of variance

Source of variation	df	TB
MT (stress levels (S)	2	NS
ST (genotypes (G)	4	NS
S x G	8	NS
SST (betaine levels) (B)	2	*
S x B	4	NS
G x B	8	NS
G x S x B	16	NS

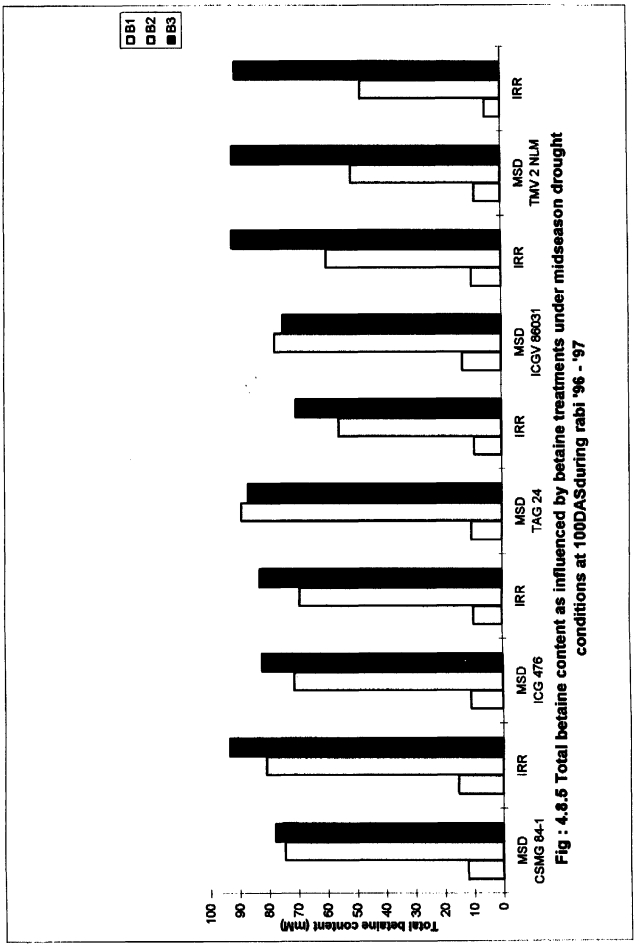


Fig : 4.8.5 Total betaine content as influenced by betaine treatments under midseason drought conditions at 100DASduring rabi '96 - '97

Table:4. 8. 6 Photosynthetic rate (Pn), relative water content (RWC), osmotic potential (OP) and light interception (LI%) as influenced by betaine Treatments under end season drought at 100 DAS during rabi '96-'97.

		Pn (μmol m^{-2} sec^{-1})			RWC (%)			OP(milli osmoles)			LI(%)		
	WD%	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
CSMG 84-1	8.65	15.2	18.7	18.7	87	90	96	327	256	245	56	41	64
	39.9	15.7	19.3	22.4	86	89	90	369	269	305	56	57	67
	67.5	13.1	15.0	13.2	55	76	67	362	305	289	41	62	41
	mean	**14.7**	**17.6**	**18.1**	**76**	**85**	**85**	**353**	**277**	**280**	**51**	**53**	**57**
ICG476	8.65	15.2	18.7	18.7	87	90	96	327	256	245	56	41	64
	39.9	16.9	16.7	19.0	76	91	83	515	423	421	77	61	41
	67.5	13.1	13.1	17.2	45	75	72	421	312	298	49	65	54
	mean	**15.1**	**16.2**	**18.3**	**69**	**85**	**84**	**421**	**330**	**321**	**61**	**56**	**53**
ICGV 86031	8.65	17.4	19.9	19.8	87	94	93	399	245	286	77	80	71
	39.9	19.2	17.7	18.6	87	93	95	298	205	214	78	83	76
	67.5	14.7	14.8	16.4	68	79	77	426	215	321	56	50	73
	mean	**17.1**	**17.5**	**18.3**	**80**	**89**	**88**	**374**	**222**	**274**	**70**	**71**	**73**
TAG24	8.65	17.0	17.4	22.8	88	97	90	352	214	263	69	71	39
	39.9	16.9	20.1	19.5	87	87	87	236	215	236	64	67	43
	67.5	15.4	16.3	15.4	54	75	68	389	354	321	52	77	49
	mean	**16.4**	**17.9**	**19.2**	**76**	**86**	**81**	**326**	**261**	**273**	**62**	**71**	**44**
TMV2NLM	8.65	21.5	19.3	19.1	88	93	95	523	421	412	87	21	35
	39.9	14.5	21.9	20.6	86	91	90	352	214	251	74	55	78
	67.5	13.7	14.1	15.5	52	73	70	263	215	210	57	47	52
	mean	**16.6**	**18.4**	**18.4**	**76**	**85**	**85**	**379**	**283**	**291**	**73**	**41**	**55**
	Gmean	16.0	17.5	18.5	76	86	85	371	274.6	287.8	63	59	56
	SeM		± 2.1			± 10.2			± 43.8			± 9.7	
	CV%		± 12.8			± 23.7			± 29.1			± 23.4	

Analysis of variance

Source of variation	df	Pn	RWC	OP	LI%
MT (stress levels (S)	2	*	**	NS	*
ST (genotypes (G)	4	NS	NS	*	NS
S x G	8	NS	NS	*	NS
SST (betaine levels) (B)	2	*	*	**	NS
S x B	4	NS	**	*	NS
G x B	8	NS	*	NS	*
G x S x B	16	NS	NS	NS	NS

Light interception (LI) (%)

Stress levels differed significantly in LI % ($P<0.05$). 8.6% WD had an LI of 58% whereas 67.5% WD had only 54% LI. Genotypes, betaine levels did not differ significantly for LI. S x G, G x B, S x B, G x S x B interactions were not significant (Table 4.8.5).

4.8.6 Total betaine content (TB) (mM)

Stress levels and genotypes did not differ significantly for TB. S x G interaction was also not significant whereas betaine levels differed significantly ($P<0.01$) (Table 4.8.6). With a spray of betaine, the TB increased from 14 mM in B1 to 90 mM in B3, showing a 542% increase in TB with B3 treatment. S x B, G x B, G x S x B interactions were not significant.

4.8.7 Correlation coefficients

Correlations of total betaine content with all the parameters were studied. At 8.65% water deficit, there was a significant positive correlation between total betaine content and RWC (0.792**), OP (0.573*), NAR (0.768**) and CGR (0.667**). There was a significant negative correlation between total betaine content and partitioning %. At 39.9%, a significant positive correlation was observed between total betaine content and CGR (0.456*) and PGR (0.621**), a significant negative correlation was there between total betaine content and OP (-0.503*). At 67.5% water deficit, there was a significant positive correlation between total betaine content and relative water content (0.727**), NAR (0.455*), PGR (0.577*). Whereas, there was a significant negative correlation between total betaine content and osmotic potential (-0.492*).

Table 4.8.7 : Correlation coefficients

	Water deficit %		
	8.65%	**39.9%**	**67.5%**
TB : CGR	0.792**	0.212^{NS}	0.727**
TB : PGR	0.573**	-0.503*	-0.492*
TB : Partition	0.768**	0.352^{NS}	0.455*
TB : NAR	0.667**	0.456*	0.262^{NS}
TB : LAD	0.022^{NS}	0.621**	0.577*
TB : Pn	-0.473*	0.222^{NS}	-0.012^{NS}

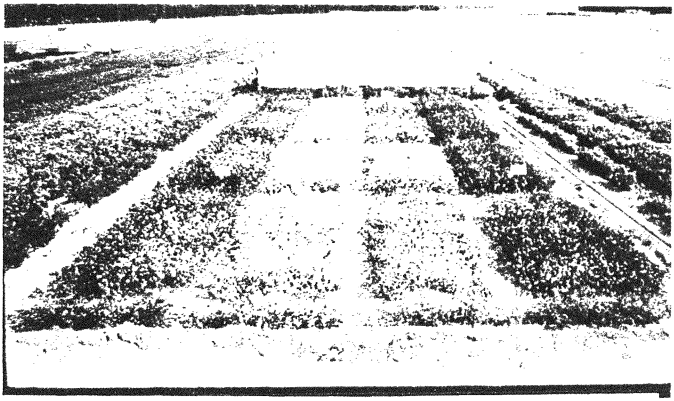


Plate 15 : Response of groundnut to water stress imposed as mid season drought from 40-80 DAS during the rainy season 1996 to study the effect of betaine to alleviate water stress.

Discussion

CHAPTER V

DISCUSSION

Legumes are the important source of dietary proteins and fat in most of the semi arid tropical (SAT) countries. In developing countries the ever increasing demand for cereal grain mitigates against the use of grain legumes in better endowed agricultural lands and often relegates them to less favourable, usually rainfed environment (Saxena *et al.*, 1993). Many of the biotic and abiotic stresses faced by grain legumes (Johansen *et al*, 1994) contribute to the large yield gap between potential yields and realized yields (*Subba Rao et al.*, 1995). Major abiotic stresses that limit the productivity of legumes in SAT are drought, salinity and high temperature. Among the grain legumes, groundnut is the major oilseed and cash crop of SAT and about 67% of global groundnut production comes from rainfed cultivation (Gibbons, 1980). The yield of groundnut crop is lower and erratic (900 kg ha $^{-1}$) mainly due to drought, diseases and pests. Drought remains as one dominant abiotic factor affecting groundnut production in India. The drought is often associated with high temperatures.

In India, salinity is also a major factor limiting the crop production. About 10% of the total cultivable soils in India suffer from salinity disorders as a result of heavy irrigation and poor drainage. It is common to see large patches of white crust of salt on the surface of the clayey soils containing sodium chloride and other salts in Haryana, Punjab, Rajasthan, and Western U. P.

Various agronomic and genetic management approaches have been studied to alleviate the complex abiotic factor such as drought (*Subba Rao et al.*, 1995). Drought is

a complex phenomenon which involves interaction of plant with environment variables such as temperature and also soil variables such as water and nutrition. The crop starts experiencing water deficit when the roots are unable to supply water to meet the environmental demand. However, environmental factors such as high temperature can accentuate the effect of drought through manipulating stomatal movement. For example, even in case of roots being able to access water from deeper soil profile and the high VPD can affect stomatal movement resulting in closure of stomata, thus leading to a build up of high temperature in the leaf. Even though, supplementary irrigation is an efficient production practice to alleviate water deficit, availability of this resource is scarce and likely to be more and more limiting in future. Hence alternate agronomic and genetic management approaches are being investigated to mitigate effects of drought on crop production. However adoption of these technologies depend on farmer's perception and his economic resource. Hence, development of seed based technologies with mechanisms to tolerate/resist major abiotic stress factors will be long lasting and sustainable.

Although extensive work was done on various biochemicals and physiological traits contributing to drought has been done, the utility of these in crop improvement programs is limiting. So far. At ICRISAT centre, physiological investigations on the effects of drought on groundnut has been extensively investigated which led to development of simple, rapid and efficient tools to assess genotypic variability for the traits contributing to superior performance of genotypes under water deficit conditions (Nageswara Rao *et al.,* 1992). It is only recently, methodologies for utilising these as indirect selection tools in breeding programs have started emerging (Wright *et al.,* 1996)

In addition to the above pursuits, it is necessary to investigate other management tools to alleviate deletorious effects of environmental factors such as drought, heat and salinity in groundnuts.

Glycine betaine is a quaternary ammonium compound, naturally occurring in many halophytes and in cultivated plants, although several crop species cannot synthesize this compound. The glycine betaine accumulation has been implicated with osmotic adjustment in many crops (Robinson and Jones, 1986; Matoh *et al*, 1987; Rhodes and Hanson, 1993). In addition to this glycine betaine was also shown to be playing a major role as a "protectant" for protein and membrane structures from high concentrations of Na^+ and Cl^- (Hanson *et al*, 1994). Several studies have shown significant yield increase with exogenous application of glycine betaine in green house and field grown crops such as tomato (Makela, 1998) tobacco (Agboma *et al.*, 1996) cotton (Naidu *et al.*, 1995). Recently, biosynthetic pathway of glycine betaine has become a target for genetic engineering approach to enhance the stress tolerance (McCue and Hanson, 1990; Rathinasabhapathi *et al.*, 1994; Holmstrom *et al.*, 1994). However there is no information on production of glycine betaine by groundnuts to our knowledge, although only one study by Muthukumaraswamy and Paneerselvam, (1997) indicated the ability of groundnuts to produce glycine betaine when fungicide - Triademefon was applied, other than this, the author is unaware of any particular study on production of betaines and their role in combating abiotic stresses in Groundnut. As the synthesis of organic solutes such as glycine betaine is bioenergetically costly, exogenous application of this compound has been suggested as an alternative approach to enrich the tissue with betaines to alleviate environmental stress effects (Makela, 1998).

The present investigation reports the effects of exogenous application of glycine betaine on sensitivity of selected groundnut genotypes to three major environmental stress factors i.e., drought, heat and salinity. Experiments have been conducted in three phases:

(a) To investigate the effects of betaine on tolerance of germinating seedlings.

(b) Effects of betaine on isolated plants growing in pots subjected to drought, heat and salinity.

(c) Effects of betaines on tolerance of genotypes to simulated drought under field conditions.

(a) **Effects of betaine on sensitivity of germinating seedlings to heat and salinity stress conditions.**

High temperature is one of the major abiotic constraints limiting the production of legume crops such as groundnut. Temperatures above 23^0 C have shown to slow down the pod growth and development (Dreyer, 1980). Several studies have shown that optimum temperature range for germination of groundnut was 21-30^0 C. The rate of growth was linearly reduced with increase in temperature beyond 32^0 C, thus high soil temperature (> 30^0 C) during the seed germination phase will be deletorious to groundnut.

Under normal circumstances, there will be slow and gradual increase in the temperatures until it reaches maximum during the midday followed by reduction, thus dynamic diurnal rhythm offers adaptive mechanism to emerging seedlings to combat high

temperature stresses. Experiments conducted with seedling systems have clearly shown that heat induction treatments resulted in improving adaptation of the growing seedlings to high temperature stress. In the seedlings with heat induction treatments (HI) growth of roots and shoot were greater by 157 and 44 % respectively than the non induced seedlings (Table 4.1.1). The inhibitory effect of NI treatments could be due to various physical (desiccation) and physiological reasons. The delecterious effects of high temperature stress on metabolism have been described in detail by Sutcliffe, (1977) and Lawlor, (1979). High temperatures may cause metabolic injury by either direct ways (desiccation) or indirectly by influencing the sequence of metabolic reactions resulting in imbalance in natural metabolic pathways. Temperatures above 31^0 C have been shown to enhance the rates of respiration in several crop plants. Prolonged exposure to high temperature can lead to thermal death. It was apparent that glycine betaine treatment with 25 mM or 50 mM concentrations resulted in a significant increase in the recovery growth of roots even under non induced treatments. The synergetic effects of HI and betaine treatments were also apparent in these experiments. These experiments also have shown that higher concentration (100 mM) of betaine was inhibitory suggesting specificity of betaine concentration in the tissues to result in positive growth. The beneficial effects of betaine could be ascribed to various reasons such as protection to proteins an their functions and in inducing new proteins (heat shock) which offer defensive mechanisms to combat heat stress.

In the present study, effect of heat stress on possible changes in protein metabolism has been investigated using gel electrophoresis techniques. As shown in the figures 4.1(a) and 4.2.1 (a), the betaine treatments resulted in quantitative as well as

qualitative changes in protein banding patterns. Nature of proteins produced consequent to the heat and betaine treatments under heat and betaine interaction has been described in detail in results chapter 4.1. In summary, HI treatment alone produced 2 additional bands of proteins with molecular weights of and 85 and 54.5 kDa. Betaine treatment under NI conditions resulted in production of 4 additional bands which included high and low molecular weight proteins (76.4, 60.6, 54.6 and 16.5 kDa). Combination of B_{25} + HI compared to B_0 + HI produced 4 additional bands which are of similar nature to those produced with B_{25} + NI treatment. B_{25} + HI treatment has produced 2 additional bands (35.6 and 32.4 kDa) which are of low molecular weight and distinctly different from those produced under B_{25} + NI.

Several studies have shown the production of heat shock proteins (HSPs) and implicated these as molecular mechanisms to enhance adaptation of the tissues to high temperature stress (Ashwni *et al.*, 1997). Several HSPs were identified with various molecular weights in different plants. Rice seedlings exposed to high and low temperatures, salinity and water stress produced HSP'S of 87 and 85 kDa collectively referred as stress associated proteins (SAP 90). (Ashwani *et al.*, 1997), HSP 104 plays a crucial role in the development of thermotolerance in yeast cells and the same protein accumulated in rice seedlings in response to heat stress.

There was no information on the influence of betaine on the protein metabolism. Results from the present study indicate production of a combination of high and low molecular weight proteins by B_{25} enrichment, whereas the heat induction apparently supported production of only high molecular weight proteins. These results indicate that betaine might be having a metbolic role by producing HSP's, these results further

substantiate from the study in which the effect of betaines was compared with other osmotically active substances such as sucrose (Plate :8) to examine if the observed changes in protein metabolism are specific to betaine alone or they could be induced by other osmoregulants. It was apparent that the effects of sucrose on root growth were not as significant as they were with betaines. The results from the gel electrophoresis also showed that sucrose treatment did not result in development of any HSP's. These results indicate that effects of glycine betaine were different to that of other osmo regulants like sucrose and the observed positive responses of betaine were manifested from the metabolic changes on the enzymes and other growth promoting proteins rather than mere osmoregulation.

Salinity stress:

The results have shown that salinity stress had significantly reduced the root and shoot growth (Table 4.2.1). Salinity induction resulted in significant enhancement in growth of root (106%) and shoot (72%) compared to that under non induced treatments. In fact, the root and shoot growth in SI treatment was comparable to that under non induced control (NI conditions). Enrichment of seeds with betaine (25 or 50 mM) resulted in significant improvement in tolerance to salinity. For example, the seeds treated with betaine upto 50 mM maintained their growth at high salinity conditions (300 mM), whereas this salinity level was inhibitory to growth of seedlings in B_0 treatment.

The effects of salinity on crop growth are documented extensively (Subba Rao and Johanson, 1994; Epstein and Rains, 1987). The major effect of high salinity levels were shown to be through damage to membrane and imbalance of osmolarity of cell sap

(Akbar and Ponnamperuma, 1980). Although management of saline soil is a major production practice, there exists a need for enhancement of salinity tolerance through genetic approaches (Tal, 1985).

The present study illustrates the role of exogenous application of betaines in alleviating salinity stress effects. Maintenance of osmotic pressure inside the cell by accumulation of solutes and exclusion of ions has been shown as an adaptive mechanism to salinity conditions. However, the present study again establishes the influence of betaine on protein metabolism. It was apparent from the results that SI is able to produce 2 additional protein bands (45.1 and 36.4 kDa) compared to NI. B_{25} treatment was able to produce 4 additional bands (65.4, 37.8, 35.4 and 16.5 kDa) in SI when compared with NI. B_{25}+ SI treatment produced 4 additional bands (45.4, 32.6, 24.8 and 18.4 kDa) when compared with 8 B_{25} + NI treatment. The proteins produced due to salinity induction and B_{25} treatments are both high and low molecular weight proteins. B_{25} + NI treatments produced 2 additional bands (46.2 and 18.5 kDa) when compared with B_0 + NI treatments.

One of the extensively characterised stress proteins in higher plants is the synthesis of stress shock proteins (SSPs). The SSPs produced under salinity stress were documented by Singh *et al.*, (1985), Ramagopal, (1987) and Esake *et al.*, (1992). The SSPs have been shown to be synthesised under mild stress. The ability of induced systems to tolerate severe levels of stress signifies the importance of stress proteins (Lin *et al.*, 1984, Vierling 1991). Unique proteins of 21 and 54 kDa were observed with 200 mM NaCl induction in fingermillet by Uma *et al.*, (1995). Similar qualitative differences were reported in maize (Ristec *et al.*, 1991) and wheat (Krishnan *et al.*, 1989). Salt stress

proteins were studied in different crops for example. 58. 18 kDa in tobacco (Singh *et al.*, 1985), 21-34 kDa in wheat (Ramagopal, 1987. Hurkman *et al.*, 1989). 14 kDa in tomato (Goday *et al.*, 1994), in citrus (Ben - Hayyim *et al.*, 1993) and 22 kDa in mustard (Reviron *et al.*, 1992).

The review and results indicate that betaine acts not as a mere osmoregulant but also had metabolic changes associated with enzymes and other growth promoting proteins like stress shock proteins called salinity stress proteins.

(b) Effects of betaines on isolated plants growing in pots subjected to salinity, drought and heat.

Salinity:

The results have shown that salinity stress had resulted in significant development (by 78% in B_0) however in B_{25} treatments the reduction was only 58% which clearly indicated that betaine treatment alleviated the adverse effects of salinity to some extent. The experimental results showed that seeds pretreated with glycine betaine heve shown increased growth of root (135%), shoot (25%) and biomass (28%) (Table 4.3.2). The increase growth and development was supported by increased photosynthetic rates by (30-35%) with B_{25} treatment.

However betaine did not influence the leaf RWC despite an increase in all the other parameters. It was apparent that the observed increment in growth due to betaine could be ascribed to presence of high betaine in the tissue. The exogenous application of betaine resulted in increase in levels of glycine betaine by 430% more than that of

untreated plants (Table 4.3.5) These results were in accordance with the work done on tomato by Smith *et al.*, (1992) and Plaut, (1995). These results showed that glycine betaine can be readily taken up by the emerging seedlings and the chemical compound was translocated to the young leaves as the leaves were analysed for total betaine content and the TB content was 430% more in plants which were from seeds treated with betaine.

The results were in agreement with studies of Naidu (1995) on the effects of glycine betaine on cotton, in which the seed treatment enhanced the germination and seedling vigour. In these studies Seedling dry matter production increased by 64-68% in response to 5% seed treatment using glycine betaine in controlled environment (Naidu, 1995) and field experiments (Naidu *et al.*, 1996, Campbell *et al.*, 1996).

The results from the present study are in support of literature and demonstrated that the glycine betaine can increase in germination, seedling vigour and yield. It was noted in the present study that the glycine betaine had a positive effect on root and shoot lengths, root and shoot development, total biomass, leaf area and crop growth rates. These responses suggest a hormone like activity for glycine betaine and similar effects have been noted in grapes (Naidu, unpublished). Wheeler (1973) in fact suggested that glycine betaine had activity similar to cytokinins. The studies suggest that either genetic or mangement practices which could result in accumulation of endogenous betaine by groundnut can enhance the seedling growth and development.

Drought stress:

Imposition of drought resulted in the reduction in above ground dry mass by 50% compared to control however in case of seed treatment with betaine the reduction was

only 12%. The present study have shown that seed treatment with glycine betaine could increase the root and shoot lengths by (25 and 15%), root and shoot development (50 and 32%) total dry matter (20%), CGR (30%) of isolated plants under water stress conditions. The increase in above ground weight following seed treatment with betaine resulted from the well known physiological function of endogenously synthesised glycine betaine that improves drought tolerance. As a cytoplasmic osmoticum, it enables the plant to maintain photosynthetic activity in osmotic stress conditions, stabilise the enzymes involved in amino acid metabolism and maintain turgor pressure even at leaf concentration of upto 500 mM (Borowitzka,, 1981; Wynjones and Storey, 1981). The overall results here indicate that treatment with glycine betaine could reduce yield losses of groundnut under water limiting conditions.

Glycine betaine treatment increased the Fv/Fm ratio under drought stress which in turn resulted in an increase in photosynthetic rate (Table 4.6.4). These findings are in support of the reports by Makela *et al.*, (1998) who showed an increase in leaf, stem, root dry weights, net photosynthetic rate in tomato under water stress.

In the present study plant water status measured as RWC, was unchanged in glycine betaine treatments and control, whereas, there was a decrease in ** values with B_{25} treatments. Our water relations data are in contrast to results of Sonoeka *et al*, 1995 who found an increase in RWC with glycine betaine accumulating maize lines grown under stress conditions compared to glycine betaine deficient genotypes. They also found that a higher leaf sap osmolarity and turgor was higher in lines which accumulate the high glycine betaine lines. Since the majority of the reported glycine betaine accumulation (6-11 mM) were predominantly located in cytoplasmic compartments

rather than vacuoles this finding indicates that glycine betaine might be playing a major metabolic role. The importance of the compartmentation of glycine betaine was even demonstrated in the osmotic adjustment of chloroplasts (Robinson and Jones, 1986) and the cytoplasm (Matoh *et al.*, 1987) of plants which are able to synthesize it naturally. However it is not known whether exogenously applied glycine betaine is accumulated in the cytoplasm or other cellular compartments such as vacuoles (Makela *et al.*, 1998). In addition to the putative role as an organic solute compatible with enzyme functioning (Rhodes and Hanson, 1993), glycine betaine treatments have also been shown to have protective effects on membranes (Yang *et al.*, 1996) and protein functions (Papageorgiou *et al.*, 1991) during stress.

High temperature stress:

The present study strongly suggested that glycine betaine accumulation offers partial protection of tissues from the injurious effects of high temperature. The results demonstrated that, as the exposure of tissues to temperature above 40^0 C results in significant reduction in root and shoot growth (Table 4.5.1). Chlorophyll fluorescence was severely disrupted in treatments where there was no glycine betaine accumulation (Table 4.5.5). Similar results were observed by Yang *et al.*, 1996 where they observed effects on high temperature membrane stability and chlorophyll fluorescence. Their results showed that chlorophyll fluorescence decreased abruptly when temperatures increased above 50^0 C in betaine deficient maize lines, whereas in betaine containing maize lines, Fv/Fm ratios increased in the heat stress treatment.

In the present study, seed treatment with betaine resulted in increase of root and shoot lengths, total dry matter and leaf area expansion rates. Root growth increased by was found to increase by 10% with B_{25} treatments. The % reduction due to high temperature stress in the TDM, CGR and LAER was reduced in B_{25} treatments compared to B_0 treatments. The benefits and stress alleviating effects of glycine betaine have been demonstrated by several workers in the past under laboratory conditions, often on isolated enzymes (Paleg *et al.*, 1985) or on whole plants in short duration experiments of Zao *et al.*, 1992.

Saneoka *et al.*, (1995) found that RWC was greater in glycine betaine accumulating maize lines grown under stressed conditions than in glycine betaine deficient ones. They also found a higher leaf sap osmolarity and higher turgor in some of the high glycine betaine lines. The results confirm the positive effects of glycine betaine under stressed conditions.

PS II plays a critical role in the responses of photosynthesis to environmental stress (Baker 1991), and several physiochemical constraints including high temperature and salinity stress can cause lesions in the reaction centre of PS II (Armond *et al.*, 1980; Cao and Govindjee, 1990). Increasing temperatures were believed to lead first to a blockage of PS II reaction centres, followed by a phase separation of non-bilayer forming lipids in thylalloid membranes (Armond *et al.*, 1980, Gounaris *et al.*, 1983). In the present study, B_0 treatments exhibit greater thermo lability from PS II function as inferred by fluorescence measurements. There were marked differences in chlorophyll fluorescence between B_{25} and B_0 treatments. These lines of evidence support the

conclusion that glycine betaine protects PS II from thermal damage as duration of exposure to 40^0 C in beyond one hour, the Fv/Fm ratio markedly decreased in the B_0 treatments under heat stress and no differences in Fv/Fm ratios were observed in B_{25} and B_0 of untreated controls. These results were similar to those described by Yang *et al.*, (1996) and Havaux (1992). It can be concluded from the present studies that high temperatures cause a complete and irreversible destruction of PS II in heat sensitive plants and B_{25} treatment appears to reduce the extent of this damage by protecting the concerned proteins and their functions. The results presented here strongly suggest a protective role for glycine betaine against heat destabilisation of plasma membranes and thylakoid membranes in groundnut. Although, the mechanism of thermoprotection Invivo is not known, several possible roles for glycine betaine have been suggested from studies where chloroplasts or membrane preparations are incubated with glycine betaine and other compatible osmolytes (Williams *et al.*, 1992).

The temperature treatments utilised in the present study suit the naturally encountered groundnut production environments, this hypothesis shows that glycine betaine can influence heat tolerance in groundnuts.

(c) Effect of betaines on tolerance of genotypes to simulated drought under field conditions.

In this field study, glycine betaine foliar application was studied in the rainy season under mid season drought conditions and glycine betaine foliar application did not result in any positive way, which is related to the tolerance of drought. As illustrated in

table 4.6.2 drought reduced the total biomass, AP, SP, TP production and growth. There was a decrease in net photosynthetic rate, RWC, LI% due to mid season drought imposed from 40-80 DAS. These results are not in agreement with results of Wynjones (1984) who examined the exogenous glycine betaine on biomass production of maize seedlings under stress. The betaine levels which were used in our experiment were 0, 3, 6 and 9 kg ha^{-1} and the betaine was foliarly applied to the plant @ 30 and 60 DAS. The leaves would have received little surface glycine betaine absorbed directly would have been greatly diluted during subsequent expansion. This finding supports the earlier work on translocation of radio labelled glycine betaine in summer turnip rape. (Makela *et al.*, 1996).

We could not detect an increase in the total biomass of plants in response to glycine betaine in the drought experiments but Makela *et al.*, (1998) have reported that the yield increases obtained by glycine betaine application are highly dependent on the growth stage of bush tomato, mid flowering stage being the most responsive in terms of increased yield. tomato has an indeterminate growth pattern (Plaut, 1995). So, glycine betaine applications might affect yield by changing the source - sink relations and assimilate might be allocated for enhancement of flower set instead of accumulation of leaf, stem or root dry weights.

Because, the levels of glycine betaine were expected to be high, and also the application of glycine betaine was very late i.e., at 30 DAS, another field experiment was done in the post rainy season of 1996-97 with 3 levels of glycine betaine (0, 3, an 6 kg ha^{-1}) and water stress was imposed as mid season and end season drought. Glycine betaine

was applied foliarly at 15 and 45 DAS, mid season drought reduced the pod dry weight, and the IRR treatment was able to produce 159% more pod dry weight, 22% more HI than that of the MSD treatment. Pegs production (both aerial and subterranean) was also found to be more in IRR conditions than that of MSD. The percentage decrease due to MSD was 35% in betaine untreated plants, whereas in plants where betaine was foliarly applied the decrease due to MSD was only 20% in B_2 and 2% in B_3 treatment (Table 4.7.2 (a)). Same trend was observed with subterranean peg and total pegs. Peg addition rates also followed the same trend, where with betaine the decrease in peg addition rates was less when compared with water sprayed plants. Crop growth rates increased by 25% with the application of betaine, the decrease due to MSD in CGR was less in betaine treated plants. PGR, Part % NAR followed the same trend. Photosynthetic rates increased by 15-20% with the application of betaine, relative water content remained unchanged.

The results are in agreement with the results obtained by Saneoka *et al.*, (1995) on isogenic lines of maize and Makela *et al.*, (1998) who have reported that the yield increases in tomato obtained by glycine betaine application. The results are highly dependent on the stage at which the glycine betaine was applied. In addition to a putative role as an organic solute compatible with enzyme functioning (Rhodes and Hanson, 1993), glycine betaine may have 'protective effects' for membranes (Yang *et al.*, 1996) and protein functions (Papageorgiou *et al.*, 1991) during stress. More specifically, glycine betaine can 'protect' the O_2 evolving machinery of chloroplasts when exposed to high NaCl concentrations (Murate *et al.*, 1992). Although these studies have used higher concentrations of glycine betaine than found in the tissues, it is possible that glycine

betaine may still play a protective role for proteins or membranes even when present at concentrations too low for an osmotic role.

When glycine betaine was applied at 15 DAS as soil application and as foliar application @ 45 DAS to plants under irrigated and end season drought conditions, similar responses were observed which were observed in the mid season drought conditions.

The increase in total dry matter following foliar application of glycine betaine to groundnut probably resulted from the well known physiological function of endogenously synthesized glycine betaine that improves drought tolerance. As a cytoplasmic osmoticum, it enables the plant to maintain photosynthetic activity in osmotic stress conditions, stabilize the enzymes involved in amino acid metabolism, and maintain turgor pressure even at leaf concentrations of upto 3 kg ha $^{-1}$ (Borowitzka, 1981; Wynjones and Storey, 1981; Laurie and Stewart, 1990). This aspect of our results is similar to that of Wynjones (1984) who examined the effects of exogenous glycine betaine on biomass production of maize seedlings under osmotic stress and found reduction in the fresh weight of the control plants upto 61%. When 1 mM of glycine betaine was applied, in our experiment, as illustrated in tables 4.8.1 to 5, drought (ESD) depressed the TDM and pod dry weights when compared with the irrigated control. Sinclair *et al.,* (1990) found decreases in maize grain yield induced by water deficits near anthesis to be closely correlated with decreases in biomass production. The significant effect of glycine betaine on the PDM could be one to more number of pods m $^{-2}$ followed by glycine betaine treatment.

Three levels of water deficit were studied (8.6, 39.9 and 67.5%). Water deficit of 8.6% was found to produce 93% more pod dry weight than that of 67.5% WD treatment. B_2 treatment, i.e., 3 kg ha^{-1} glycine betaine application produced a pod dry weight of 442 g m^{-2} whereas, it was only 383 g m^{-2} in water sprayed plants i.e., control plants. (Table 4.8.1). Peg addition rates increased from 2.88 to 6.62 with betaine treatment. The percentage reduction due to ESD was 87% in control plants whereas it was only 22% in betaine treated plants. Similar trend was observed for SP and TP addition rates, betaine treatments were found to increase the CGR by 20%, PGR by 33%, HI by 15% and NAR by 28%. On an average, net photosynthetic rate was 15.9 μ mol 2 m^{-2} sec^{-1} whereas with betaine treatments it increased upto 18.9 μ mol m^{-2} sec^{-1}. RWC was only 75% in B0 and 86% in B_2 treatments (Table 4.8.4). Mc Donnell *et al.*, (1988) and Naidu *et al.*, (1990) reported that glycine betaine was accumulated during progressive stress development in wheat the increase in PDM could have compensated by an increase in PGR and no. of pods m^{-2}. The relatively high residual glycine betaine concentrations (Table 4.8.5), 60-90 mM in betaine treated plants confirm its stability in plant systems, as reported by Storey (1976), Hanson and Wyse (1982) and Agboma *et al.*, (1996). This implies that drought protection for the treated plants can last for a considerable time. The apparently high residual leaf glycine betaine level would have resulted from the plant's ability to translocate glycine betaine.

In these field studies, aqueous glycine betaine, foliarly applied to drought stressed groundnut at their critical growth stages, improved dry matter, production, pod yield. Three weeks after application, residual concentrations of leaf tissue glycine betaine in

groundnut were comparable to levels in notable glycine betaine accumulating species (Wynjones and Storey 1981). The stability of glycine betaine in plants could mean that treated plants are drought tolerant for a long time after treatment. The overall results from the field indicate the application of glycine betaine could reduce yield losses of groundnut grown under water limited conditions.

It is now a well known fact that glycine betaine plays a protective role for proteins or membranes even when present at too low concentrations for an osmotic role.

Other solutes may play a protective role in plants suffering drought or salinity stress. Salt stressed tomato has previously been shown to accumulate proline, glucose and sucrose (Hever and Feigin, 1993; Alarcon *et al.*, 1994a, Bolarin *et al.*, 1995, Balibree *et al.*, 1997). The adaptive significance of proline accumulation in non halophytes has been questioned by several workers (Rabe 1990). For tomato, Bolarin *et al.*, 1995 have argued that, since proline accumulation occurs only after high levels of sugar accumulate, proline accumulation is a consequence of reduced protein synthesis. Further more, proline accumulation has been reported to be higher in the leaves of salt sensitive rather than salt tolerant tomato genotypes (Balibree *et al.*, 1997).

Larhar *et al.*, (1996) found that glycine betaine supply to leaf discs of osmotically stressed turnip rape resulted in lower accumulation of proline. Makela *et al.* (1998), found that exogenously applied glycine betaine did not affect the levels of protein in the leaves, so that the physiological effects of glycine betaine absorbed from foliar applications was not linked to changes in tissue proline concentration.

In conclusion, the results of these experiments suggest that plants when treated with glycine betaine confer increased tolerance to high temperature, water stress and salinity. Either seed treatment or foliar application of glycine betaine can increase the yield increasing parameters in the groundnut plants and will reduce the yield losses under limiting or stress conditions.

However, many higher plants do not accumulate glycine betaine and this has led to interest in the metabolic engineering of the glycine betaine biosynthesis pathway a an approach for enhancing stress resistance.

Summary

CHAPTER VI

Summary

Many of the biotic and abiotic stresses faced by grain legumes contributr to the large yield gap between potential and realzed yields. Abiotic stresses occurring at critical growth stages in groundnut affect productivity by reducing the total dry matter, pod yield and quality. Present study investigates the role of glycine betaine in alleviating effects of the three major abiotic stresses i. e., drought, heat and salinity on selected groundnut genotypes. The investigation was conducted in 3 phases (a) effect of betaine on tolerance of groundnut seedlings to heat and salinity stress conditions, (b) effect of betaine on isolated plants growing in pots subjected to drought, heat and salinity, and (c) effect of betaine on tolerance of groundnut genotypes to simulated drought under field conditions. The experiments were conducted during 1996- 98 period at **ICRISAT** centre, Patancheru in laboratory, glass house, growth chamber and field. (a) The seedlings were subjected to high temperature and salinity stress conditions in laboratory with and without glycine betaine treatment, under high temperature stress conditions, the seedlings with betaine treatment are able to produce root and shoot lengths (34 and 40%) than seedlings without betaine treatment, in the non induced treatments, there was a 122% greater growth in betaine treated seedlings compared to untreated ones, correspondingly the gel electrophoresis results indicated that betaine treatment was able to produce four new proteins with molecular weights of 76.4, 60.6, 54.6 and 16.5 kDa. Similarly under salinity stress conditions, the betaine treatment was able to produce 30 and 32% more root and shoot growths. The protein profiles indicated that betaine treatment was able to produce four new proteins with molecular weights of 65.4, 37.8, 35.4 and 16.5 kDa. These stress shock proteins which are produced under high temperature and

salinity stress conditions were implicated as molecular mechanisms to enhance the adaptation of the tissues to stress conditions. (b) isolated plants of groundnut which are pretreated with glycine betaine were studied in glass house and growth chamber under heat, drought and salinity stress conditions. Under salinity stress conditions, the betaine treatment could enhance the growth in root, shoot and total biomass by 135%, 25% and 28% respectively when compared with B_0. Correspondingly the net photosynthetic rate was observed to increase by 35% with betaine treatment. Under heat stress conditions, seed treatment with glycine betaine could increase the root and shoot development by 150 and 32% and total dry matter by 20%. There was a relative increase in net photosynthetic rate and Fv/Fm ratios, decrease in leaf water potential. Similarly with high temperature stress conditions, the seed treatment with glycine betaine could increase the root and shoot development by 22 and 43%, total dry matter was increased by 23%. There was a relative increase in relative water content by 10%, decrease in leaf water potential by 25%. The empirical fluorescence parameter (Fv/Fm) which is an index of PSII quantum yield was reduced in stresses plants without betaine when compared with betaine treated stressed plants. These results indicate that glycine betaine accumulation confers protection against the photochemical reaction of PS II in vivo. (c) In field studies, glycine betaine was foliarly applied to plants (at 3, 6 and 9 kg ha^{-1} and a control)in mid season and end season drought conditions to establish whether its application could ameliorate the effects of drought on the. yield of groundnut. Drought significantly reduced the biomass production ($P<0.01$). Pod dry matter was also significantly depressed ($P<0.01$) by drought. The percent decrease in growth rates due to mid season and end season droughts was greatly reduced by the betaine application at 3 kg ha^{-1}.This corresponds to a high residual tissue glycine betaine

concentrations in betaine treated plants at 100DAS. The positive effects of glycine betaine treatment appear to be linked not only to its physiological role as a plant osmoticum that improves drought tolerance but also to a protective role for proteins and membranes even at low concentrations. The results of the present study suggest that foliar application of glycine betaine may be used to improve stress tolerance and economic yield of groundnut.

While agronomic applications of glycine betaine are exciting, genetic variation in natural accumulating ability could be used in plant improvement research. It may be possible to select, breed or genetically engineer cultivars for higher glycine betaine content to increase crop performance in saline, dryland and high temperature conditions in groundnut.

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