

**Improving drought tolerance in maize (*Zea mays* L.) by
exogenous application of thiourea**



By

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Declaration

I hereby declare that contents of the thesis “Improving drought tolerance in maize (*Zea mays* L.) by exogenous application of thiourea” are product of my own research and no part has been copied from any published source (except the references, standard mathematical or genetic models/equations /formulae/ protocols etc.). I further declare that this work has not been submitted for award of any other diploma/degree. The university may take action if the information provided is found inaccurate at any stage. (In case of any default, the scholar will be proceeded against as per HEC plagiarism policy).

Babar Hussain Babar

2001-ag-1027

I humbly

DEDICATE

This effort

to

MY PARENTS

*I am indebted for their unconditional support and encouragement
throughout my life.*

LIST OF ABBREVIATIONS

Abbreviation	Full
AOSA	Association of Official Seed Analysis
%	percent
cm	centimeter
cm ²	square centimeter
DAS	days after sowing
d ⁻¹	per day
EC	electrical conductivity
g	gram
ha ⁻¹	per hectare
TU	thiourea
K	potassium
kg	kilogram
LSD	least significant difference
m ²	per square meter
N	nitrogen
P	phosphorus
K	potassium
Plant ⁻¹	per plant
t ha ⁻¹	ton per hectare
TDM	total dry matter
CP	crude protein
LAI	leaf area index
LA	leaf area
RL	root length
SL	shoot length
RDW	root dry weight
SDW	shoot dry weight
WP	water potential
OP	osmotic potential
TP	turgor potential
RWC	relative water contents
MDA	malondialdehyde
EL	electrolyte leakage
SOD	superoxide dismutase
POD	peroxidase
CAT	catalase

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ABSTRACT

Drought stress is one of the most important constraints in crop production, particularly in arid and semi-arid regions of the world. Seed priming and foliar application of growth hormones, osmolytes and nutrients is an important tool to decrease the drastic effects of drought stress. The effects of thiourea in improving drought tolerance in maize were studied from 2010-12. Four green house and one field experiment was carried out at Research Area of Agronomy Department. First experiment was screening trial in which seven maize hybrids (32F10, 32B33, 33H25, 3335, 34N43, 6142, 6525) were sown under three water regimes (80% WHC, 60% WHC, 40% WHC). Among seven hybrids, 34N43 recorded vigorous germination, maximum growth and improved water relations under stress as well as under well watered conditions and hybrid 32F10 recorded least vigorous germination, growth and water relations. So on the basis of screening trial hybrid 32F10 was selected as sensitive and hybrid 34N43 as tolerant to drought stress for further studies. Three green house experiments with different levels of thiourea application were conducted. In first experiment four levels of thiourea (200mg/l, 400mg/l, 600mg/l, 800mg/l) was applied through seed treatment under two water regimes (80% WHC, 40% WHC). Seed treatment with TU increased growth under normal and drought stress conditions and improved water relations, increased proline contents, decreased MDA content and electrolyte leakage and increased the activities of antioxidant enzymes in both the hybrids, however effect was more pronounced in hybrid 34N43 than 32F10 and 800 mg/l TU proved to be best for seed treatment. In rooting medium again TU increased shoot length, root dry weight, shoot dry weight, leaf area, improved water relations and increased proline contents, decreased electrolyte leakage and MDA content. In third experiment three thiourea levels (500mg/l, 1000mg/l, 1500mg/l) were applied through foliar application. Foliar application of TU increased plant biomass, increased water potential, turgor potential and relative contents. One field experiment was conducted in which tensiometers were used for measuring soil water potential and irrigation was made on the basis of tensiometer reading. Thiourea levels and its method of application for field experiment were decided on the basis of green house experiments. From seed treatment experiment, 800 mg/l TU was best treatment and from foliar application, 1500mg/l TU was best. These two treatments were used in field experiment along with dry seed sowing and distilled water foliar application using as control. The experiment was laid out in randomized complete block design with split-split arrangement using four replications. Data regarding various agronomic, physiological and biochemical traits of crop were recorded using standard procedures available in the literature. The present study suggests that seed priming with TU and its foliar application not only improved water relations, increased growth, nutrients uptake and yield of maize crop under water stress but also improved performance of maize under normal availability of water.

Chapter 1

INTRODUCTION

Water scarcity imposes a considerable reduction in crop yield and is one of the major limitations to crop production. Scenarios for global environmental change suggest a future increase in aridity and in the frequency of extreme events in many areas of the earth (IPCC, 2001). The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands, and moisture storing capacity of soils (Wery *et al.*, 1994). Because the world's water supply is limiting, future food demand for rapidly increasing population pressures is likely to further aggravate the effects of drought (Somerville and Briscoe, 2001). Scarcity of water resources is considered the single most critical threat to world food security in near future.

Plants are subjected to several biotic and abiotic factors that affect growth in higher plants (Lichtenthaler, 1996). The environmental stresses such as drought, extreme temperature, salinity, air pollution, heavy metals, pesticides and soil pH are major limiting factors in crop production because, they affect almost all plant functions (Lawlor, 2002; Hernandez *et al.*, 2001). Of these, drought is a major abiotic factor that limits agricultural crop production (Nemeth *et al.*, 2002; Chaves and Oliveria, 2004; Lea *et al.*, 2004; Ramachandra *et al.*, 2004). Plants experience drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. These two conditions often coincide under arid and semi-arid climates (Ramachandra *et al.*, 2004). However, decreasing water supply either temporarily or permanently adversely affects morphological and physiological processes in plants.

Most important and primary effects of water deficit are the hampered leaf water status (Taiz and Zeiger, 2010; Farooq *et al.*, 2010). Moreover, Medici *et al.* (2003) found that maize hybrid P-6875 showed water potential of -0.78 MPa under control condition and water potential of this hybrid decreased up to -0.96 MPa under water stress condition. Claudio *et al.* (2006) found that osmotic potential of leaves of well watered plants increased up to -0.90 MPa 40 DAS, while under water stress it decreased up to -1.20 MPa. Claudio *et al.* (2006) observed that the leaf turgor potential decreased from 0.54 MPa to 0.18 MPa with the increase of water stress.

Shirinzadeh *et al.* (2010) reported that under stress condition relation of turgor potential was significant with plant water contents of maize hybrid. Mohammady and Hasannejad (2006) screened out maize hybrids on the basis of leaf water contents. The results showed that the relationship of leaf water content was significant with osmotic or turgor potential under drought.

The adverse effects of drought stress on growth and development of crop plants are of multifarious nature. They may be due to inhibited cell expansion and reduced biomass production (Ashraf and Mehmood, 1990), alteration in different metabolic activities of plants (Lawlor and Cornic, 2002), inhibition of enzymatic activities (Ashraf *et al.*, 1995), ionic imbalance (Kidambi *et al.*, 1990), disturbances in solute accumulation (Khan *et al.*, 1999) or a combination of all these factors. Three main mechanisms reduce crop yield by soil water deficit including reduced canopy absorption of photosynthetically active radiation, decreased radiation-use efficiency and reduced harvest index (Earl and Davis, 2003). Low supply of water during different phases of plant growth particularly at the reproductive stage is very harmful for seed development (Boutraa and Sanders, 2001). Lowered absorption of the inorganic nutrients can result from interference in nutrient uptake and the unloading mechanism, and reduced transpirational flow (Garg, 2003; McWilliams, 2003).

The availability of nutrients in the soil decreases under water deficit conditions due to their less solubility as well as precipitation of salts that result in altered physiological processes including low absorption and uptake of nutrients in plants grown under such conditions (Garg, 2003; Fageria *et al.*, 2002). This decrease in absorption of nutrients in plants generally results due to a substantial decrease in transpiration rate, reduced active transport as well as membrane permeability (Baligar *et al.*, 2001; Gunes *et al.*, 2006) that results in diminished tissue nutrient concentration (Garg, 2003; McWilliams, 2003). Under drought stress, root system development plays an important role in plant water status and internal water deficit conditions (Fageria *et al.*, 2002; Samarah *et al.*, 2004). However, in a number of reports, it has been observed that plant species and cultivars within a species differ in absorbing nutrients from soil and transporting them to root and then from root to shoot under water deficit conditions (Garg, 2003; Gunes *et al.*, 2006; Ali *et al.*, 2008).

Proline and quaternary ammonium compounds, e.g. glycinebetaine, choline, prolinebetaine are key osmolytes contributing towards osmotic adjustment (Huang *et al.*, 2000 and Kavikishore *et al.*, 2005). Plants perceive the stress conditions and signal to alter the metabolic flux for the activation/ synthesis of defense mechanisms (Mehdy, 1994; Chaves and Oliveria, 2004; Ramachandra *et al.*, 2004). Many molecules / elements, for example, calcium, jasmonic acid, ethylene and salicylic acid have been suggested as signal transducers or messengers (Klessing and Malamy, 1994; Chaves *et al.*, 2003).

These compatible organic solutes are low molecular weight and highly soluble compounds that are usually nontoxic at high cellular concentrations. Normally, they protect plants from different abiotic stresses in a number of ways including their role in cellular osmotic adjustment, scavenging of reactive oxygen species (ROS), protection of cellular membranes, and stabilization of proteins/enzymes and activities of enzymes (Shulaev *et al.*, 2008; Gill and Tuteja 2010). Furthermore, because some of these solutes also protect cellular components from dehydration injury during stress, so they are commonly referred to as osmoprotectants. Although a large number of efforts have been devoted to produce genetically modified plants for the increased synthesis of these osmoprotectants, a little success is achieved for desired levels of these organic osmolytes in plants.

Maize (*Zea mays* L.) is an important crop grown all over the world and is the third most important cereal grain after wheat and rice. It contributes 62 % in the total cereal production (Frova *et al.*, 1999). Maize is relatively a short duration crop and is grown twice a year as a spring and autumn crop. In Pakistan, maize is grown on an area of 950,000 ha with the production of 3,487,000 tones and average grain yield of 3671 kg ha⁻¹ (Government of Pakistan, 2010). Among cereals, maize (*Zea mays* L.) not only has a sufficient amount of carotenoids, tocopherols, and oil but also has a reasonable amount of starch and protein content compared with other major food crops such as rice and wheat. Although maize is principally cultivated for carbohydrate production, in the past several years, it has gained great significance as a source of vegetable oil for the food industry (Balasundram *et al.*, 2006). Maize oil is considered as a best vegetable oil due to its large amount of unsaturated fatty acids, most predominantly being oleic and linoleic acids ranging from 65 to 85% (Goffman and Böhme, 2001). Despite a large amount of unsaturated fatty acids, the

oil is a rich source of phenolics, flavonoids, and different types of tocopherols (Balasundram *et al.*, 2006). Phenolic compounds being secondary metabolites are considered non-essential for nutrition, but the interest in the appraisal of their antioxidant and bioactive properties has amplified due to their considerable role in human and animal health (Schussler and Westgate, 1995; Balasundram *et al.*, 2006). However, yield potential of maize is highly prone to abiotic stresses (Drought, salinity, extreme temperatures, flooding, pollutants and poor or excessive irradiation) which are important factors towards limiting the crop productivity (Misovic, 1985; Lawlor, 2002). Among the abiotic stresses, drought is the most severe limitation to maize production (Sallah *et al.*, 2002).

Under mild drought stress, the reduction in maize crop is up to 10-13%, but under severe drought, the loss may increase many times. The problem becomes more alarming under arid conditions covering an area of 25-30% of major crops planted in Pakistan (Farooq *et al.*, 2007). Drought stress at vegetative stage in maize caused 25-60 % reduction in yield (Atteya *et al.*, 2003). Kamara *et al.* (2003) reported that drought stress at reproductive stage reduced 63-87% yield in maize.

In maize, drought reduces leaf area, leaf chlorophyll contents, photosynthesis and ultimately lowers the grain yield (Athar and Ashraf, 2005). At flowering, drought widens the anthesis silking interval (ASI) in maize, which severely reduces the kernel set (Emeadeas *et al.*, 2000). Under drought leaf senescence is also accelerated to decrease the canopy size (Moony and Duplesis, 1970) severely affecting the crop yield. However delayed leaf senescence affects positively for reducing the harmful effects of drought on crop yield (Rivero *et al.*, 2007).

Drought at flowering commonly results in barrenness. A major cause of this, though not the only one, was a reduction in assimilate flux to the developing ear below some threshold level necessary to sustain optimal grain growth (Yadav *et al.*, 2004).

Many strategies are being practised in the world to cope with this global issue, the more important amongst them include use of cultural practices to increase the availability of stored soil moisture, to improve water use efficiency, or to exploit other methods by which plant can yield reasonably well under water deficit conditions (Boyer, 1982). Adjustment of agronomic practices such as sowing time,

plant density and soil management is done to ensure that sensitive crop stages occur at the time when likelihood of drought is minimal (Farooq *et al.*, 2009).

Different approaches for the provision of irrigation water and use of cultural methods to prevent drought are cost and labor-intensive, inconvenient, and require specific knowledge for their proper use. However, in spite of these methods a number of biological approaches are being used for better crop production under water limited conditions. These biological approaches are considered much efficient in achieving effective water use in crop production. The biological approach is being used efficiently these days include the production of stress resistant plants through selection of stress tolerant crop varieties through traditional breeding (Munns *et al.*, 2003; Slafer, 2003; Khan *et al.*, 2010), genetic engineering (Vettakkorumakankav *et al.*, 1999; Araus *et al.*, 2008; Ashraf, 2010), mutant selection (Ahloowalia *et al.*, 2004; Farooq *et al.*, 2008), marker-assisted selection (Baum *et al.*, 2003; Foolad, 2004; Forster *et al.*, 2004; Talame *et al.*, 2004; Ashraf, 2010), in vitro-selection, and exogenous application of a variety of growth regulators (Fletcher and Hofstra, 1988; Ashraf and Foolad, 2007).

Use of plant growth hormones (ABA, GA, cytokinin, SA), antioxidants (ascorbic acid, H₂O₂), osmoprotectants inorganic salts and fertilizers as foliar application and seed treatment is a pragmatic approach for increasing drought tolerance under water deficit conditions (Senaratna *et al.*, 2000; Farooq *et al.*, 2009). Seed priming is one of the most viable and pragmatic approaches to reduce the deleterious effects of drought stress especially on seed germination and early seedling growth, high vigor, uniform stand establishment (Harris *et al.*, 2002 and 2004) and better yield especially in vegetable and field crops (Khalil *et al.*, 2001). It is a technique by which seeds are prepared for germination by starting the germination related metabolic processes. During this process the seeds are soaked in water (hydro priming) or in a chemical solution of specific concentration for a specific time to start the germination up to a point, but the concentration and soaking time vary from crop to crop and genotypes within a species (Farooq *et al.*, 2006; Ashraf and Foolad, 2007). In view of a large number of studies it is evident that foliar application of those nutrients that become deficit in plants under stress, could reduce the deficiencies of these nutrients in plants that lead to osmotic adjustment

and finally play a role in growth and yield improvement (Irshad *et al.*, 2002; Akram *et al.*, 2007; Ashraf *et al.*, 2007).

Inducing drought tolerance in crop plants by exogenous application of hormones and different salts like thiourea TU is cost effective method for increasing yield under arid and semiarid conditions.

Sahu *et al.* (1993) reported that foliar spray of TU significantly increased growth and yield of maize, most probably via improvement in canopy photosynthesis under drought conditions. Thiourea has been reported to significantly improve growth yield and water use efficiency of wheat under arid and semi arid conditions (Sahu and Singh, 1995). Thiourea (TU) treated plants performed better in terms of plant growth in spite of experiencing slightly more water deficit due to stabilization of lipoprotein structure and less malondialdehyde production compared to control (Mojtaba and Karr, 2001). However, information about how Thiourea affects water relations antioxidant, enzymes and osmoprotectants in maize under drought stress is still lacking. Therefore, this study was carried out to:

- 1) Assess whether exogenous application of thiourea could alleviate the inhibitory effects of drought on the growth of maize plants.
- 2) Determine the extent and pattern of changes in various metabolites in exogenously treated maize plants with thiourea under drought conditions.

Chapter 2

REVIEW OF LITERATURE

Plant growth and drought stress

The adverse effects of water stress takes place at all levels of plant life, ranging from morphological to molecular levels that are observable at all phenological growth stages during plant life cycle at whatever stage the drought takes place (Jajarmi, 2009; Farooq *et al.*, 2010). The impairment of germination that leads to the poor stand establishment is the foremost effect of water stress (Harris *et al.*, 2002; Kaya *et al.*, 2006; Jajarmi, 2009). It has been reported that water stress severely reduces the seed germination and seedling stand establishment that leads to reduced final production (Kaya *et al.*, 2006). In a study on observing seed germination behavior in different cultivars of pea under drought stress, Okcu *et al.* (2005) reported that water stress reduced the seed germination and early seedling growth. Furthermore, in another study Zeid and Shedeed (2006) reported that polyethylene glycol-induced drought stress reduced the germination potential, hypocotyl length, shoot and root fresh and dry weights in alfalfa (*Medicago sativa*), while increased root length. However, in rice a significant reduction in plant growth and development was observed when drought stress was applied at the vegetative growth stage (Tripathy *et al.*, 2000; Manikavelu *et al.*, 2006). The rate of plant growth depends upon a number of important events such as cell division, enlargement and cell differentiation as well as genetic, morphological, physiological, ecological events along with their complex interactions that are severely affected by water stress (Taize and Zeiger, 2010). Cell growth is one of the physiological processes that is most drought sensitive due to reduction in turgor pressure (Taize and Zeiger, 2010). It has been reported that under severe water stress, inhibition of cell elongation takes place due to interruption of water flow from xylem to surrounding elongating cells. Therefore, the impairment takes place in cell expansion, elongation and process of mitosis resulting in reduced leaf area, plant height and reduced crop growth under water deficit conditions (Kaya *et al.*, 2006; Hussain *et al.*, 2008).

Changes in morphological characters are the ultimate determinants of stress effects on plants (Farooq *et al.*, 2009; Jaleel *et al.*, 2009). It has been reported that

the plant height of single cross maize hybrid was affected when deficit water was applied at different growth stages (Abo-El-Kheir and Mekki, 2007). Such effect of drought stress might be up to 32.8% (Golbashy *et al.*, 2010). Olaoye (2009) reported that at 100% FC maximum leaf area was recorded, he also concluded that with decrease in field capacity, leaf area of different maize hybrids decreased significantly. Granier *et al.* (2006) and Abo-El-Kheir and Mekki (2007) reported that leaf area of maize genotypes was affected by different stress levels. They concluded that intensity of the soil water deficit largely influenced a genotype's leaf area. The adverse effects of drought stress upon plant growth are well reported in a number of studies (Bajji *et al.*, 2001; Lizana *et al.*, 2006; Ali *et al.*, 2007; Ali and Ashraf, 2011) which suggests that reduction in plant biomass production was associated with decreased leaf biomass due to reduction in leaf area, transpiration rate as well as plant photosynthesis. The reduction in leaf area could be explained in terms of reduction in production of new leaves and reduction in leaf size (Hessini *et al.*, 2009). Moreover, this reduction in plant biomass production would be due to increased abscission and senescence of the older leaves, as it has been observed in a number of plant species (Brevedan and Egli, 2003; Mahajan and Tuteja, 2005). However, a reduction in plant leaf area under water deficit condition may be beneficial because this reduction in plant leaf area overall results in reduced plant transpiration rate (Chaves *et al.*, 2003; Hessini *et al.*, 2009), which is considered a good response of plants under water deficit conditions. Furthermore, Hessini *et al.* (2009) also found that the shoot to root ratio (on the basis of fresh or dry weights) in *S. alterniflora* decreased by water stress, indicating that shoot growth is more sensitive to drought stress than root growth as it has been earlier described by Ashraf and Foolad (2007). Rahman *et al.* (2004) have reported water stress-induced growth reduction by inhibiting root/shoot length and dry mass production in different maize cultivars. These results also indicate that among different morphological traits dense and deep rooting system is the most important and early response of the plants to water stress. Under water deficit conditions, greater production of plant fresh and dry weights is an important desirable character. The reduction in fresh and dry biomass production of plants under water stress conditions is a common phenomenon (Farooq *et al.*, 2009). In a study on different sunflower cultivars (Tahir and Mehid, 2001) it was found that water stress reduced the biomass of almost all cultivars of sunflower under study. However, in some cultivars this reduction in biomass was more than in

the others. Furthermore, Mohammadian *et al.* (2005) reported more reduction in shoot biomass than root biomass in different sugar beet cultivars under severe water stress. Reduced biomass was also reported under water deficit conditions in crops such as in soybean (Specht *et al.*, 2001), seedlings of *Poncirus trifoliatae* (Wu *et al.*, 2008), green gram and common bean (Webber *et al.*, 2006), *Petroselinum crispum* (Petropoulos *et al.*, 2008) and maize (Ali and Ashraf, 2011). In a number of studies, a significant positive relationship between grain yield and biomass production has been reported in wheat (Munir *et al.*, 2007; Semenov and Shewry, 2010), barley, soybean (Mirakhori *et al.*, 2009), rice (Guan *et al.*, 2010) and maize (Khan *et al.*, 2001; Rashidi and Sayfi, 2007).

Drought stress and water relations

To survive under drought stress plants use a number of mechanisms. Of these, the most common are curtailed water loss by enhanced water uptake due to deep and prolific root system, increased diffusion resistance, and the reduction in leaf area to decrease the transpiration (Farooq *et al.*, 2010). Relative water contents, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are important characteristics that influence plant water relations. Relative water contents of wheat leaves were higher initially during leaf development and decreased as the dry matter accumulated and leaf matured (Siddique *et al.*, 2001). A conservative influence of decreased stomatal conductance in non-irrigated plants was negated by a leaf-to-air vapor pressure difference caused by the associated higher leaf temperature. Transpiration rates were similar in both treatments and the lower total water use of the non-irrigated stand resulted entirely from a smaller leaf area index (Craufurad *et al.*, 2000). In another study on *Hibiscus rosasinensis*, relative water contents, turgor potential, transpiration, stomatal conductance and water-use efficiency were decreased under drought stress (Egilla *et al.*, 2005). The ratio between dry matter produced and water consumed is termed as water-use efficiency at the whole-plant level (Monclus *et al.*, 2006). Abbate *et al.* (2004) concluded that under limited supply, water-use efficiency of wheat was greater than in well-watered conditions. They correlated this higher water-use efficiency with stomatal closure to reduce the transpiration. In another study on clover (*Trifolium alexandrinum*), water-use efficiency was increased due to lowered water loss under drought stress, primarily by decreased transpiration rate and leaf area, and relatively

lesser reduction in yield (Lazaridou and Koutroubas, 2004). Lazaridou *et al.* (2003) further reported that leucern (*Medicago sativa*) grown under drought had greater water-use efficiency than that under irrigated conditions, for the same leaf water potential. In fact, although components of plant water relations are affected by reduced availability of water, stomatal opening and closing is more strongly affected. Moreover, change in leaf temperature may be an important factor in controlling leaf water status under drought stress. Drought tolerant species maintain water use efficiency by reducing the water loss. However, in the events where plant growth was hindered to a greater extent, water-use efficiency was also reduced significantly.

Most important and primary effects of water deficit are the hampered leaf water status (Taiz and Zeiger, 2010; Farooq *et al.*, 2010). Moreover, Medici *et al.* (2003) found that maize hybrid P-6875 showed water potential of -0.78 MPa under control condition and water potential of this hybrid decreased up to -0.96 MPa under water stress condition. Claudio *et al.* (2006) found that osmotic potential of leaves of well watered plants increased up to -0.90 MPa 40 DAS, while under water stress it decreased up to -1.20 MPa. Claudio *et al.* (2006) observed that the leaf turgor potential decreased from 0.54 MPa to 0.18 MPa with the increase of water stress. Shirinzadeh *et al.* (2010) reported that under stress condition relation of turgor potential was significant with plant water contents of maize hybrid. Mohammady and Hasannejad (2006) screened maize hybrids on basis of leaf water content. The results showed that the relationship of leaf water content was significant with osmotic or turgor potential under drought. In a study on maize, Li-ping *et al.* (2006) observed the responses of plants to different levels of water stress (moderate and severe water stress) applied at different timings of growth (leaf stage to maturity) in relation to plant water status, antioxidant enzyme activities and lipid peroxidation. They further revealed that the effects of drought stress depend upon the intensity and duration of stress. Severe water stress resulted in serious effects. Moderate water stress at blister and silking stages showed no effects on leaf relative water contents (RWC) but changed the leaf relative conductivity (LRC) while severe water stress at later growth stages (tasseling, blister and milk stages) resulted in reduced RWC with an increased LRC and decreased antioxidant activities. Furthermore, in another study, while observing the effects of drought on different maize cultivars in relation to plant water relations, Subramanian *et al.* (2006) found that water stress applied following

tasselling (75-95 days after sowing) significantly decreased the leaf water potential (WP) with a concomitant decrease in transpiration rate, stomatal resistance and green leaf area. Decrease in water potential was severe observed in sensitive genotypes than that in drought tolerant ones.

Water potential (ψ_w) is considered a reliable parameter for measuring plant water stress response. It varies greatly, depending on the type of plant and on environmental conditions. Decrease in water potential causes decrease in grain number, size and yield (Saini and Westgate, 2000). Relative water contents is integrated measure of plant water status. Higher RWC is necessary for proper growth and function of plant. The reduction of RWC in stressed plants may be associated with the decrease in plant vigour and was observed in many plant species (Halder and Burrage, 2003; Lopez *et al.*, 2002). RWC is an integrative index of plant water status which is used to evaluate tolerance to water deficit. Reduction in RWC under water stress leads to the stomatal closure (Gindaba *et al.*, 2004) which results in decreased CO₂ assimilation. Water stress induces biochemical and cellular changes in plants due to variable decline in RWC which has been reported in various plants imposed to drought stress (Taiz and Zeiger, 2010; Bai *et al.*, 2006; Masoumi *et al.*, 2010). Various studies showed that decline in RWC leads to a reduction in photosynthetic activity under water stress (Sage and Zhu, 2011).

Membrane permeability and MDA

As cell membranes are the first targets of many plant stresses, active oxygen species (AOS) may destroy normal metabolism through peroxidation of membrane lipids (Arora *et al.*, 2002). Lipid peroxidation of biological membranes might lead to structural alterations such as denaturalization of proteins and nucleic acids in drought-stressed plants. Experimental evidences suggest that lipid peroxidation reactions of cellular membranes may play an important role in radical mediated cell injury in view of malondialdehyde (MDA) accumulation (Zhang *et al.*, 2007). Therefore, activity of antioxidant enzymes and MDA content may act as efficient determinant criteria in the toxic degree to drought stressed plants (Arora *et al.*, 2002; Aslam *et al.*, 2006). Cell membranes are one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants. The degree of cell membrane injury induced by water stress may easily be

estimated through measurements of electrolyte leakage from the cells (Bajji *et al.*, 2002). . The cascade of studies on physiological adjustment to crops revealed that the change patterns of production, RWC and activities of SOD, POD, CAT as well as MDA content were associated with cultivar and development stage (Sairam and Srivastava, 2001; Ramachandra *et al.*, 2004; Chutipajit *et al.*, 2009). It has been reported that membranes are subject to damage rapidly with increasing water stress. This leakiness of membranes is caused by an uncontrolled increase in free radicals, which cause lipid peroxidation. Further damage to fatty acids could then produce small hydrocarbon fragments including malondialdehyde (MDA) (Alscher *et al.*, 2002). It is hypothesized that modulation of the activities of antioxidative enzymes, MDA content and membrane permeability at seedling stage is an important criteria to select drought-tolerant and susceptible maize genotypes (Moussa and Aziz, 2008).

Under stress conditions, reactive oxygen species (ROS), such as superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals can be produced in large amounts. Hydrogen peroxide and superoxide radicals are relatively unreactive, but they can form hydroxyl radicals, which can damage proteins, lipids and DNA (Del *et al.*, 2002). Peroxidation of lipids, commonly taken as an indicator of oxidative stress, disrupts the membrane integrity of the plant cell. This means that essential solutes leak out from the organelles and from the cell and cause the damage of membrane function and metabolic imbalances (Blokhina *et al.*, 2003). Peroxidation of lipids, commonly taken as an indicator of oxidative stress, disrupts the membrane integrity of the plant cell. This means that essential solutes leak out from the organelles and from the cell and cause the damage of membrane function and metabolic imbalances. Cell membrane stability, reciprocal to cell membrane injury, is a physiological index widely used for the evaluation of drought tolerance (Quan *et al.*, 2004). Also Quan *et al.* (2004) found higher electrolyte leakage in drought stressed maize plants than in plants grown under controlled conditions.

Antioxidant enzymes

Crucial changes in water homeostasis can lead to osmotic stress, which are primary effects of drought stress. Such free radicals and other active derivatives of oxygen may produce inevitably as by-products of physiological redox reactions. It has been suggested that drought stress caused elevated levels of active oxygen species (AOS) (Arora *et al.*, 2002), and the production of active oxygen exceeded the

capacity of scavenging systems, resulting in oxidative damage (Taiz and Zeiger, 2010). The increased levels of AOS can inactivate enzymes, damage important cellular components, which induced plant growth arrest, and even death finally (Arora *et al.*, 2002). To mitigate the deleterious effect of DS on regular metabolism, plants have evolved various strategies to contend with this problem. By necessity, plants possess a number of antioxidants such as superoxide dismutase (SOD: EC 1.15.1.1), peroxidase (POD: EC 1.11.1.7), and catalase (CAT: EC 1.11.1.6) in order to protect cellular membranes and organelles from the damaging effects of toxic concentrations of AOS and maintain their integrity and stability under drought-stressed conditions (Arora *et al.*, 2002). Plants protect cellular and sub-cellular system from the cytotoxic effects of active oxygen radicals with anti-oxidative enzymes such as SOD, POD and CAT as well as metabolites like glutathione, ascorbic acid, tocopherol and carotenoids (Alscher *et al.*, 2002). The production of ROS takes place in many cell compartments, but the chloroplast is the most important one. Under water deficit conditions, their production occurs due to inhibition of photosynthetic ability of tissues that takes place due to an imbalanced utilization of absorbed light (Foyer and Noctor, 2000; Reddy *et al.*, 2004). This imbalance results in down-regulation of PS II and less utilization of generated electrons that ultimately change the quantum yield. This imbalance in utilization of generated electrons in the PS II core and antenna center results in the dissipation of excess light energy in drought stressed plants, thereby causing the production of ROS (Peltzer *et al.*, 2002). The production of ROS also takes place in other compartments when O₂ produced in the photosynthetic machinery interacts with the reduced components of electron transport chain (ETC) in mitochondria as well as in peroxisomes during photorespiration in the oxidation of glycolate to glyoxylic acid (Fazeli *et al.*, 2007). These ROS include the hydroxyl radicals (OH), superoxide radicals (O²⁻), hydrogen peroxide (H₂O₂), alkoxy radical (RO) and singlet oxygen (Munne-Bosch and Penueles, 2003). Production of these ROS under water stress conditions is highly dangerous, because they impair the normal functions of cells due to their oxidative reaction with membrane proteins, lipids, deoxyribonucleic acid as well as the inactivation of enzyme activities (Foyer and Fletcher, 2001; Blokhina *et al.*, 2003; Sairam *et al.*, 2005).

Detoxification of ROS in plant cells can be categorized as enzymatic as well as non-enzymatic that exist in almost all plants (Mittler, 2002). The non-enzymatic antioxidant includes ascorbic acid, tocopherols, flavonoids, phenolics and carotenoids (Ashraf, 2010; Ali and Ashraf, 2011), however, in the enzymatic detoxification of ROS, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase and ascorbate peroxidase are the important ones. The increase in the activities and amount of these antioxidant enzymes under water deficit conditions differ among plant species and even within cultivars of a same species (Nikolaeva *et al.*, 2010). Many stress situations cause an increase in antioxidant activities in different plant parts (Pastori *et al.*, 2000; Bian and Jiang, 2009). Many studies have shown an enhanced drought tolerance due to increased activities and over-production of these antioxidant enzymes (Singh and Usha, 2003; Luna *et al.*, 2004; Jaleel *et al.*, 2007; Bian and Jiang, 2009). While appraising drought tolerance on the basis of antioxidant enzyme activities in different species and cultivars within species, Csiszer *et al.* (2007) reported that drought tolerance is associated with higher activities of SOD, POD and CAT in shoots and roots and is positively correlated with their biomass production. However, in maize, Ti-da *et al.* (2006) reported enhanced drought tolerance with their increased antioxidant enzyme activities in leaves and roots with a reduced MDA contents. While working with different wheat cultivars Nikolaeva *et al.* (2010) reported a sharp increase in the activities of different antioxidant enzymes at early growth stages under water deficit conditions and then a decrease at later stages. Furthermore, they found a positive correlation of antioxidant enzyme activities, proline accumulation and leaf chlorophyll contents with drought tolerance of wheat cultivars differing in drought tolerance. Furthermore, in rapeseed Abedi and Pakniyat (2010) found a positive relationship of drought tolerance of different cultivars with their increased antioxidant enzyme activities. It is known that plants have a well-organized defense system against ROS under stress conditions and SOD constitutes the first line of defense via detoxification of superoxide radicals (Sairam *et al.*, 2000). It is suggested that the higher concentrations of catalase and ascorbate peroxidase may remove the $O_2^{\cdot-}$ radicals and its product H_2O_2 induced by water stress (Sairam *et al.*, 2000; Gupta and Gupta, 2005). Nayar and Kaushal (2002) also reported that the increased activity of CAT and POX enzymes constitute potential defense mechanism against chilling induced oxidative damage in germinating wheat grains. Plants protect cellular and

sub-cellular system from the cytotoxic effects of active oxygen radicals with anti-oxidative enzymes such as SOD, POX and CAT as well as metabolites like glutathione, ascorbic acid, tocopherol and carotenoids (Alscher *et al.*, 2002).

Drought stress and osmolytes

Under water deficit conditions to cope with the stress, cellular osmotic adjustment (OA) is considered as a key physiological adaptation. During this cellular osmotic adjustment under water deficit conditions, accumulation of the organic metabolites takes place due to reduced cell water potential that reduces the cell osmotic potential and in turn results in uptake of water into the cell to maintain cell turgor potential (Martinez *et al.*, 2007). Osmotic adjustment (OA) is a part of drought avoidance mechanisms to counteract the loss of turgor by increasing and maintaining higher amount of intracellular compatible solutes in cytosol and vacuole and has been proved to be particularly significant among all the stress adaptation mechanisms (Cushman, 2001).

For osmotic adjustment, accumulation of osmolytes under water limiting conditions is compulsory but it depends upon water status, crop growth stage and cultivar (Shao *et al.*, 2006). Despite their role as osmotic adjustment, they also play an important role in protection of membranes by scavenging of ROS (Ashraf and Foolad, 2007), reduce the protein denaturation and preserve the enzyme structure and their activity (Hamilton and Heckathorn, 2001). It has been widely reported that plants accumulate a variety of compatible solute such as proline and betaine, as an adaptive mechanism of tolerance to salinity and drought (Hasegawa *et al.*, 2000; Ashraf and Harris, 2004). These compatible solutes protect and stabilize 3D structure of proteins and photosynthetic apparatus, regulate cellular osmotic adjustment (Subbarao *et al.*, 2001) in response to abiotic stresses. These organic metabolites include a variety of proteins, sugars (trehalose, sucrose, mannitol etc.), amino acids (proline, glycinebetain), glycerol, polyols and some other low molecular weight metabolites (Hong-Bo *et al.*, 2006; Ashraf and Foolad, 2007). Accumulation of these osmolytes in response to water stress has been reported in wheat (Hong-Bo *et al.*, 2006), sunflower (Manivannin *et al.*, 2007), and maize (Ali and Ashraf, 2011).

Upon relief from stress, these solutes are metabolized and are considered as an important energy source for recovery from stress. Much attention has been paid on the role of proline in stress tolerance as a compatible osmolyte. Proline and

soluble sugars are the key osmolytes contributing towards osmotic adjustment (Mundree *et al.*, 2002). They can also improve stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions. Proline accumulation helps maintaining cell water status, sub-cellular structures and protecting membranes and proteins from denaturing effect of the osmotic stress (Ashraf and Foolad, 2007). Glycinebetaine (GB) is found to be actively associated in plants as defensive response to adverse conditions of salinity, drought, chilling or heat stress (Farooq *et al.*, 2008). Furthermore, Ali and Ashraf (2011) reported that more accumulation of GB and proline was associated with increased biomass and antioxidant activities under water stress conditions.

NPK uptake

The availability of nutrients in the soil decreases under water deficit conditions due to their less solubility as well as precipitation of salts that result in altered physiological processes including low absorption and uptake of nutrients in plants grown under such conditions (Garg, 2003; Fageria *et al.*, 2002). This decrease in absorption of nutrients in plants generally results due to a substantial decrease in transpiration rate, reduced active transport as well as membrane permeability (Baligar *et al.*, 2001; Gunes *et al.*, 2006) that results in diminished tissue nutrient concentration (Garg, 2003; McWilliams, 2003). Under drought stress, root system development plays an important role in plant water status and internal water deficit conditions (Fageria *et al.*, 2002; Samarah *et al.*, 2004). However, in a number of reports, it has been observed that plant species and cultivars within a species differ in absorbing nutrients from soil and transporting them to root and then from root to shoot under water deficit conditions (Garg, 2003; Gunes *et al.*, 2006; Ali *et al.*, 2008). Generally, it has been observed that under drought stress an increase in N uptake takes place with concomitant decrease in P uptake without affecting K⁺ uptake (Garg, 2003). Normally, the decrease in nutrient uptake under water deficit conditions takes place due to a reduction in transpiration rate (Ali *et al.*, 2008; Jabeen *et al.*, 2008), but this is not the only way of controlling nutrient uptake from soil to root and then root to leaves (Peuke *et al.*, 2006). Effects of drought stress on plant nutrition may also be due to unavailability of sufficient energy for the assimilation of available nutrients such as PO₄⁻, SO₄⁻², NH₄⁺ and NO₃⁻, because before the use of these ions for growth and development of plants, their conversion is necessary into

energy-dependent processes (Grossman and Takahashi, 2001). It is generally accepted that due to low nutrient availability, a significant decrease in crop yield takes place under water deficit conditions (McWilliams, 2003; Lopez *et al.*, 2008). Of different micro and macro minerals that are necessary for plant growth and metabolic functions, potassium (K^+) plays a key role in plant osmotic adjustment, activation of a number of enzymes involved in respiration and photosynthesis. It also controls the opening of stomata and helps to transport sugars from phloem to other parts. Further, it plays a role in water and nutrient transport, protein and starch synthesis, and finally improves crop quality in terms of final grain yield (Lopez *et al.*, 2008). Under water deficit conditions K^+ uptake increases, but it depends upon the type of crop and the stage at which water stress is applied (Gunes *et al.*, 2006; Lopez *et al.*, 2008). Nitrogen availability in plants regulates many physiological and biochemical processes due to the reason that it is a constituent of many plant cell components including amino acids and nucleic acids. Therefore, low availability of nitrogen under water deficit conditions directly affects plant growth, final yield and yield quality (D'Andrea *et al.*, 2008; Moser *et al.*, 2006). Like nitrogen, P is also an important nutrient that takes part in growth and development of plants. It is the part of many cell components such as nucleic acids, phospholipids and sugar phosphate. Under water deficient conditions, its severe deficiency may occur which can affect plant metabolism and growth, which is one of the earliest affect on soil-grown plants. Jabeen *et al.* (2008) reported that water stress significantly reduced the growth, photosynthesis and water content of plants, however, leaf chlorophyll contents and shoot N and K increased, while shoot P contents remained un-affected. In another study while assessing the drought tolerance of different chickpea cultivars Gunes *et al.* (2006) found that drought tolerant cultivars accumulated more N, P, K, Ca, Zn, Mn and B in shoots and roots. In summary, conclusion may be drawn that water stress reduces the uptake, translocation and metabolism of nutrients. Drought-induced reduction in transpiration rate decreases the nutrient absorption and efficiency of their utilization.

Maize yield and drought stress

Maize crop during its growth period is subjected to drought stress frequently (Tai *et al.*, 2011). Arora *et al.* (2002) reported that pre-pollination and post-pollination water deficit reduced the kernel number and kernel size of the corn.

Drought stress at grain filling stage caused 79-81 % yield reduction in maize (Monneveux *et al.*, 2005). In maize, water stress reduced yield by delaying silking, thus increasing the anthesis-to-silking interval. This trait was highly correlated with grain yield, specifically ear and kernel number per plant (Cattivelli *et al.*, 2008). A considerable increase in final yield can be obtained with an increase in growth rate (Aslam and Tahir, 2003; Kumaga *et al.*, 2003). However, in a study to observe the response of different maize inbred lines to drought stress in relation to biomass production and final grain yield, Zhang *et al.* (2007) reported that final yield of all maize inbred lines decreased due to water stress that was positively correlated with the smaller leaf area, thinner stalk and reduced biomass production.

Adverse environmental conditions such as drought adversely affects the assimilate partitioning from source to sink, either to roots or reproductive parts, but depends upon the growth stages. Normally, at early stages allocation of dry matter takes place to the roots due to drought stress that enhances the water uptake (Leport *et al.*, 2006). Secondly, the movement of assimilates from source to sink also depends upon the concentration of sucrose and photosynthetic rate (Komor, 2000). Drought stress disrupts the carbohydrate metabolism and sucrose contents in leaves by decreasing the photosynthetic rate and by lowering the activity of acid invertase (Kim *et al.*, 2000). This reduction in photosynthesis and limited accumulation of sucrose in the leaves due to water stress limits the sucrose movement to the sink and finally reduces the development of reproductive organs that increases the chances of reproductive abortion due to the enhanced endogenous abscisic acid concentration that decreases the seed ability to use incoming sucrose (Setter *et al.*, 2001). Secondly, a reduced activity of acid invertase enzyme reduces the development of reproductive tissues due to improper phloem unloading (Goetz *et al.*, 2001). These adverse effects of drought on assimilate partitioning from source to sink results in the reduction of final crop yield as well as the quality of seed. Andersen *et al.* (2002) reported that under water deficit conditions invertase activity in young ovaries lowered that decreased the hexose to sucrose ratio that resulted in a reduction in mitotic cell divisions in young embryos resulting in reduced final yield. So it can be summarized that drought stress affects the crop yield by inhibiting the assimilate partitioning from source to sink as well as the seed quality in terms of its nutritional value.

Short-term approaches to induce drought tolerance in crop plants

In addition to selection, conventional breeding and molecular approaches, exogenous application of various organic (plant growth regulators) and inorganic (fertilizers and salts) chemicals can help to improve drought tolerance. These chemicals can be applied as seed dressing, pre-sowing seed treatment (seed priming), through rooting medium as well as foliar spray at various growth stages.

Pre-sowing seed treatment (Seed priming)

Seed priming is one of the most viable and pragmatic approaches to reduce the deleterious effects of drought stress especially on seed germination and early seedling growth, high vigor, uniform stand establishment (Harris *et al.*, 2002, 2004) and better yield especially in vegetable and field crops (Khalil *et al.*, 2001) . It is a technique by which seeds are prepared for germination by starting the germination related metabolic processes. During this process the seeds are soaked in water (hydro priming) or in a chemical solution of specific concentration for a specific time to start the germination up to a point, but the concentration and soaking time vary from crop to crop and among genotypes within a species (Farooq *et al.*, 2006; Ashraf and Foolad, 2007). In a number of crops this approach has been employed for reducing the adverse effects of water stress on germination, growth and final yield (Chiu and Sung, 2002; Harris *et al.*, 2002; Khan *et al.*, 2008). In a study to examine the effects of different inorganic osmotica on the germination behavior of rice, Du and Tuong (2002) reported that saturated CaHPO₄ and 4% KCl solution were found to be effective in reducing the germination time, better crop establishment and increased yield under water deficit conditions. Similarly, seed priming effects on promoting germination and plant growth under water deficit conditions have also been observed in wheat by Ajouri *et al.* (2004). They reported a 44% increase in WUE of plants raised from primed seeds as compared with unprimed seeds.

Furthermore, it is also observed that seed priming not only increases the germination and growth under stress conditions but also reduces the reproductive period by early flowering thereby resulting in increased yield (Kaur *et al.*, 2005; Kaya *et al.*, 2006). In a field trial with sunflower, Hussain *et al.* (2006) reported that priming sunflower seed with different inorganic osmotica enhanced the germination, increased the growth and achene yield and achene oil content. Similar effects of seed osmo-priming on germination, growth and final yield were also found in mung bean

grown under water deficit conditions (Khan *et al.*, 2008). Like inorganic salts some organic compounds are also used for seed priming. These organic compounds include growth regulators such as hormones and compatible osmolytes including GB, proline, trehalose, mannitol, PEG and some others. Osmopriming with these osmolytes was found to be effective in reducing the adverse effects of water stress on germination, growth and final yield (Naidu and Willium, 2004; Bardhan *et al.*, 2007; Hussain *et al.*, 2008). Naidu and Willium (2004) performed a variety of experiments to examine the effects of betaine, proline, trehalose, spermine and spermidine as pre-sowing seed treatment in rice cultivars. They reported a 9 to 27% increase in seedling growth and found that 0.5, 2, 0.5, 0.05, and 0.05 mM levels for proline, betaine, trehalose, spermidine and spermine, respectively to be more effective. Similarly, Wahid and Shabbir (2005) observed the effects of pre-sowing seed treatment of different levels of GB for reducing the adverse effects of stress on seed germination, seedling growth and some physiological and biochemical attributes of *Hordeum vulgare* L. They reported that 20 mM concentration of GB was very effective in enhancing seed germination, shoot fresh and dry weights, and shoot water content. They also reported increased shoot fresh and dry weights, net photosynthetic rate, and reduced relative membrane permeability of plants raised from seeds pre-treated with 20 mM GB.

Foliar application of inorganic salts/fertilizers

In view of a large number of studies it is evident that foliar application of those nutrients that become deficit in plants under stress, could maintain the deficiencies of these nutrients in plants that lead to osmotic adjustment and finally play a role in growth and yield improvement (Irshad *et al.*, 2002; Akram *et al.*, 2007; Ashraf *et al.*, 2007). For example, to induce drought tolerance in chickpea, different concentrations of K (0, 100, 200 mgL⁻¹) were applied as foliar spray (Bardhan *et al.*, 2007). The results showed that 200 mg L⁻¹ K was found very effective in increasing the plant health in terms of increased plant height, plant dry weight, 100-seed weight and grain yield. While assessing the performance of different rice cultivars in a field trial Quampah *et al.* (2011) used two K levels (0 and 180 kg/ha) as foliar spray. Foliar-applied K enhanced the performance of rice cultivars under low irrigation in terms of improved WUE, growth and grain yield. Hua *et al.* (2007) reported that foliar application of different nutrients such as N, P and K significantly increased the

biomass production in maize and enhanced the tissue nutrient contents that resulted in enhanced grain yield. Similarly, Sajedi *et al.* (2009) applied foliar selenium and “Biomim” (mixture of Fe, Zn, Cu, Mn B, Mo and Mg) to maize plants and they observed a significant effect of these substances on plant WUE, biomass production and final crop yield.

Thiourea (NH₂-CS-NH₂)

Thiourea, a sulphhydryl compound is known for breaking dormancy and stimulating germination. (Polyakoff-Mayber and Mayer, 1960). Pre-sowing soaking of barley seed in 500 mg L⁻¹ solution of thiourea derivative effectively enhanced the seedling growth (Uppal and Banerji, 1985). Thiourea may play an important role in the active accumulation of starch and phloem transport of soluble sugars (Giaquinta, 1976). Thiourea has also been reported to suppress the speed of chlorophyll decay (Liu *et al.*, 2002). Better performance of thiourea treated plants in terms of plant growth in spite of experiencing slightly more water deficit could be due to stabilization of lipoprotein structure and less malondialdehyde production compared to control (Mojtaba and Karr, 2001). Sahu *et al.* (1993) reported that foliar spray of thiourea (TU) significantly increased growth and yield of maize, most probably via improvement in canopy photosynthesis. Thiourea has been reported to significantly improve growth yield and water use efficiency of wheat under arid and semi-arid conditions (Sahu and Singh, 1995). In cluster bean (*Cyamopsis tetragonoloba* L.), thiourea increase seed yield under rainfed conditions due to enhanced photosynthetic efficiency and more efficient N metabolism (Garg *et al.*, 2006). Seed treatment and foliar application of thiourea improved growth, yield and WUE of pearl millet (*Pennisetum glaucum* L.) under arid and semi arid conditions (Parihar *et al.*, 1998). Combined application of thiourea and phosphorus also significantly improved plant growth and seed yield of clusterbean under water stress (Burman *et al.*, 2003).

The adversaries of abiotic stresses provoke finding pragmatic means of coping with them. Evolving new or screening of the existing crop varieties holding promise against environmental adversaries, although pay rich dividends, are expensive and long-term ventures. Currently, emphasis has been placed on the exogenous use of stress alleviating compounds either seed or foliar application (Farooq *et al.*, 2009a). Many organic and inorganic salts, natural and synthetic plant growth regulators and stress signaling molecules have been used based on their

specific properties and roles in improving germination and subsequent growth in a number of grain, forage and horticultural crops (Sivritepes *et al.*, 2005; Wahid and Shabbir, 2005). Moreover, their application at various phenological stages of growth is important, since growth stage may modulate the stress tolerance tendency of any species (Wilson *et al.*, 2000). The plant stress tolerance can be improved with the exogenous use of stress alleviating chemicals (Wahid and Shabbir, 2005; Wahid *et al.*, 2007b; Farooq *et al.*, 2009b).

Among the stress alleviating compounds, thiourea is an important molecule with two functional groups; ‘thiol’ is important to oxidative stress response and ‘imino’ partly fulfils the N requirement. It is highly water soluble and easily absorbed in the living tissues. Although role of thiourea in abiotic stress tolerance is not much investigated, available reports show that it relieves salinity induced seed dormancy in *Allenrolfea occidentalis* at lower concentration (Gul and Weber, 1998). Under conditions of water stress or high temperature, external use of thiourea can increase K⁺ uptake by chickpea and reduce ABA biosynthesis (Aldasoro *et al.*, 1981). Recently, Srivastava *et al.* (2010) reported that thiourea treatment coordinately bioregulates different signaling and effector mechanisms and alleviates the adverse effects of high salinity in salt-treated *Brassica juncea* seeds. Keeping in view the above review the present study was proposed to investigate the possible role of TU in improving drought tolerance in maize and to explore the biochemical basis of drought tolerance.

Chapter 3

MATERIALS AND METHODS

Experimental site and design

Studies were carried out at the Research Area of Department of Agronomy, University of Agriculture Faisalabad and analytical work was done in the Analytical Laboratory, Department of Agronomy and Seed Physiology Laboratory, Department of Crop Physiology, University of Agriculture, Faisalabad. The green house experiments were laid out in completely randomized design (CRD) and field experiment was laid out in randomized complete block design (RCBD) with four replications.

Experimental details

Experiment 1:

Screening maize hybrids for drought tolerance

The experiment was conducted in plastic pots to screen the available maize germplasm (32F10,32B33, 33H25, 3335, 34N43, 6142, 6525) for drought tolerance in green house. The water stress levels (80%, 60%, 40% water holding capacities) were maintained by applying measured quantity of water. Screening was done based on the performance of above mentioned maize hybrids under drought stress. One sensitive and one tolerant hybrid was selected from this experiment.

EXPERIMENT 2:

Improving drought tolerance in maize (*Zea mays* L.) by application of thiourea through seed treatment

Experiment was conducted to see the response of maize hybrids to seed treatment with thiourea under drought. Studies were carried out in the pots (green house) at Research Area of Department of Agronomy. Experimental treatments were

Treatments

Factor A (Hybrids)

H₁ = (Drought sensitive 32F10; Selected from experiment 1)

H₂ = (Drought tolerant 34N43; Selected from experiment 1)

Factor B (Moisture stress levels)

F₁ = 80% Water holding capacity

F₂ = 40% Water holding capacity

Factor C: (Seed treatment with thiourea 'TU')

T₁ = Control (seed was treated with distilled water)

T₂ = Dry seed was used

T₃ = 200mg/l

T₄ = 400mg/l

T₅ = 600mg/l

T₆ = 800mg/l

Experiment 3:

Improving drought tolerance in maize (*Zea mays* L.) by application of thiourea through rooting medium

Experiment was conducted to see the response of maize hybrids by application of thiourea through rooting medium under drought. Studies were carried out in the pots. Nutrient solution was used as growth medium in the pots (green house). Moisture stress was created by using PEG 8000. Experimental treatments were

Treatments

Factor A (Hybrids)

H₁ = (Drought sensitive 32F10; Selected from experiment 1)

H₂ = (Drought tolerant 34N43; Selected from experiment 1)

Factor B (Moisture stress levels)

Moisture stress was created by using PEG 8000

S₁ = Control

S₂ = -0.4 MPa

S₃ = -0.6 MPa

S₄ = -0.8 MPa

Factor C: (Rooting medium)

Thiourea (TU) levels

- T₁ = Control (distilled water)
- T₂ = 200mg/l
- T₃ = 400mg/l
- T₄ = 600mg/l
- T₅ = 800mg/l

Experiment 4:

Improving drought tolerance in maize (*Zea mays* L.) by foliar application of thiourea

Experiment was conducted to see the response of maize hybrids to foliar application of thiourea under drought. Studies were carried out in the pots (green house). Experimental treatments were

Treatments:

Factor A (Hybrids)

- H₁ = (Drought sensitive 32F10; Selected from experiment 1)
- H₂ = (Drought tolerant 34N43; Selected from experiment 1)

Factor B (Moisture stress levels)

- F₁ = 80% Water holding capacity
- F₂ = 40% Water holding capacity

Factor C: Foliar application

Thiourea levels

- T₁ = Control (no application of Thiourea)
- T₂ = 500mg/l
- T₃ = 1000mg/l
- T₄ = 1500mg/l

Field experiment

Improving drought tolerance in maize (*Zea mays* L.) by exogenous application of thiourea

Experiment was conducted to see the response of hybrid maize to exogenous application of Thiourea under drought. Studies were carried out under field conditions at Research Area of Department of Agronomy. Tensiometers were used for measuring soil water potential and irrigation was made on the basis of tensiometer reading.

Experimental treatments were as under

Factor A (Hybrids)

H₁ = Drought sensitive 32F10; Selected from experiment 1

H₂ = Drought tolerant 34N43; Selected from experiment 1

Factor B

For field experiment, thiourea levels and method of application were decided on the basis of green house experiments. From seed treatment experiment, 800 mg/l TU was best treatment and from foliar application experiment, 1500 mg/l TU foliar application was best treatment. So these two were used in field experiment along with dry seed sowing and foliar application of distilled water as control.

Factor C: Moisture stress levels

S₁ = - 15 kPa

S₂ = - 30 kPa

S₃ = - 45 kPa

Experimental conditions

Treated and control seeds were sown in Research Area of Agronomy Department, University of Agriculture, Faisalabad, and foliar application of thiourea and distilled water was made 30 days after sowing.

Irrigation was made when soil water potential decreased below the level mentioned in respective treatments of water stress.

Procedure for recording the data

Procedure to record data on various physiological and biochemical characteristics are given as under:

Seedling establishment

The data for the following observations were recorded:

1. Time taken to 50% emergence (E_{50}) [days]
2. Mean emergence time (MET) [days]
3. Emergence energy (EE) [%]
4. Final emergence percentage (FEP) [%]
5. Coefficient of uniformity of emergence (CUE)

1. Time taken to 50 % emergence (E_{50}) [Days]

The experiments were visited daily. Number of emerged seeds was recorded daily according to the seedling evaluation Handbook of Association of Official Seed Analysts (1990). Time taken to 50% emergence of seedlings (E_{50}) was calculated according to the following formulae of Coolbear *et al.* (1984) modified by Farooq *et al.* (2005):

$$E_{50} = t_i + \left[\frac{N/2 - n_i}{n_j - n_i} \right] (t_j - t_i)$$

Where 'N' is the number of final emergence count and n_i , n_j cumulative number of seeds emerged at adjacent days t_i and t_j when $n_i < (N+1)/2 < n_j$.

2. Mean emergence time (MET) [Days]

Mean emergence time (MET) was calculated according to following equation of Ellis and Roberts (1981):

$$MET = \frac{\sum Dn}{\sum n}$$

Where 'n' is the number of seeds, which will emerge on day D, and D is the number of days that will be counted from the beginning of emergence.

3. Emergence energy (EE) [%]

Energy of germination was recorded at 4th day after planting. It is the percentage of emerged seedlings 4 days after planting relative to the total number of seeds tested (Farooq *et al.*, 2006).

$$EE (\%) = \frac{\text{Number of seedlings emerged 4 days after sowing}}{\text{Total number of seeds sown}} \times 100$$

4. Final emergence percentage (FEP) [%]

Final emergence percentage was taken at the end of experiment. It represents the ratio, in percentage, of number of emerged seedlings to total seeds planted.

$$FEP (\%) = \frac{\text{Final number of seedlings emerged}}{\text{Total number of seeds sown}} \times 100$$

5. Coefficient of uniformity of emergence (CUE)

The coefficient of uniformity of emergence (CUE) was calculated using the following formulae of Bewley and Black (1985):

$$CUE = \sum n / \sum \left[(\bar{t} - t)^2 . n \right]$$

Where 't' is the time in days, starting from day 0, the day of sowing, and 'n' is the number of seeds completing emergence on day 't' and ' \bar{t} ' is equal to MET.

Seedling vigor

Stem length (cm)

Stem length was measured with the help of scale. Three plants were randomly selected for measuring stem length and then average was taken after 45 days of sowing.

Root length (cm)

Root length was measured with the help of scale. Three plants were randomly selected for measuring stem length and then average was taken after 45 days of sowing.

Seedling dry weight (mg)

For dry weight seedlings were separated into roots and shoot and kept at 70°C till constant weight in an oven.

Water relations

Relative water content

Fresh leaves (0.5 g; W_f) were rinsed in water until the weight of the leaves is constant. The saturated leaves were weighed (W_s) and then dried for 24 h at 80°C for determination of the dry weight (W_d). Relative water contents (RWC) were calculated by the following formula (Barr and Weatherley, 1962):

$$RWC = (W_f - W_d)/(W_s - W_d) \times 100$$

Leaf water potential (-MPa)

The third leaf from top (fully expanded youngest leaf) was excised at 6:30 a.m. to 8:30 a.m. to determine the leaf water potential with a Scholander type pressure chamber (arimad -2 –Japan, ELE international).

Leaf osmotic potential (- MPa)

The same leaf used for water potential measurement was frozen in a freezer below -20 °C for more than seven days, after which the frozen leaf material was

thawed and the sap was extracted by pressing the material with a glass rod. The sap was used directly for the determination of osmotic potential in a vapor pressure osmometer (Vapro, 5520).

Leaf turgor potential (MPa):

The turgor potential was calculated as the difference between osmotic potential (ψ_s) and water potential (ψ_w) values.

$$\psi_p = \psi_w - \psi_s$$

Estimation of nitrogen

Nitrogen was estimated by micro – Kjeldahl's method (Bremner, 1965). Following reagents were used for N determination: Boric acid solution (3%), sulphuric acid standard (0.01 N), mixed indicator of bromocresol green and methylene red.

Phosphorus determination

Phosphorus (P) was determined spectrophotometrically. The extracted material (2 mL) was dissolved in 2 mL of Barton's reagent and total volume was made 50 mL. These samples were kept for half an hour before analyzing phosphorus. The Barton's reagent was prepared as described below;

Barton's reagent

Solution A: 25 g of ammonium molybdate was dissolved in 400 mL of distilled water.

Solution B: Ammonium metavanadate (1.25 g) was dissolved in 300 mL of boiling water, cooled, and 250 ml of conc. HNO_3 was added. The solution was again cooled at room temperature.

The solutions A and B were mixed and the volume was maintained up to one liter. It was stored at room temperature.

Determination of potassium

The leaf sap was diluted as required by adding distilled water. The dilution factor was correlated with the original value and potassium concentrations were measured by using Sherwood 410 Flame photometer with the help of self prepared standard solutions using reagent grade salt of KCl.

Biochemical analysis

Extraction and determination of antioxidants

Total extractable SOD activity was measured following the protocol described by McCord and Fridovitch (1969). The inhibition of the colour formation (measured at 560 nm) was determined by addition of 0-50 μL of the extract to a reaction mixture containing 50 mM HEPES/KOH buffer (pH 7.8), 0.05 units' xanthine oxidase, 0.5 mM nitroblue tetrazolium, and 4 mM xanthine. One unit of SOD activity is equivalent to the volume of extract needed to cause 50% inhibition of the colour reaction. Catalase (CAT) activity was determined according to modified method of Luck (1974). Enzyme extract (50 μL) was added to 3 mL of hydrogen-peroxide-phosphate buffer (pH 7.0). The time required for the decrease in the absorbance from 0.45 to 0.40 was noted. Enzyme solution containing hydrogen peroxide-free phosphate buffer was used as control. Ascorbate peroxidase (APX) activity was determined following the method of Nakano and Asada (1987) with slight modification. Ascorbate oxidation to dehydro ascorbate (DHA) approximately was followed at 265 nm in 1 mL reaction mixture containing 50 mM HEPES/KOH (pH 7.6), 0.1 mM EDTA, 0.05 mM ascorbate, 10 μL extract and 0.1 mM H_2O_2 .

Proline determination

Proline from the third leaf from top was estimated according to the method of the Bates *et al.* (1973). Reagents used in this method were (i) sulfosalicylic acid (3 % Sulfosalicylic acid solution was prepared in distilled water). (ii) Acidic ninhydrin (Ninhydrin 1.25 g) was dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M orthophosphoric acid, thereafter stored and cooled at 4 °C). (iii) Glacial acetic acid (GAA) (iv) Toluene.

Procedure

A sample of 0.5 g fresh leaf tissue was homogenized in 10 ml of 3 % sulfosalicylic acid and the homogenate was filtered through Whatman No. 2 filter

paper. Then 2.0 ml of the filtrate was mixed with 2.0 ml acid ninhydrin, 20 ml 6 M orthophosphoric acid and 2 ml of glacial acetic acid in a test tube. This mixture was incubated at 100°C for 60 minutes and then cooled in an ice bath. Finally, 4.0 ml of toluene was added to the solution and mixed vigorously by passing a continuous stream of air for 1-2 min. The chromophore containing toluene was aspirated from the aqueous phase, warmed at room temperature and the absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated on fresh weight basis as follows:-

Proline $\mu\text{mole g}^{-1}$ fresh weight = $(\mu\text{g proline ml}^{-1} \times \text{ml of toluene}/115.5)/(\text{g of sample})$

Determination of MDA/lipid peroxidation

The level of lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). A fresh leaf sample (0.5 g) was homogenized in 10 mL of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 rpm for 10 min. To 2 mL aliquot of the supernatant, 4 mL of 0.5% thiobarbituric acid and 20% TCA were added. The mixture was heated at 95°C for 30 minutes and then quickly cooled in an ice bath and centrifuged at 10,000 rpm for 10 minutes, the absorbance of supernatant was recorded at 532 and 600 nm. After subtracting the non-specific absorbance at 600 nm, the MDA content was calculated using its molar extinction coefficient ($155 \text{ mM}^{-1} \text{ cm}^{-1}$) and the result was expressed as $\mu\text{mol (MDA) g}^{-1}$ fresh weight.

Electrolyte leakage (%)

Membrane permeability was estimated by electrolyte leakage (EL) according to Valentovic *et al.* (2006) with a few modifications. Leaves samples (0.5g) were excised, washed with deionized water and placed in test tubes containing 20 ml distilled deionized water and incubated at 25°C. The electrical conductivity of bathing solution (L_1) was determined. The samples were then autoclaved at 120°C for 20 min to release all electrolytes, cooled to 25°C and final electrical conductivity (L_2) was determined. The EL was determined using the following formula

$$\text{EL} = (L_1/L_2) \times 100$$

Growth Parameters

Plant height at maturity (cm)

Five plants were harvested at random from each plot from ground level. Their height was measured with the help of measuring tape and then average height was calculated.

Crop growth rate (g m⁻²d⁻¹)

Dry matter (DM) accumulation was determined at fortnight interval by selecting five plants randomly from each plot. The sampling was started 30 days after sowing (DAS) and terminated at the harvest of crop. Soon after harvest samples were weighed to determine fresh weight. Each plant sample was chaffed, thoroughly mixed and then sun dried. Afterwards samples were placed in oven at 70 °C to dry the plant material to constant dry weight. Then dry weight per m⁻² was calculated and used to estimate crop growth rate as proposed by Hunt (1978).

$$\text{CGR} = \frac{W_2 - W_1}{T_2 - T_1}$$

Where,

CGR = Crop growth rate

W₂ = dry weight per unit land area (g m⁻²) at second harvest

W₁ = dry weight per unit land area (g m⁻²) at first harvest

T₂ = time corresponding to second harvest

T₁ = time corresponding to first harvest

Yield and yield components

1000 grain weight

Thousand (1000) grain weight was taken at random from the grain lot of each sub plot and was weighed by means of electric balance.

Biological yield (kg ha⁻¹)

After harvesting the crop from each plot, whole material was sundried. Then this total weight of cobs and stalks per lot was determined with the help of electric balance and then converted into kg ha⁻¹.

Grain yield (kg ha⁻¹)

All the cobs in each plot were shelled, sundried and weighed. Then weight of grains per plot was converted in to kilograms per hectare.

Crude protein determination

Nitrogen determined by Kjeldahl's method as described earlier was converted into protein content by multiplying with factor 6.25 (Anonymous, 2002).

Statistical analysis

Data regarding all the parameters were analyzed by using Fisher's analysis of variance technique and LSD test at 5 % probability was used to compare the differences among treatments' means (Steel *et al.*, 1997).

Chapter 4

RESULTS AND DISCUSSION

Experiment 1:

Screening maize hybrids for drought tolerance

Table 4.1: Effect of irrigation regimes on time taken for 50% emergence (days) of maize

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	6.65 a	6.18 b	4.87 d	5.22 a	3.47 g	4.05 e	3.76 f	4.89 c
I₂ 60% WHC	6.71 a	6.53 b	6.46 c	6.33 d	4.19 g	5.74 e	5.09 f	5.86 b
I₃ 40% WHC	7.36 a	7.30 b	7.21 c	7.07 d	5.57 g	6.89 e	6.82 f	6.89 a
Means (Hybrids)	6.91 a	6.67 b	6.18 d	6.21 c	4.41 g	5.56 e	5.22 f	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.01 Irrigation (I) = 0.01 H x I = 0.02

Maize hybrids differed significantly ($P \leq 0.05$) for time taken for 50% emergence (E_{50}). Irrigation regimes also differed significantly. Interaction between maize hybrids and irrigation regimes for E_{50} was also significant (Table 4.1). Increasing water stress increased E_{50} as revealed from data. Maize hybrid 32F10 had maximum E_{50} (7.36days) under 40 % water holding capacity (WHC) whereas hybrid 34N43 had minimum E_{50} (5.57days) under same moisture status.

Table 4.2: Effect of irrigation regimes on emergence energy (%) of maize hybrids

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	29.39 g	42.76 f	57.84 e	51.97 d	66.46 a	61.26 c	65.12 b	53.54 a
I₂ 60% WHC	27.95 g	32.16 f	36.66 d	39.54 e	60.43 a	45.92 c	54.36 b	42.43 b
I₃ 40% WHC	18.74 g	20.13 f	21.25 e	23.48 d	48.33 a	24.75 c	26.44 b	26.16 c
Means (Hybrids)	25.36 g	31.69 f	38.58 d	38.33 e	58.40 a	43.97 c	48.64 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.01 Irrigation (I) = 0.01 H x I = 0.03

Maize hybrids differed significantly ($P \leq 0.05$) for emergence energy (EE) under different irrigation regimes (Table 4.2). Increasing water stress decreased EE as is clear from data. Maximum EE (66.46%) was observed under 80 % WHC in hybrid 34N43 and in hybrid 32F10 it was 29.39%.

Table 4.3: Effect of irrigation regimes on coefficient of uniformity of emergence of maize hybrids

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	0.48 g	0.63 f	0.78 d	0.72 e	0.89 a	0.82 c	0.85 b	0.74 a
I₂ 60% WHC	0.45 g	0.52 f	0.55 e	0.59 d	0.80 a	0.66 c	0.75 b	0.62 b
I₃ 40% WHC	0.12 g	0.16 f	0.23 e	0.28 d	0.69 a	0.34 c	0.38 b	0.31 c
Means (Hybrids)	0.35 g	0.44 f	0.52 e	0.53 d	0.79 a	0.61 c	0.66 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.007 Irrigation (I) = 0.005 H x I = 0.006

Irrigation regimes differed significantly ($P \leq 0.05$) for coefficient of uniformity of emergence (CUE). Maize hybrids also differed significantly for CUE. Interaction between maize hybrids and irrigation regimes for this parameter was also significant (Table 4.3). Maximum CUE (0.89) was observed under 80 % WHC for hybrid 34N43 and minimum (0.48) was observed for hybrid 32F10.

Table 4.4: Effect of irrigation regimes on emergence index of maize hybrids

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	4.95 g	6.65 f	9.42 d	8.41 e	10.41 a	10.12 c	10.24 b	8.60 a
I₂ 60% WHC	4.74 g	5.43 f	5.84 e	6.25 d	9.72 a	7.42 c	8.79 b	6.89 b
I₃ 40% WHC	3.21 g	3.51 f	3.93 e	4.07 d	7.90 a	4.26 c	5.04 b	4.56 c
Means (Hybrids)	4.30 g	5.20 f	6.40 d	6.24 e	9.34 a	7.87 c	8.02 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.01 Irrigation (I) = 0.009 H x I = 0.02

Maize hybrids differed significantly ($P \leq 0.05$) for emergence index (EI) under different irrigation regimes. Increasing moisture level increased emergence index of all the maize hybrids as revealed from data (Table 4.4). Maximum EI (10.41) was observed in hybrid 34N43 under 80 % WHC whereas minimum (4.95) was observed in hybrid 32F10 under same moisture contents.

Table 4.5: Effect of irrigation regimes on mean emergence time (days) of maize hybrids

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	8.35 a	7.53 b	6.15 d	6.63 c	5.11 g	5.55 e	5.47 f	6.40 c
I₂ 60% WHC	8.78 a	8.13 b	7.88 c	7.73 d	5.83 g	7.33 e	6.39 f	7.44 b
I₃ 40% WHC	10.38 a	10.28 b	10.16 c	9.67 d	6.84 g	9.48 e	9.04 f	9.41 a
Means (Hybrids)	9.17 a	8.66 b	8.06 c	8.01 d	5.93 g	7.45 e	6.97 f	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.01 Irrigation (I) = 0.01 H x I = 0.02

Maize hybrids differed significantly ($P \leq 0.05$) for mean emergence time (MET). Irrigation regimes had also a significant effect on MET. Similarly, interaction between irrigation regimes and maize hybrids for MET was also significant (Table 4.5). Increasing water stress increased MET for all the maize hybrids as is clear from data. Maximum MET (10.38d) was recorded for hybrid 32F10 under 40% WHC and minimum MET (6.84d) was recorded for hybrid 34N43 under same moisture percentage. Similar trend was observed under 60% WHC as well as under 80% WHC.

Table 4.6: Effect of irrigation regimes on final emergence percentage of maize hybrids

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	75.72 g	79.34 f	86.94 d	84.76 e	92.53 a	89.80 c	90.86 b	85.71 a
I₂ 60% WHC	74.77 g	76.97 f	77.74 e	78.44 d	88.45 a	81.16 c	85.63 b	80.45 b
I₃ 40% WHC	70.27 g	71.14 f	71.83 e	72.54 d	81.72 a	73.20 c	73.94 b	73.52 c
Means (Hybrids)	73.59 g	75.81 f	78.84 d	78.58 e	87.56 a	81.38 c	83.48 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.014 Irrigation (I) = 0.009 H x I = 0.025

Maize hybrids differed significantly ($P \leq 0.05$) for final emergence percentage (FEP) under different irrigation regimes (Table 4.6). Increasing water stress decreased FEP for all the hybrids as revealed from data. Hybrid 34N43 attained maximum FEP at all irrigation levels as against minimum in maize hybrid 32F10.

Table 4.7: Effect of irrigation regimes on root length (cm) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	13.00 c	13.05 c	13.50 c	13.87 c	20.70 a	14.50 b	14.60 b	14.74 c
I₂ 60% WHC	15.80 gh	17.75 fg	17.82 de	18.62 cd	24.00 ab	19.42 cd	21.25 bc	19.23 b
I₃ 40% WHC	17.37 fg	18.75 ef	18.32 de	20.75 cd	34.67 a	24.37 b	25.12 b	22.76 a
Means (Hybrids)	15.39 e	16.51 d	16.55 d	17.75 c	26.45 a	19.43 b	20.32 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 1.592 Irrigation (I) = 1.042 H x I = 2.758

Maize hybrids differed significantly ($P \leq 0.05$) under different irrigation regimes for root length (Table 4.7). Increased water stress level increased root length as revealed by higher values in columns. Maximum root length (34.67cm) was recorded in hybrid 34N43 under 40% water holding capacity (WHC) whereas minimum root length (17.37 cm) with same moisture level was recorded in hybrid 32F10. Trend was similar with other water holding capacities.

Table 4.8: Effect of irrigation regimes on shoot length (cm) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids							Means
	32F10	32B33	33H25	3335	34N43	6142	6525	(Irrigation)
I₁ 80% WHC	16.30	18.17	18.37	19.95	27.50	21.07	22.80	20.59 a
I₂ 60% WHC	15.12	16.85	16.87	17.87	22.04	18.57	19.00	18.04 b
I₃ 40% WHC	12.05	12.50	13.77	13.75	18.90	13.97	16.95	14.55 c
Means (Hybrids)	14.49 F	15.84 e	16.34 de	17.19 cd	22.81 a	17.87 c	19.58 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 1.341 Irrigation (I) = 0.878 H x I = NS

Maize hybrids differed significantly ($P \leq 0.05$) with respect to shoot length. Similarly irrigation levels had significant effect on shoot length. Interaction between hybrids and irrigation regimes was non-significant (Table 4.8). Mean values showed that maximum shoot length (20.59 cm) was recorded with 80 % WHC while minimum (14.55 cm) with 40 % WHC. Among hybrids, 34N43 attained maximum shoot length (22.81cm) against minimum in hybrid 32F10 (14.49 cm).

Table 4.9: Effect of irrigation regimes on root dry weight (g) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids							Means
	32F10	32B33	33H25	3335	34N43	6142	6525	(Irrigation)
I₁ 80% WHC	1.12	1.16	1.27	1.24	1.84	1.42	1.79	1.41 a
I₂ 60% WHC	0.77	1.00	1.00	1.10	1.82	1.24	1.28	1.17 b
I₃ 40% WHC	0.31	0.33	0.34	0.80	1.07	0.57	0.87	0.61 c
Means (Hybrids)	0.73 d	0.83 d	0.87 D	1.05 c	1.58 a	1.08 c	1.31 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.173 Irrigation (I) = 0.113 H x I = NS

Irrigation regimes have significant ($P \leq 0.05$) effect on root dry weight. Similarly, hybrids differed significantly for root dry weight. Interaction between hybrids and irrigation regimes was non-significant (Table 4.9). Maximum root dry weight (1.41g) was recorded at 80 % WHC and minimum (0.61g) was recorded at 40 % WHC. Hybrid 34N43 had more root dry weight (1.58 g) and hybrid 32F10 had minimum (0.73 g) which was statistically at par with hybrids 32B33 and 33H25.

Table 4.10: Effect of irrigation regimes on shoot dry weight (g) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids							Means
	32F10	32B33	33H25	3335	34N43	6142	6525	(Irrigation)
I₁ 80% WHC	0.91 d	0.93 d	1.38 c	1.45 c	2.94 a	1.46 c	2.22 b	1.61 a
I₂ 60% WHC	0.78 f	0.79 f	0.80 e	0.95 d	2.09 a	1.24 b	1.10 c	1.07 b
I₃ 40% WHC	0.41 d	0.45 d	0.42 d	0.56 bc	1.05 a	0.61 b	0.71 b	0.60 c
Means (Hybrids)	0.70 d	0.72 d	0.87 d	0.98 c	2.02 a	1.03 c	1.34 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.205 Irrigation (I) = 0.134 H x I = 0.355

Maize hybrids differed significantly ($P \leq 0.05$) under different irrigation regimes. Increase in water stress decreased shoot dry weight whereas more moisture level increased shoot dry weight in all maize hybrids (Table 4.10). Maximum shoot dry weight (2.94 g) was recorded in hybrid 34N43 and minimum (0.91 g) was recorded in hybrid 32F10 which was statistically at par with hybrids 32B33.

Table 4.11: Effect of irrigation regimes on leaf area (cm²) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	443.40 g	513.95 f	623.12 e	698.52 d	927.10 a	751.54 c	849.11 b	686.68 a
I₂ 60% WHC	428.62 g	458.69 f	512.68 e	559.07 d	873.99 a	626.58 c	701.74 b	594.48 b
I₃ 40% WHC	213.31 e	248.03 d	258.32 c	264.85 c	412.58 a	286.46 b	304.82 b	284.05 c
Means (Hybrids)	361.77 f	406.89 e	464.70 d	507.48 d	737.89 a	554.86 c	618.55 b	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 44.288 Irrigation (I) = 28.994 H x I = 76.710

Maize hybrids differed significantly ($P \leq 0.05$) under different irrigation regimes for leaf area (Table 4.11). Leaf area of all hybrids decreased with decrease in moisture percentage. However, this decrease was non significant from 80% WHC to 60% WHC but significant at 40% WHC. Performance of hybrid 34N43 was best at all moisture levels in attaining maximum leaf area.

Table: 4.12 Effect of irrigation regimes on water potential (-MPa) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	0.54 a	0.51 b	0.45 c	0.41 d	0.31 g	0.40 e	0.33 f	0.42 c
I₂ 60% WHC	0.81 a	0.79 b	0.74 c	0.69 d	0.59 g	0.66 e	0.62 f	0.69 b
I₃ 40% WHC	1.02 a	1.00 b	0.96 c	0.93 d	0.85 g	0.90 e	0.89 f	0.93 a
Means (Hybrids)	0.79 a	0.71 b	0.76 c	0.67 d	0.58 g	0.65 e	0.61 f	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.002 Irrigation (I) = 0.001 H x I = 0.003

Maize hybrids differed significantly ($P \leq 0.05$) for water potential. Similarly, irrigation regimes had significant effect on water potential. Interaction between maize hybrids and irrigation regimes was also significant (Table 4.12). Maximum water potential (-0.31MPa) was recorded in hybrid 34N43 under 80% WHC and minimum (-0.54MPa) was recorded in hybrid 32F10 under same WHC. Similar trend was observed under 40 % WHC as well as 60 % WHC.

Table 4.13: Effect of irrigation regimes on osmotic potential (-MPa) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids							Means
	32F10	32B33	33H25	3335	34N43	6142	6525	(Irrigation)
I₁ 80% WHC	0.99 a	0.98 b	0.98 b	0.97 c	0.93 f	0.95 d	0.94 e	0.96 c
I₂ 60% WHC	1.13 a	1.10 b	1.08 c	1.07 d	0.99 g	1.02 e	1.01 f	1.06 b
I₃ 40% WHC	1.29 a	1.25 b	1.24 c	1.22 d	1.19 g	1.21 f	1.20 e	1.22 a
Means (Hybrids)	1.14 a	1.11 b	1.10 c	1.08 d	0.98 g	1.06 e	1.05 f	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.001 Irrigation (I) = 0.001 H x I = 0.002

Maize hybrids differed significantly ($P \leq 0.05$) under different irrigation regimes with respect to osmotic potential (Table 4.13). Increase in moisture level increased osmotic potential. Maximum osmotic potential was recorded in hybrid 34N43 and minimum was recorded in hybrid 32F10 under all irrigation levels.

Table 4.14: Effect of irrigation regimes on turgor potential (MPa) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids							Means (Irrigations)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	0.41 g	0.48 f	0.52 e	0.55 d	0.64 a	0.62 b	0.57 c	0.54 a
I₂ 60% WHC	0.24 g	0.32 f	0.36 e	0.37 d	0.45 a	0.40 c	0.42 b	0.37 b
I₃ 40% WHC	0.21 f	0.26 e	0.29 d	0.29 d	0.34 a	0.30 c	0.32 b	0.29 c
Means (Hybrids)	0.29 g	0.35 f	0.39 e	0.40 d	0.48 a	0.44 b	0.44 c	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 0.001 Irrigation (I) = 0.001 H x I = 0.003

Irrigation regimes had significant ($P \leq 0.05$) effect on turgor potential (TP). Maize hybrids also differed significantly for turgor potential. Interaction between hybrids and irrigation regimes was also significant (Table 4.14). With respect to TP performance of maize hybrid 34N43 was statistically superior than other hybrids at all levels of WHC, although a considerable decrease in TP was observed with decrease in percent WHC.

Table 4.15: Effect of irrigation regimes on RWC (%) of maize hybrids at 45 DAS

Irrigation levels (I)	Maize hybrids (H)							Means (Irrigation)
	32F10	32B33	33H25	3335	34N43	6142	6525	
I₁ 80% WHC	85.42	86.61	88.95	90.51	91.42	90.92	91.17	89.28 a
I₂ 60% WHC	72.10	72.73	73.29	73.68	76.51	74.00	75.42	73.98 b
I₃ 40% WHC	61.55	61.78	62.75	62.76	64.78	63.76	64.11	63.07 c
Means (Hybrids)	73.02 d	73.70 d	75.00 bc	75.65 bc	77.57 a	76.23 ab	76.90 a	

Means not sharing the same letter within a row differ significantly at $P \leq 0.05$

LSD (5%): Hybrids (H) = 2.680 Irrigation (I) = 1.755 H x I = NS

Mean values showed that maize hybrids as well as irrigation regimes differed significantly ($P \leq 0.05$) for relative water contents (RWC), however their interactive effect was non significant on this parameter (Table 4.15). Maximum RWC (89.28%) was recorded under 80% WHC and minimum (63.07%) was recorded under 40 % WHC. Hybrid 34N43 had maximum RWC (77.57%) which was statistically at par with RWC of hybrids 6142 and 6525 as against the minimum in 32F10 (73.02%).

Discussion:

A) Emergence parameters

Water stress reduced the germination efficiency of maize hybrids. Under 80 % WHC, lowest time for 50 % emergence and mean emergence time was recorded and water availability increased emergence energy, coefficient of uniformity of emergence, emergence index and final emergence percentage. However, increase rate was different for different hybrids (Tables 4.1-4.6). Hybrid 34N43 improved germination more as compared to all other hybrids whereas hybrid 32F10 recorded less improvement in germination to all other hybrids. Similarly, water stress decreased emergence energy, emergence index, uniformity of emergence, final emergence percentage and increased time taken for 50 % emergence and mean emergence time in all maize hybrids. There are many biochemical and physiological processes involved in seed germination i.e imbibition of seed with water which helps in making seed coat soft and facilitates the emergence of embryo parts. Activation of hydrolysis enzymes i.e, α - and β - amylase which play a key role in conversion of complex sugars to simpler ones. Mobilization of food reserves from storage parts to embryo. And for all these processes, availability of adequate water is obligatory (Taiz and Zeiger, 2010; Wahid and Farooq, 2012). However, response to drought stress was different in different hybrids. Hybrid 32F10 was more sensitive to drought stress as compared to all other hybrids and its germination was affected more adversely and hybrid 34N43 was more tolerant to drought stress and its germination efficiency was comparatively better than all other hybrids. Different genotypes respond differently to availability of water and water stress so that some are more sensitive to water stress and some are relatively tolerant (Farhad *et al.*, 2011).

B) Growth parameters

More root length was observed under drought stress as compared to well watered conditions. Among hybrids, maximum root length was recorded in hybrid 34N43 at 40 % WHC and under same moisture conditions, root length in hybrid 32F10 was less as compared to other hybrids (Table 4.7). Root to shoot ratio is increased when plants face water stress. Moreover root proliferation is an important

parameter to assess drought tolerance in different genotypes so that tolerant genotypes under drought stress has more root proliferation to explore water from more depth as compared to sensitive ones (Farooq *et al.*, 2009). More shoot length was recorded under well watered condition as compared to water stress. However, interaction between irrigation regimes and hybrids for shoot length was non significant (Table 4.8). Among hybrids, more shoot length was recorded in hybrid 34N43 and minimum was recorded in hybrid 32F10. Changes in morphological characters reflect the effects of drought stress on plants (Farooq *et al.*, 2009; Jaleel *et al.*, 2009). Olaoye (2009) reported that water stress decreased the plant height of maize hybrids whereas more height of maize hybrids was recorded under well watered conditions. Both root dry weight (RDW) and shoot dry weight (SDW) was increased under 80 % WHC in all hybrids. However, both parameters expressed a decreasing trend under water stress. Less RDW and SDW was recorded for hybrid 32F10 and maximum was recorded for hybrid 34N43 (Tables 4.9-4.10). Water stress disrupts homeostasis in plants. Major changes in water status can cause molecular damage, growth inhibition and resultantly death of plant cells. It has already accepted that different crop cultivars hold different responses to different level of water stress in view of water status and plant growth (Li Xin *et al.*, 2011). Leaf area (LA) of maize hybrids varied significantly under varying water stress levels. Drought caused maximum decrease in LA for hybrid 32F10 and this decrease was less in hybrid 34N43 as compared to all other hybrids (Table 4.11). Intensity of soil water deficit largely influences LA of a genotype (Abo-El-Kheir and Mekki, 2007; Farhad *et al.*, 2011).

C) Water relations

Maximum water potential (WP) was recorded in maize hybrids under well watered conditions. However, WP decreased significantly under water stress. Among hybrids, more negative WP recorded in 32F10 while 34N43 sustained less negative WP under water stress (Table 4.12). The primary and most important effect of water deficits in plants is hampered water status (Farooq *et al.*, 2010; Taiz and Zeiger, 2010). Medici *et al.* (2003) observed that maize hybrid P-6875 showed a WP of -0.78MPa under controlled condition and WP of this hybrid decreased to -0.96MPa under water stress conditions. Osmotic potential (OP) of leaves decreased as water contents of soil decreased. Claudio *et al.* (2006) reported that OP of leaves increased

upto -0.90 MPa under normal water availability and it decreased to -1.20 MPa under water stress conditions. Turgor potential (TP) of maize hybrids was increased under well watered conditions. The first response of plants to water stress is that cells lose turgidity so that cell size is reduced resultantly decreasing leaf area. Because of this adaptation, less surface is available for water loss and plants maintain minimum water status for survival (Taiz and Zeiger, 2010). Claudio *et al.* (2006) found that leaf TP decreased from 0.54 MPa to 0.18 MPa under water stress. Maximum relative water contents (RWC) recorded at 80 % WHC (Table 4.15). Decrease in RWC indicates a loss of turgor that results in limited water availability for the cell extension process in crop plants (Li Xin *et al.*, 2011).

Conclusion

From screening trial, it was found that hybrid 32F10 is sensitive to water stress while hybrid 34N43 is tolerant. Both, sensitive and tolerant hybrids will be used in further studies to examine the role of thiourea under water stress and normal availability of water for improving emergence, growth, water relations and osmolytes accumulation.

Experiment 2: Improving drought tolerance in maize by application of thiourea through seed treatment.

Table 4.16: Effect of thiourea seed treatment on time taken (days) for 50% emergence of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	4.13 b	3.96 b	5.93 b	6.00 b	5.00 b
T ₂ : dry Seed	4.43 a	4.27 a	6.13 a	6.07 a	5.22 a
T ₃ : 200 mg/l thiourea	3.83 c	3.70 c	5.84 c	5.63 c	4.75 c
T ₄ : 400 mg/l thiourea	3.54 d	3.39 d	5.47 d	5.28 d	4.42 d
T ₅ : 600 mg/l thiourea	3.25 e	3.13 e	5.15 e	4.96 e	4.12 e
T ₆ : 800 mg/l thiourea	2.97 f	2.88 f	4.76 f	4.62 f	3.81 f
Means (H×I)	3.69	3.55	5.56	5.41	
Means (Irrigation)	5.48 a		3.62 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD_{0.05} for, Irrigation = 0.007, H×I = NS, T = 0.01, H×I×T = 0.02

Table 4.17: Effect of thiourea seed treatment on emergence energy (%) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) \ Thiourea levels (T)	32F10	34N43	32F10	34N43	
T₁: control (Distilled water)	70.20 e	33.43 e	35.94 e	16.82 e	53.48 e
T₂: Dry Seed	63.57 f	28.87 f	30.26 f	15.02 f	47.61 f
T₃: 200 mg/l thiourea	77.44 d	79.35 d	38.23 d	41.41 d	59.11 d
T₄: 400 mg/l thiourea	80.14 c	81.28 c	43.64 c	46.46 c	62.88 c
T₅: 600 mg/l thiourea	82.35 b	83.63 b	49.54 b	52.74 b	67.07 b
T₆: 800 mg/l thiourea	84.70 a	85.40 a	55.45 a	59.17 a	71.18 a
Means (H×I)	76.40 b	78.62 a	41.53 d	44.33 c	
Means (Irrigation)	77.51 a		42.93 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.008, H×I = 0.01, T = 0.01, H×I×T = 0.03

Time taken for 50% emergence (days)

Irrigation regimes, hybrids as well as thiourea (TU) seed treatments differed significantly ($P \leq 0.05$) for time taken (days) for 50 % emergence (E_{50}). Three way interaction was also significant. Drought stress increased E_{50} in both hybrids 34N43 (Tolerant) and 32F10 (Sensitive). However, TU treatment decreased E_{50} as compared to dry seed sowing under drought as well as under well watered conditions (Table 4.16). Seed treatment with 800 mg/l TU gave best results in decreasing time for 50 % emergence under drought stress as well as under well watered conditions. Maximum E_{50} (6.13d) was recorded for hybrid 32F10 with 40 % water holding capacity under dry seed sowing conditions, however it was decreased to (4.76 d) with 800 mg/l TU. Similarly hybrid 34N43 had less E_{50} (6.07d) as compared to 32F10 with 40 % water holding capacity under dry seed sowing conditions. With 800 mg/l TU seed treatment for hybrid 34N43 under 40 % water holding capacity E_{50} (4.62d) was recorded. With 80 % WHC hybrid 34N43 had E_{50} (4.27d) whereas hybrid 32F10 had (4.43d) under dry seed sowing but with 800 mg/l TU seed treatment hybrid 34N43 had E_{50} (2.88d) and hybrid 32F10 had E_{50} (2.97d) under 80 % WHC.

Emergence energy (%)

Thiourea levels, irrigation regimes and hybrids differed significantly ($P \leq 0.05$) for energy of emergence. All the interactions were significant. Means for three way interactions show (Table 4.17) that minimum emergence energy was recorded for both the hybrids under 80 % water holding capacity as well as 40 % water holding capacity (WHC) under dry sowing conditions. Maximum emergence energy (85.40%) was recorded in hybrid 34N43 with 800 mg/l TU priming under 80 % WHC whereas with same moisture level and same TU treatment emergence energy of hybrid 32F10 was 84.70%. Water stress decreased emergence energy. With 40 % WHC and 800 mg/l TU seed treatment emergence energy of hybrid 32F10 was 55.45% and that of hybrid 34N43 was 59.17%.

Table 4.18: Effect of thiourea seed treatment on coefficient of uniformity of emergence of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	0.94 e	0.96 e	0.43 e	0.47 e	0.70 e
T ₂ : Dry Seed	0.85 f	0.92 f	0.32 f	0.37 f	0.62 f
T ₃ : 200 mg/l thiourea	0.99 d	1.03 d	0.53 d	0.56 d	0.78 d
T ₄ : 400 mg/l thiourea	1.07 c	1.13 c	0.61 c	0.65 c	0.86 c
T ₅ : 600 mg/l thiourea	1.15 b	1.18 b	0.70 b	0.74 b	0.94 b
T ₆ : 800 mg/l thiourea	1.21 a	1.23 a	0.79 a	0.82 a	1.01 a
Means (H×I)	1.03	1.08	0.56	0.60	
Means (Irrigation)	1.05 a		0.58 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.004, H×I = NS, T = 0.007, H×I×T = 0.01

Table 4.19: Effect of thiourea seed treatment on emergence index of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	10.46 e	10.77 e	5.16 e	5.72 e	8.03 e
T ₂ : Dry Seed	9.84 f	10.16 f	4.19 f	4.54 f	7.18 f
T ₃ : 200 mg/l thiourea	10.97 d	11.18 d	6.08 d	6.82 d	8.76 d
T ₄ : 400 mg/l thiourea	11.46 c	11.64 c	7.34 c	8.21 c	9.66 c
T ₅ : 600 mg/l thiourea	11.85 b	12.15 b	8.52 b	8.85 b	10.34 b
T ₆ : 800 mg/l thiourea	12.37 a	12.55 a	9.26 a	9.55 a	10.93 a
Means (H×I)	11.16 b	11.41 a	6.76 d	7.28 c	
Means (Irrigation)	11.28 a		7.02 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.005, H×I = 0.008, T = 0.01, H×I×T = 0.02

Co-efficient of uniformity of emergence

Thiourea levels, irrigation regimes and hybrids differed significantly ($P \leq 0.05$) for coefficient of uniformity of emergence (CUE). Interaction between the irrigation regimes and hybrids for CUE was non-significant whereas all other interactions were significant. All the thiourea seed treatments improved the uniformity of emergence as compared to dry seed sowing. Even priming with distilled water (Hydro Priming) improved CUE as compared to dry seed sowing (Table 4.18). Under 80 % WHC with hydro priming CUE in hybrid 32F10 was 0.94 and under 40 % WHC it was 0.43 which is more as compared to dry seed sowing i.e (0.85 and 0.32 respectively). Similarly, for hybrid 34N43 with hydro priming CUE was 0.96 under 80 % WHC and under 40 % WHC it was 0.47 which is more as compared to dry seed sowing i.e (0.92) and (0.37) respectively.

Emergence index

Thiourea levels, irrigation regimes and hybrids differed significantly ($P \leq 0.05$) for emergence index (EI) also known as emergence vigour. All the interactions were significant. Means for three way interaction show that hybrid 34N43 recorded maximum EI under both the water holding capacities and all seed sowing treatments as compared to hybrid 32F10 (Table 4.19) . Maximum EI (12.55) with 800 mg/l TU seed treatment was recorded in hybrid 34N43 under 80 % WHC whereas it was reduced to 10.16 with dry seed sowing under same water holding capacity. But under 40 % WHC, EI was (9.55) and (4.54) with 800 mg/l TU seed treatment and dry seed sowing respectively. For hybrid 32F10 under 80 % WHC with 800 mg/l TU seed treatment, EI was 12.37 and with dry seed sowing it was 9.84 and with 40 % WHC it was 9.26 and 4.19 with 800 mg/l TU seed treatment and dry seed sowing, respectively.

Table 4.20: Effect of thiourea seed treatment on mean emergence time (days) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	6.52 b	6.27 b	9.39 b	9.19 b	7.84 b
T ₂ : Dry Seed	7.19 a	6.79 a	9.84 a	9.55 a	8.34 a
T ₃ : 200 mg/l thiourea	5.94 c	5.64 c	8.90 c	8.68 c	7.29 c
T ₄ : 400 mg/l thiourea	5.36 d	5.18 d	8.44 d	8.28 d	6.82 d
T ₅ : 600 mg/l thiourea	5.03 e	4.79 e	7.96 e	7.81 e	6.40 e
T ₆ : 800 mg/l thiourea	4.62 f	4.44 f	7.65 f	7.47 f	6.04 f
Means (H×I)	5.77 c	5.52 d	8.70 a	8.50 b	
Means (Irrigation)	8.60 a		5.65 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.009, H×I = 0.01, T = 0.01, H×I×T = 0.03

Table 4.21: Effect of thiourea seed treatment on final emergence percentage of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	92.73 e	93.44 e	76.64 e	77.38 e	85.05 e
T ₂ : Dry Seed	90.62 f	92.04 f	75.12 f	76.33 f	83.53 f
T ₃ : 200 mg/l thiourea	94.25 d	95.14 d	78.34 d	79.84 d	86.89 d
T ₄ : 400 mg/l thiourea	95.76 c	96.57 c	80.35 c	81.54 c	88.55 c
T ₅ : 600 mg/l thiourea	97.20 b	97.72 b	84.21 b	85.83 b	91.24 b
T ₆ : 800 mg/l thiourea	98.17 a	98.69 a	87.37 a	89.21 a	93.36 a
Means (H×I)	94.79 b	95.60 a	80.34 d	81.69 c	
Means (Irrigation)	95.19 a		81.01 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.008, H×I = 0.01, T = 0.01, H×I×T = 0.02

Mean emergence time (days)

Irrigation regimes, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for mean emergence time (MET). All the interactions were significant. According to three way interaction data (Table 4.20), it is revealed that drought stress increased mean emergence time in both hybrids in all seed treatments. Seed treatment with 800 mg/l TU decreased MET in both hybrids under drought stress and under normal water availability. Maximum MET (9.84d) was recorded in hybrid 32F10 under 40 % WHC under dry seed sowing and it was reduced to 7.65d with 800 mg/l TU seed treatment under same moisture percentage. Similarly, minimum MET (4.44d) was recorded in hybrid 34N43 under 80 % WHC with 800 mg/l TU seed treatment and it increased to 6.79d with dry seed sowing under same moisture conditions.

Final emergence percentage

Irrigation regimes, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for final emergence percentage (FEP). All the interactions were significant. Means for three way interaction show that increasing moisture percentage increased the FEP. However seed treatment with TU caused additional improvement in FEP (Table 4.21). Maximum FEP (98.69) was recorded in hybrid 34N43 under 80 % WHC with 800 mg/l TU seed treatment and it was 92.04% with dry seed sowing under same water holding capacity. Similarly for same hybrid under 40 % WHC with 800 mg/l TU seed treatment, FEP was (89.21) and it was 76.33. For hybrid 32F10 under 80 % WHC with 800 mg/l TU seed treatment, FEP was (98.17) and with dry seed sowing it was (90.62) and under 40 % WHC with 800 mg/l TU seed treatment, FEP was 87.37 and it was 75.12 with dry seed sowing.

Table 4.22: Effect of thiourea seed treatment on root length (cm) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	10.11 e	10.57 e	16.55 e	16.82 e	13.51 e
T ₂ : Dry Seed	8.27 f	9.77 f	14.52 f	15.02 f	11.90 f
T ₃ : 200 mg/l thiourea	10.82 d	11.00 d	17.02 d	17.65 d	14.13 d
T ₄ : 400 mg/l thiourea	11.82 c	11.97 c	17.85 c	18.05 c	14.92 c
T ₅ : 600 mg/l thiourea	12.70 b	12.80 b	18.10 b	18.77 b	15.59 b
T ₆ : 800 mg/l thiourea	13.82 a	14.02 a	20.07 a	23.17 a	17.77 a
Means (H×I)	17.35 b	11.26 d	18.25 a	11.69 c	
Means (Irrigation)	17.80 a		11.47 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.23, H×I = 0.33, T = 0.40, H×I×T = 0.80

Table 4.23: Effect of thiourea seed treatment on shoot length (cm) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) \ Thiourea levels (T)	32F10	34N43	32F10	34N43	
T₁: control (Distilled water)	16.87 e	18.97 e	10.31 e	11.12 e	14.32 e
T₂: Dry Seed	16.07 f	16.27 f	8.77 f	10.12 f	12.81 f
T₃: 200 mg/l thiourea	19.70 d	19.87 d	11.17 d	12.15 d	15.72 d
T₄: 400 mg/l thiourea	21.27 c	21.45 c	13.27 c	13.77 c	17.44 c
T₅: 600 mg/l thiourea	23.30 b	24.10 b	13.97 b	14.00 b	18.84 b
T₆: 800 mg/l thiourea	24.80 a	26.25 a	14.75 a	15.30 a	20.27 a
Means (H×I)	20.33	12.04	21.15	12.74	
Means (Irrigation)	20.74 a		12.39 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.19, H×I = NS, T = 0.32, H×I×T = 0.65

Root length (cm)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for root length (RL). All the interactions were significant. Data for three-way interaction show that increasing water stress increased RL (Table 4.22). Maximum RL (23.17cm) was recorded in hybrid 34N43 under 40 % WHC with 800 mg/l TU seed treatment, and it was (20.07cm) for hybrid 32F10 under same moisture level and same TU seed treatment. Similarly, minimum RL (8.27cm) was recorded for hybrid 32F10 at 80 % WHC with dry seed sowing and it was 9.77cm for hybrid 34N43 under same sowing treatment and water holding capacity.

Shoot length (cm)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for shoot length. All other interactions were significant except interaction between irrigation and hybrids. Means for three-way interaction show that drought stress decreased shoot length. However, seed treatment with TU, increased shoot length under drought stress as well as under well watered conditions (Table 4.23). Maximum shoot length (26.25cm) was recorded in hybrid 34N43 with 800 mg/l TU seed treatment under 80 % WHC whereas it was 24.80cm in hybrid 32F10 under same seed treatment and water holding capacity. Under 40 % WHC, with 800 mg/l TU, shoot length in hybrid 34N43 was 15.30cm and in hybrid 32F10 it was 14.75cm.

Table 4.24: Effect of thiourea seed treatment on root dry weight (g) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	0.96 e	1.03 e	0.31 e	0.32 e	0.65 e
T ₂ : Dry Seed	0.90 f	0.96 f	0.17 f	0.20 f	0.56 f
T ₃ : 200 mg/l thiourea	1.11 d	1.29 d	0.33 d	0.40 d	0.78 d
T ₄ : 400 mg/l thiourea	1.38 c	1.49 c	0.45 c	0.47 c	0.95 c
T ₅ : 600 mg/l thiourea	1.68 b	1.80 b	0.76 b	0.78 b	1.25 b
T ₆ : 800 mg/l thiourea	2.62 a	3.00 a	0.84 a	0.84 a	1.82 a
Means (H×I)	1.44 b	0.47 d	1.59 a	0.50 c	
Means (Irrigation)	86.29 a		66.57 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.01, H×I = 0.01, T = 0.02, H×I×T = 0.04

Table 4.25: Effect of thiourea seed treatment on shoot dry weight (g) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	0.60	0.64	0.17	0.21	0.40 e
T ₂ : Dry Seed	0.55	0.58	0.14	0.15	0.35 f
T ₃ : 200 mg/l thiourea	0.72	0.80	0.23	0.25	0.50 d
T ₄ : 400 mg/l thiourea	0.88	1.04	0.28	0.31	0.63 c
T ₅ : 600 mg/l thiourea	1.13	1.27	0.43	0.46	0.82 b
T ₆ : 800 mg/l thiourea	1.74	1.97	0.51	0.52	1.18 a
Means (H×I)	0.94	0.29	1.05	0.32	
Means (Irrigation)	0.99 a		0.30 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.06, H×I = NS, T = 0. 12, H×I×T = NS

Root dry weight (g)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for root dry weight (RDW). All the interactions were significant (Table 4.24). Drought stress decreased root dry weight. However, seed treatment with TU increased RDW in both irrigation regimes as compared to dry seed sowing. Minimum RDW (0.17g) was recorded in hybrid 32F10 with dry seed sowing under 40 % WHC that was increased to 0.84g with 800 mg/l TU seed treatment under same water percentage. Similarly, in hybrid 34N43, RDW was 0.20g with dry seed sowing under 40 % WHC which was increased to 0.84g with 800 mg/l TU seed treatment under same water holding capacity. Under 80 % WHC, RDW for hybrid 34N43 was 3.00g with 800 mg/l TU seed treatment that was decreased to 0.96g with dry seed sowing whereas for hybrid 32F10 RDW was 2.62g with 800 mg/l TU seed treatment which was decreased to 0.90g with dry seed sowing under same moisture percentage.

Shoot dry weight (g)

Irrigation regimes, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for shoot dry weight (SDW). All the interactions were non significant except interaction between hybrids and thiourea. Means values showed that maximum SDW (1.18g) was recorded with 800 mg/l TU seed treatment and minimum SDW (0.35g) was recorded with dry seed sowing. Similarly under 80 % WHC, SDW was 0.99g and under 40 % WHC, it was 0.30g (Table 4.25).

Table 4.26: Effect of thiourea seed treatment on leaf area (cm²) of maize hybrids after 45 DAS

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T₁: control (Distilled water)	543.44	623.33	167.24	216.40	387.60 e
T₂: Dry Seed	509.07	520.24	149.61	162.54	335.34 f
T₃: 200 mg/l thiourea	626.36	666.52	248.03	262.75	450.91 d
T₄: 400 mg/l thiourea	702.12	746.71	269.02	304.82	505.67 c
T₅: 600 mg/l thiourea	784.97	861.12	417.68	428.57	623.08 b
T₆: 800 mg/l thiourea	878.49	931.98	451.25	458.08	679.95 a
Means (H×I)	674.07	283.79	724.98	305.53	
Means (Irrigation)	699.53 a		294.66 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD \leq 0.05 for, Irrigation = 18.89, H×I = NS, T = 32.72, H×I×T = NS

Table 4.27: Effect of thiourea seed treatment on water potential (-MPa) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	0.57 b	0.53 b	0.99 b	0.95 b	0.76 b
T ₂ : Dry Seed	0.66 a	0.61 a	1.03 a	1.02 a	0.83 a
T ₃ : 200 mg/l thiourea	0.50 c	0.45 c	0.92 c	0.89 c	0.69 c
T ₄ : 400 mg/l thiourea	0.41 d	0.40 d	0.87 d	0.84 d	0.63 d
T ₅ : 600 mg/l thiourea	0.39 e	0.34 e	0.80 e	0.76 e	0.57 e
T ₆ : 800 mg/l thiourea	0.33 f	0.31 f	0.72 f	0.68 f	0.51 f
Means (H×I)	0.48 c	0.89 a	0.44 d	0.86 b	
Means (Irrigation)	0.46 b		0.87 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.0007, H×I = 0.001, T = 0.001, H×I×T = .002

Leaf area (cm²)

Irrigation regimes, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for leaf area (LA). All the interactions were non significant. Means for irrigation regimes show that maximum LA (699.53cm²) was recorded at 80 % WHC and it was reduced to 294.66 cm² under 40 % WHC. While means for seed treatment showed that with 800 mg/l TU seed treatment LA was 679.95 cm² whereas it was 335.34 cm² with dry seed sowing (Table 4.26).

Water potential (-MPa)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for water potential (WP). All the interactions were significant. Data for three way interaction show that drought stress decreased the WP and TU seed treatment improved it as compared to dry seed sowing and 800 mg/l TU seed treatment proved to be best treatment in this regard (Table 4.27). Maximum WP (-0.31MPa) was recorded in hybrid 34N43 with 800 mg/l TU seed treatment under 80 % WHC and it was reduced to -0.61MPa with dry seed sowing under same moisture percentage. At 40 % WHC, with 800 mg/l TU seed treatment WP was -0.68MPa and it was reduced to -1.02MPa with dry seed sowing for same hybrid. Similarly, WP for hybrid 32F10 was -0.33MPa with 800 mg/l TU seed treatment under 80 % WHC and it was reduced to -0.66MPa with dry seed sowing. Under 40 % WHC, WP was -0.72MPa and it was decreased to -1.03MPa with dry seed sowing for same hybrid.

Table 4.28: Effect of thiourea seed treatment on osmotic potential (-MPa) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	0.99 b	0.98 f	1.23 b	1.21 b	1.10 b
T ₂ : Dry Seed	1.01 a	1.00 a	1.28 a	1.24 a	1.13 a
T ₃ : 200 mg/l thiourea	0.97 c	0.97 c	1.20 c	1.19 c	1.08 c
T ₄ : 400 mg/l thiourea	0.96 d	0.94 d	1.18 d	1.14 d	1.05 d
T ₅ : 600 mg/l thiourea	0.93 e	0.92 e	1.11 e	1.09 e	1.01 e
T ₆ : 800 mg/l thiourea	0.91 f	0.90 f	1.07 f	1.03 f	0.98 f
Means (H×I)	0.96 c	1.18 a	0.95 a	1.15 b	
Means (Irrigation)	0.96 b		1.16 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD \leq 0.05 for, Irrigation = 0.0006, H×I = 0.0008, T = 0.001, H×I×T = .002

Table 4.29: Effect of thiourea seed treatment on turgor potential (MPa) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	0.41 e	0.44 e	0.24 e	0.26 e	0.34 e
T ₂ : Dry Seed	0.35 f	0.39 f	0.22 f	0.23 f	0.30 f
T ₃ : 200 mg/l thiourea	0.47 d	0.52 d	0.27 d	0.29 d	0.39 d
T ₄ : 400 mg/l thiourea	0.53 c	0.54 c	0.29 c	0.30 c	0.42 c
T ₅ : 600 mg/l thiourea	0.55 b	0.58 b	0.30 b	0.32 b	0.431 b
T ₆ : 800 mg/l thiourea	0.58 a	0.59 a	0.34 a	0.35 a	0.47 a
Means (H×I)	0.48 b	0.28 d	0.51 a	0.29 c	
Means (Irrigation)	0.50 a		0.29 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.0009, H×I = 0.001, T = 0.001, H×I×T = .003

Osmotic potential (-MPa)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for osmotic potential (OP). All the interactions were significant. Drought stress decreased the OP in both hybrids and with all seed treatments. Means for three way interaction show that under 40 % WHC, OP in hybrid 34N43 was -1.03MPa with 800 mg/l TU seed treatment and in hybrid 32F10 it was -1.07MPa with same seed treatment and water holding capacity. However, with dry seed sowing under 40 % WHC, OP in hybrid 34N43 was -1.24MPa and in hybrid 32F10 it was -1.28MPa. Similarly, under 80 % WHC, in hybrid 34N43 maximum OP -0.90MPa was recorded with 800 mg/l TU seed treatment that decreased to -1.00MPa. For hybrid 32F10, under 80 % WHC and with 800 mg/l TU seed treatment OP was -0.91MPa and it was reduced to -1.01MPa with dry seed sowing under same moisture percentage.

Turgor potential (MPa)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for turgor potential. All the interactions were significant. More the availability of water more was TP as revealed from data of three way interaction (Table 4.29). However, TU seed treatment improved TP in well watered conditions as well as under water stress. Maximum TP (0.59MPa) was recorded in hybrid 34N43 with 800 mg/l TU seed treatment under 80 % WHC and it was 0.35MPa with same seed treatment under 40 % WHC. Whereas in hybrid 32F10, TP was 0.58 MPa under 80 % WHC with 800 mg/l TU seed treatment and under 40 % WHC, TP was 0.34MPa for same seed treatment.

Table 4.30: Effect of thiourea seed treatment on relative water contents (%) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T₁: control (Distilled water)	80.11	85.38	61.93	62.95	72.59 e
T₂: Dry Seed	75.26	76.11	60.33	61.21	68.23 f
T₃: 200 mg/l thiourea	86.51	87.40	63.14	64.41	75.80 d
T₄: 400 mg/l thiourea	89.45	90.00	65.12	67.78	78.08 c
T₅: 600 mg/l thiourea	90.51	90.97	71.69	72.88	81.51 b
T₆: 800 mg/l thiourea	91.05	92.75	73.29	74.12	82.80 a
Means (H×I)	85.48	65.92	87.10	67.22	
Means (Irrigation)	86.29 a		66.57 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD \leq 0.05 for, Irrigation = 0.87 H×I = NS T = 1.51 H×I×T = NS

Table 4.31: Effect of thiourea seed treatment on proline contents (umol/g FW) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distl water)	2.86 e	3.56 e	10.85 e	11.28 e	6.47 e
T ₂ : Dry Seed	1.36 f	2.12 f	9.54 f	9.93 f	6.40 f
T ₃ : 200 mg/l thiourea	4.24 d	4.75 d	11.79 d	12.47 d	8.31 d
T ₄ : 400 mg/l thiourea	5.34 c	6.16 c	13.17 c	14.08 c	9.69 c
T ₅ : 600 mg/l thiourea	6.81 b	7.64 b	14.96 b	16.70 b	11.53 b
T ₆ : 800 mg/l thiourea	8.46 a	9.04 a	15.86 a	17.52 a	12.72 a
Means (H×I)	4.85 d	5.55 c	12.69 b	13.66 a	
Means (Irrigation)	5.20 b		13.18 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.01, H×I = 0.02, T = 0.02, H×I×T = 0.04

Relative water contents (%)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for relative water contents (RWC). Except interaction between irrigation regimes and TU, all other interactions were non significant (Table 4.30). Maximum RWC (86.29%) were recorded at 80 % WHC and RWC at 40 % WHC were 66.57%. Among seed treatments, maximum RWC (82.80%) were recorded with 800 mg/l TU and with dry seed sowing RWC were 68.23%.

Proline contents (umol/g FW)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for proline. All the interactions were significant. There was less synthesis of proline under normal availability of water and increased under drought stress as revealed from three way interaction data (Table 4.31). Hybrid 34N43 increased synthesis of proline as compared to 32F10 with 800 mg/l TU seed treatment as well as with dry seed sowing under drought stress. Maximum proline contents (17.52umol/g FW) were recorded in hybrid 34N43 under 40 % WHC with 800 mg/l TU whereas in hybrid 32F10, proline contents were 15.86umol/g FW with same seed treatment and water holding capacity. With dry seed sowing under 40 % WHC, proline contents in hybrid 32F10 were 9.54umol/g FW and in hybrid 34N43 proline contents were 9.93umol/g FW.

Table 4.32: Effect of thiourea seed treatment on malondialdehyde contents (umol/g FW) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	8.33 b	7.73 b	14.91 b	14.55 b	11.38 b
T ₂ : Dry Seed	9.44 a	8.70 a	15.75 a	15.24 a	12.28 a
T ₃ : 200 mg/l thiourea	6.14 c	5.56 c	14.16 c	13.41 c	9.82 c
T ₄ : 400 mg/l thiourea	4.95 d	4.40 d	12.87 d	12.53 d	8.69 d
T ₅ : 600 mg/l thiourea	3.85 e	2.68 e	11.94 e	11.09 e	7.27 e
T ₆ : 800 mg/l thiourea	3.24 f	2.25 f	10.62 f	10.24 f	6.70 f
Means (H×I)	5.99 c	5.22 d	13.45 a	12.76 b	
Means (Irrigation)	5.60 b		13.11 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD \leq 0.05 for, Irrigation = 0.008, H×I = 0.01, T = 0.01, H×I×T = 0.03

Table 4.33: Effect of Thiourea seed treatment on electrolyte leakage (%) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	22.17 b	20.77 b	39.17 b	38.42 b	30.13 b
T ₂ : Dry Seed	24.67 a	23.94 a	41.66 a	40.95 a	32.80 a
T ₃ : 200 mg/l thiourea	19.52 c	18.39 c	37.75 c	35.58 c	27.81 c
T ₄ : 400 mg/l thiourea	17.24 d	16.64 d	34.38 d	32.14 d	25.07 d
T ₅ : 600 mg/l thiourea	15.10 e	14.45 e	30.44 e	29.84 e	21.84 e
T ₆ : 800 mg/l thiourea	13.80 f	12.30 f	28.04 f	26.39 f	20.74 f
Means (H×I)	18.86 c	17.64 d	35.52 a	33.59 b	
Means (Irrigation)	18.25 b		34.55 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.09, H×I = 0.13, T = 0.16, H×I×T = 0.32

Malondialdehyde contents (umol/g FW)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for malondialdehyde (MDA). All the interactions were significant. Drought stress increased the MDA production in both the hybrids under all seed treatments. However, MDA production was more in hybrid 32F10 as compared to hybrid 34N43. Similarly, MDA production was more with dry seed sowing as compared to 800 mg/l TU seed treatment under drought stress. Maximum MDA (15.75umol/g FW) was recorded in hybrid 32F10 under 40 % WHC with dry seed sowing and in hybrid 34N43 MDA was 15.24umol/g FW. With 800 mg/l TU, MDA in hybrid 32F10 was 10.62umol/g FW and in hybrid 34N43 was 10.24umol/g FW under 40 % WHC.

Electrolyte leakage (%)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for electrolyte leakage (EL). All the interactions were significant. More the drought stress more was electrolyte leakage as revealed from data of three way interaction (Table 4.33). Maximum EL (41.66%) was recorded in hybrid 32F10 with dry seed sowing under 40 % WHC and in hybrid 34N43 it was (40.95%) with same seed treatment and water holding capacity. With 800 mg/l TU seed treatment under 40 % WHC, EL in hybrid 32F10 was (28.04%) and in hybrid 34N43 it was (26.39%).

Table 4.34: Effect of thiourea seed treatment on superoxide dismutase (U /mg protiens) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	136.68 e	161.95 e	204.37 e	217.43 e	180.11 e
T ₂ : Dry Seed	132.27 f	154.80 f	201.49 f	212.91 f	175.37 f
T ₃ : 200 mg/l thiourea	170.41 d	171.63 d	218.17 d	219.35 d	194.59 d
T ₄ : 400 mg/l thiourea	172.55 c	175.63 c	230.83 c	235.35 c	203.59 c
T ₅ : 600 mg/l thiourea	182.88 b	184.24 b	253.07 b	271.21 b	222.85 b
T ₆ : 800 mg/l thiourea	194.90 a	199.45 a	259.93 a	275.76 a	232.51 a
Means (H×I)	164.95 d	174.62 c	227.97 b	238.67 a	
Means (Irrigation)	169.78 b		233.32 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD_{0.05} for, Irrigation = 0.09 H×I = 0.13 T = 0.16 H×I×T = 0.32

Table 4.35: Effect of thiourea seed treatment on peroxidase (U /mg proteins) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	25.37 e	31.70 e	38.24 e	40.71 e	34.00 e
T ₂ : Dry Seed	24.66 f	28.65 f	37.95 f	39.56 f	32.70 f
T ₃ : 200 mg/l thiourea	31.88 d	32.17 d	41.36 d	42.43 d	36.96 d
T ₄ : 400 mg/l thiourea	32.18 c	32.84 c	42.95 c	43.80 c	37.94 c
T ₅ : 600 mg/l thiourea	33.76 b	34.43 b	47.05 b	50.39 b	41.41 b
T ₆ : 800 mg/l thiourea	36.51 a	37.08 a	48.62 a	51.32 a	43.38 a
Means (H×I)	30.73 d	32.81 c	42.69 b	44.70 a	
Means (Irrigation)	31.70 b		43.77 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD_{0.05} for, Irrigation = 0.02 H×I = 0.03 T = 0.04 H×I×T = 0.08

Superoxide dismutase (U /mg protiens)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for antioxidant enzyme superoxide dismutase (SOD). All the interactions were significant. Under drought stress hybrid 34N43 increased synthesis of SOD as compared to hybrid 32F10 with dry seed sowing as well as TU seed treatment. Maximum SOD (275.76U/mg proteins) was recorded in hybrid 34N43 under 40 % WHC with 800 mg/l TU seed treatment and with dry seed sowing it was (212.91U/mg proteins) under same water holding capacity. Whereas, in hybrid 32F10 under 40 % WHC with dry seed sowing SOD was 201.49U/mg proteins and with 800 mg/l TU seed treatment it was 259.93U/mg proteins.

Peroxidase (U /mg protiens)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for antioxidant enzyme peroxidase (POD). All the interactions were significant. Drought stress increased the synthesis of POD as compared to normal availability of water. Furthermore, in hybrid 34N43 there was more synthesis of antioxidant enzyme POD as compared to hybrid 32F10 under drought stress and TU seed treatment additionally increased the synthesis of POD. In hybrid 32F10 under 40 % WHC with dry seed sowing POD was 37.95U/mg proteins and with 800 mg/l TU seed treatment POD was 48.62U/mg proteins. Hybrid 34N43 being tolerant synthesized more POD (51.32U/mg proteins) with 800 mg/l TU seed treatment and with dry seed sowing POD was 39.56U/mg proteins under same moisture status.

Table 4.36: Effect of thiourea seed treatment on catalase (U /mg protiens) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	19.88 e	20.40 e	35.53 e	41.20 e	29.25 e
T ₂ : Dry Seed	18.68 f	19.90 f	35.15 f	39.71 f	28.36 f
T ₃ : 200 mg/l thiourea	21.51 d	22.71 d	42.40 d	42.85 d	32.37 d
T ₄ : 400 mg/l thiourea	23.67 c	24.50 c	43.16 c	48.50 c	34.95 c
T ₅ : 600 mg/l thiourea	25.41 b	29.09 b	56.85 b	58.93 b	42.57 b
T ₆ : 800 mg/l thiourea	30.37 a	33.12 a	58.54 a	65.63 a	46.92 a
Means (H×I)	23.25 d	24.95 c	45.27 b	49.47 a	
Means (Irrigation)	24.10 b		47.37 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.03 H×I = 0.04 T = 0.05 H×I×T = 0.11

Catalase (U /mg protiens)

Irrigation regimes, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for antioxidant enzyme catalase (CAT). All the interactions were significant. There was more synthesis of CAT under drought stress as compared to well watered conditions. Seed treatment with TU increased the synthesis of CAT under drought stress. Maximum CAT (65.63U/mg proteins) was synthesized in hybrid 34N43 under 40 % WHC with 800 mg/l TU seed treatment and with dry seed sowing CAT was 39.71U/mg proteins. In hybrid 32F10 under 40 % WHC with dry seed sowing, CAT was 35.15U/mg proteins and with 800 mg/l TU seed treatment CAT was 58.54U/mg proteins under same field capacity.

Discussion:

A) Emergence parameters

Drought stress decreased the germination performance of maize under drought stress. However, seed treatment with thiourea (TU) improved the germination indicating that TU has successfully induced drought tolerance. Moreover, seed priming with TU improved the performance both under normal and stress conditions. Seed treatment with 800 mg/l TU proved to be most suitable treatment that significantly improved the emergence. Seed treatment with 800 mg/l TU decreased time taken for 50 % emergence (E_{50}), increased emergence energy (EE), improved coefficient of uniformity of emergence (CUE), increased emergence index (EI), reduced the mean emergence time (MET) and effectively increased the final emergence percentage under water stress as well as well watered conditions as compared to dry seed sowing (Tables 4.16-21). Hybrid 34N43 being tolerant increased the performance in all emergence parameters with TU seed treatment as compared with hybrid 32F10, a sensitive hybrid under drought stress as well as under normal availability of water.

The improved germination is result of many profound physiological and biochemical changes taking place in seed before it is sown in the soil that were enhanced by seed treatment with TU. Seed treatment enhances the mobilization of reserves from the storage part of the seed (e.g., cotyledons and endosperms) for partitioning to embryo. The activation of enzymes particularly of sugar hydrolysis *i.e.*, α - and β - amylase is the first to be activated for the conversion of complex sugars to simple ones for mobilization to embryo (Farooq *et al.*, 2009; Taiz and Zeiger, 2010). The embryo following activation is set for rapid cell division and emergence of embryonic tissues through rupture of seed coat. The seed priming and seed hardening are critical to these processes and primed seeds are on an advantage as compared to non-primed ones (Farooq *et al.*, 2006). Similarly, Farooq *et al.* (2008) have reported that application of glycinebetaine through seed treatment improved emergence energy, uniformity of emergence, emergence index, final emergence percentage and decreased time taken for 50 % emergence and mean emergence time under chilling stress in maize.

B) Growth Parameters:

Under normal availability of water i.e 80 % WHC, Thiourea (TU) seed treatment improved shoot length, root dry weight, shoot dry weight and leaf area as compared to dry seed sowing. However, increase in growth parameters was more pronounced in hybrid 34N43 (Tolerant hybrid) as compared to 32F10 (Sensitive hybrid) (Tables 4.23-4.26). Seed treatment under well watered conditions increases shoot length, leaf area and plant biomass of maize plants as compared to control (Basra *et al.*, 2011). Water stress i.e 40 % WHC decreased shoot length, root dry weight, shoot dry weight in both hybrids. However, decrease was less in hybrid 34N43 as compared to 32F10. Seed treatment with TU increased shoot length, root dry weight, shoot dry weight and leaf area under water stress and this increase was more significant in hybrid 34N43 as compared to hybrid 32F10. Seed priming is one of the most viable and pragmatic approaches to reduce the deleterious effects of drought stress especially on seed germination and early seedling growth, high vigor, uniform stand establishment (Harris *et al.*, 2004, 2007) and better yield especially in vegetable and field crops (Khalil *et al.*, 2001) . The embryo following activation is set for rapid cell division and emergence of embryonic tissues through rupture of seed coat. The seed priming and seed hardening are critical to these processes and primed seeds are on an advantage as compared to non primed ones (Farooq *et al.*, 2006). Better performance of maize plants raised after seed priming might be due to the maintenance of tissue water contents, increase in antioxidant activities and carbohydrate metabolism under water stress (Farooq *et al.*, 2008). Similarly, seed priming effects on promoting germination and plant growth under water deficit conditions have also been observed in wheat by Ajouri *et al.* (2004).

C) Water relations

Under 80 % WHC, maximum water potential, osmotic potential, turgor potential and relative water contents were recorded with 800 mg/l TU seed treatment in both the hybrids. Water stress decreased water potential, osmotic potential, turgor potential and relative water contents in two hybrids. TU seed treatment improved water relations under water stress. However, effect of TU seed treatment was more significant in hybrid 34N43 as compared to hybrid 32F10 (Tables 4.27-4.30). RWC were also decreased significantly, possibly because of

decreased metabolites and osmotica available to hold the water within the cells under water stress. Plant water potential, osmotic potential and turgor potential is reduced under water stress (Farhad *et al.*, 2011). Seed treatment and foliar application of thiourea improve growth, yield and WUE of pearl millet (*Pennisetum glaucum* L.) under arid and semi arid conditions (Parihar *et al.*, 1998).

D) Proline, MDA and membrane stability

Water stress stimulated the synthesis of proline in maize hybrids as compared to well watered conditions. Hybrid 34N43 being tolerant synthesized more proline. TU seed treatment increased the synthesis of proline under water stress. Effect was more in hybrid 34N43 but TU seed treatment had also good effect on hybrid 32F10 for proline synthesis (Table 4.31). For osmotic adjustment, accumulation of osmolytes under water limiting conditions is compulsory but it depends upon water status, crop growth stage and cultivar (Shao *et al.*, 2006). It has been widely reported that plants accumulate a variety of compatible solutes such as proline and betaine, as an adaptive mechanism of tolerance to salinity and drought (Hasegawa *et al.*, 2000; Ashraf and Harris, 2004; Ashraf and Foolad, 2007). Water stress increased the MDA content and electrolyte leakage under stress in maize hybrids. There was more electrolyte leakage and MDA content in hybrid 32F10 as compared to hybrid 34N43. Electrolyte leakage and increased MDA content indicate that membrane stability is disturbed. Peroxidation of lipids, commonly taken as an indicator of oxidative stress, disrupts the membrane integrity of the plant cell. This means that essential solutes leak out from the organelles and from the cell and cause the damage of membrane function and metabolic imbalances (Blokina *et al.*, 2003). Also Quan *et al.* (2004) found higher electrolyte leakage in drought stressed maize (*Zea mays* L.) plants than in plants grown under controlled conditions. However TU seed treatment decreased the electrolyte leakage and MDA content by stabilizing cell membrane. Thiourea (TU) treated plants performed better in terms of plant growth in spite of experiencing slightly more water deficit due to stabilization of lipoprotein structure and less malondialdehyde production compared to control (Mojtaba and Karr, 2001).

E) Antioxidant enzymes:

Activity of antioxidant enzymes i.e superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) significantly increased under water stress as compared to normal availability of water. Crucial changes in water homeostasis can lead to osmotic stress, which are primary effects of drought stress. Such free radicals and other active derivatives of oxygen may produce inevitably by-products of physiological redox reactions. It has been suggested that water stress caused elevated levels of active oxygen species (AOS) (Arora *et al.*, 2002) and the production of active oxygen exceeded the capacity of the scavenging systems, resulting in oxidative damage (Taiz & Zeiger, 2010). The increased levels of AOS can inactivate enzymes, damage important cellular components, which induced plant growth arrest, and even death finally (Arora *et al.*, 2002). To mitigate the deleterious effects of water stress on regular metabolism and ensure crops under optimal growth conditions, plants have evolved various strategies to contend with this problem. By necessity, plants possess a number of antioxidants such as superoxide dismutase (SOD: EC 1.15.1.1), peroxidase (POD: EC 1.11.1.7), and catalase (CAT: EC 1.11.1.6) in order to protect cellular membranes and organelles from the damaging effects of toxic concentrations of AOS and maintain their integrity and stability under drought-stressed conditions (Arora *et al.*, 2002). Ramaswamy *et al.* (2007) reported that seed soaking of pearl millet in TU solution increased the activities of superoxide dismutase, glutathione reductase and glutathione-S-transferase under water stress as compared to control. Seed treatment with TU accelerated the antioxidant system in wheat under water stress so that significantly increased activities of superoxide dismutase, catalase and ascorbate peroxidase was observed in TU treated plants as compared to control (Nathawat *et al.*, 2007).

Conclusion:

Seed treatment with TU improved emergence, growth, relative water contents, osmolytes accumulation, increased the synthesis of antioxidant enzymes in both the hybrids under water stress, thus indicating the significance of TU seed treatment but implications when TU is present in soil solution is yet to be explored. For this purpose, a solution culture experiment was designed to study the significance of TU applied through rooting medium.

**Experiment 3: Improving drought tolerance in maize (*Zea mays* L.)
by application of thiourea through rooting medium.**

Table 4.37: Effect of thiourea on root length (cm) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	5.69 e	5.94 e	9.88 e	10.34 e	13.66 e	13.95 e	17.16 e	17.52 e	11.77 e
T ₃ : 200 mg/l thiourea	6.36 d	6.81 d	10.59 d	11.05 d	14.43 d	14.74 d	17.77 d	18.10 d	12.48 d
T ₄ : 400 mg/l thiourea	7.46 c	7.76 c	11.46 c	11.74 c	15.07 c	15.53 c	18.39 c	18.73 c	13.27 c
T ₅ : 600 mg/l thiourea	8.22 b	8.55 b	12.11 b	12.54 b	15.87 b	16.2 b	19.05 b	19.65 b	14.03 b
T ₆ : 800 mg/l thiourea	8.94 a	9.63 a	12.85a	13.37 a	16.46 a	16.82 a	19.97 a	20.30 a	14.79 a
Means (H×I)	7.39 h	7.74 g	11.38f	11.81 e	15.10 d	15.46 c	18.47 b	18.86 a	
Means (Irrigation)	7.33 e		11.59 d		15.28 b		18.66 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Stress = 0.007, H×S = 0.01, T = 0.008, H×S×T = 0.02

Table 4.38: Effect of thiourea on shoot length (cm) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	18.95 e	19.36 e	15.16 e	15.46 e	11.82 e	12.14 e	8.17 e	8.53 e	10.00 e
T ₃ : 200 mg/l thiourea	19.73 d	20.04 d	15.78 d	16.33 d	12.41 d	12.76 d	8.87 d	9.14 d	12.23 d
T ₄ : 400 mg/l thiourea	20.29 a	20.52 a	16.67 c	16.97 c	13.04 c	13.52 c	9.64 c	10.06 c	16.00 c
T ₅ : 600 mg/l thiourea	20.84 b	21.11 b	17.44 b	17.86 b	13.86 b	14.22 b	10.50 b	10.77 b	18.32 b
T ₆ : 800 mg/l thiourea	21.45 a	21.91 a	18.27 a	18.58 a	14.57 a	14.94 a	10.97 a	11.34 a	18.95 a
Means (H×I)	17.11 a	16.84 b	16.76 c	16.50 d	15.12 e	14.71 f	12.04 g	11.72 h	
Means (Irrigation)	16.93 a		16.67 b		14.91 c		11.88 d		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Stress = 0.007, H×S = 0.01, T = 0.008, H×S×T = 0.02

Root length (cm)

Osmotic stress levels, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for root length (RL). All the interactions were significant. Data for three-way interaction show that increasing osmotic stress increased RL (Table 4.38). TU applied through rooting medium increased RL in both the hybrids under osmotic stress and 800 mg/l gave best results in this regard. Hybrid 34N43 had more increase in RL as compared to hybrid 32F10 with application of TU under osmotic stress. Maximum RL (20.30cm) was recorded in hybrid 34N43 under -0.8MPa with 800 mg/l TU applied through rooting medium, and it was 19.97cm for hybrid 32F10 under same stress level and same TU treatment. Under osmotic stress -0.6MPa with 800 mg/l TU application RL in hybrid 34N43 was 16.82cm and in hybrid 32F10 RL was 16.46cm and RL was 13.66 cm, 13.95cm in hybrids 32F10 and 34N43 respectively where distilled water was used without TU as growth medium. Similarly, minimum RL (5.69cm) was recorded for hybrid 32F10 under control with distilled water and it was 5.94cm for hybrid 34N43 under same sowing treatment and stress level.

Shoot length (cm)

Osmotic stress levels, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for shoot length. All the interactions were significant. Means for three-way interaction show that osmotic stress decreased shoot length but availability of water increased the shoot length. TU applied through rooting medium, increased shoot length under drought stress as well as under well watered conditions (Table 4.39). Maximum shoot length (21.91cm) was recorded in hybrid 34N43 with 800 mg/l TU applied through rooting medium whereas it was 21.45cm in hybrid 32F10 under same TU treatment where there was no osmotic stress. Under -0.8MPa osmotic stress, with 800 mg/l TU, shoot length in hybrid 34N43 was 11.34cm and in hybrid 32F10 it was 10.97cm. However, without TU application, under same osmotic stress, shoot length in hybrid 32F10 was 8.17cm and in hybrid 34N43 shoot length was 8.53cm.

Table 4.39: Effect of thiourea on root dry weight (g) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	2.46	2.50	1.88	1.91	1.11	1.21	0.23	0.31	0.63 e
T ₃ : 200 mg/l thiourea	2.55	2.60	1.98	2.04	1.28	1.36	0.40	0.47	1.15 d
T ₄ : 400 mg/l thiourea	2.65	2.72	2.10	2.15	1.43	1.48	0.54	0.62	2.16 c
T ₅ : 600 mg/l thiourea	2.76	2.81	2.23	2.28	1.58	1.65	0.74	0.80	2.29 b
T ₆ : 800 mg/l thiourea	2.86	3.31	2.35	2.40	1.72	1.81	0.88	0.95	2.40 a
Means (H×I)	2.05	1.99	1.98	1.92	1.69	1.62	1.48	1.10	
Means (Irrigation)	2.02 a		1.95 b		1.65 c		1.29 d		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Stress = 0.32, H×S = NS, T = 0.35, H×S×T =NS

Table 4.40: Effect of thiourea on shoot dry weight (g) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T₁: control (Distilled water)	2.05 e	2.12 e	1.42 e	1.48 e	0.73 e	0.79 e	0.18 e	0.22 e	0.43 e
T₃: 200 mg/l thiourea	2.17 d	2.21 d	1.56 d	1.63 d	0.86 d	0.92 d	0.27 d	0.32 d	1.85 d
T₄: 400 mg/l thiourea	2.29 c	2.35 d	1.68 c	1.75 c	1.02 c	1.06 c	0.39 c	0.45 c	1.54 c
T₅: 600 mg/l thiourea	2.38 b	2.44 b	1.81 b	1.88 b	1.14 b	1.21 b	0.49 b	0.52 b	1.94 b
T₆: 800 mg/l thiourea	2.49 a	2.56 a	1.92 a	1.96 a	1.28 a	1.33 a	0.58 a	0.67 a	2.06 a
Means (H×I)	1.72 a	1.65 b	1.65 b	1.60 c	1.36 d	1.31 e	0.78 f	0.83 g	
Means (Irrigation)	1.69 a		1.63 b		1.34 c		0.80 d		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Stress = 0.003, H×S = 0.0004, T = 0.003, H×S×T = 0.009

Root dry weight (g)

Osmotic stress levels and thiourea levels differed significantly ($P \leq 0.05$) for root dry weight (RDW). All the interactions were non significant (Table 4.40).

Shoot dry weight (g)

Osmotic stress levels, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for shoot dry weight (SDW). All the interactions were significant. Osmotic stress decreased shoot dry weight. However, TU applied through rooting medium increased SDW in moisture levels as compared to distilled water (Table 4.41). Minimum SDW (0.18g) was recorded in hybrid 32F10 with distilled water under -0.8MPa osmotic stress that was increased to 0.58g with 800 mg/l TU applied through rooting medium under same stress level. Similarly in hybrid 34N43, SDW was 0.22g with distilled water under -0.8MPa osmotic stress that increased to 0.83g with 800 mg/l TU applied through rooting medium under same osmotic stress. Under control i.e normal availability of water, SDW for hybrid 34N43 was 2.56g with 800 mg/l TU applied through rooting medium which was decreased to 2.12g with distilled water whereas for hybrid 32F10 RDW was 2.49g with 800 mg/l TU applied through rooting medium which was decreased to 2.05g with distilled water under same moisture percentage.

Table 4.41: Effect of thiourea on leaf area (cm²) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	521.22 e	529.11 e	390.91 e	400.25 e	244.12 e	256.72 e	80.75 e	95.51 e	157.30 e
T ₃ : 200 mg/l thiourea	539.14 d	551.87 d	415.77 d	432.07 d	273.57 d	292.94 d	117.46 d	128.84 d	265.04 d
T ₄ : 400 mg/l thiourea	559.44 c	566.10 c	447.69 c	463.89 c	307.12 c	318.46 c	140.34 c	159.07 c	413.82 c
T ₅ : 600 mg/l thiourea	576.92 b	586.01 b	475.73 b	483.52 b	333.86 b	348.06 b	173.20 b	185.72 b	494.40 b
T ₆ : 800 mg/l thiourea	596.73 a	612.59 a	495.44 a	512.85 a	363.77 a	378.24 a	198.11 a	225.67 a	516.79 a
Means (H×I)	447.24 a	430.66 b	430.25 b	420.02 c	376.09 d	360.17 e	251.91 f	239.40 g	
Means (Irrigation)	438.95 a		425.14 b		368.13 c		345.66 d		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD_{0.05} for, Stress = 0.62, H×S = 0.88, T = 0.70, H×S×T = 1.98

Table 4.42: Effect of thiourea on water potential (-MPa) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	0.53 a	0.52 a	0.66 a	0.65 a	0.82 a	1.01 a	1.00 a	0.92 a	0.92 a
T ₃ : 200 mg/l thiourea	0.51 b	0.49 b	0.63 b	0.62 b	0.77 b	0.76 b	0.99 b	0.98 b	0.79 b
T ₄ : 400 mg/l thiourea	0.47 c	0.45 c	0.61 c	0.60 c	0.74 c	0.71 c	0.95 c	0.93 c	0.64 c
T ₅ : 600 mg/l thiourea	0.43 d	0.41 d	0.58 d	0.57 d	0.70 d	0.69 d	0.91 d	0.89 d	0.54 d
T ₆ : 800 mg/l thiourea	0.40 e	0.36 e	0.56 e	0.55 e	0.68 e	0.67 e	0.87 e	0.84 e	0.51 e
Means (H×I)	0.58 h	0.60 g	0.61 f	0.63 e	0.69 d	0.70 c	0.82 b	0.83 a	
Means (Irrigation)	0.59 d		0.62 c		0.69 b		0.82 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Stress = 0.0007, H×S = 0.001, T = 0.0008, H×S×T = 0.002

Leaf area (cm⁻²)

Osmotic stress levels, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for leaf area (LA). All the interactions were significant. Maximum LA (612.59cm²) was recorded in hybrid 34N43 with 800 mg/l TU applied through rooting medium under control i.e normal availability of water and it was reduced to (529.11cm²) without TU application under same availability of water (Table 4.42). In hybrid 32F10, LA with 800 mg/l TU application under control was 596.73cm² and it was reduced to (521.22cm²) without TU application under same water availability. Under -0.8MPa osmotic stress with 800 mg/l TU applied through rooting medium LA was 225.67 cm² whereas it was reduced to 95.51 cm² with distilled water under same osmotic stress. Whereas for hybrid 32F10 under -0.8MPa osmotic stress with 800 mg/l TU applied through rooting medium LA was 198.11 cm² and it was decreased to 80.75 cm² without TU application under same osmotic stress.

Water potential (-MPa)

Osmotic stress levels, thiourea levels, and hybrids differed significantly ($P \leq 0.05$) for water potential (WP). All the interactions were significant. Means for three way interaction show that drought stress decreased the WP while TU applied through rooting medium improved it as compared to distilled water and 800 mg/l TU proved to be best treatment in this regard (Table 4.43). Maximum WP (-0.36MPa) was recorded in hybrid 34N43 with 800 mg/l TU applied through rooting medium under control and it was reduced to -0.52MPa with distilled water under same moisture percentage. Under -0.8MPa osmotic stress, with 800 mg/l TU applied through rooting medium WP was -0.84MPa and it was reduced to -0.92MPa with distilled water for same hybrid. Similarly, WP for hybrid 32F10 was -0.40MPa with 800 mg/l TU applied through rooting medium under control and it was reduced to -0.53MPa with distilled water. Under -0.8MPa osmotic stress, WP was -0.87MPa with 800 mg/l TU application and it was decreased to -1.00MPa with distilled water for same hybrid.

Table 4.43: Effect of thiourea on osmotic potential (-MPa) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43 3	32F10	34N43	
T ₁ : control (Distilled water)	0.90 a	0.86 a	1.00 a	0.99 a	1.85 a	1.69 a	2.56 a	2.51 a	2.24 a
T ₃ : 200 mg/l thiourea	0.85 b	0.85 b	0.99 b	0.97 b	1.57 b	1.45b	2.46 b	2.39 b	1.61 b
T ₄ : 400 mg/l thiourea	0.84 c	0.83 c	0.96 c	0.96 c	1.34 c	1.27 c	2.32 c	2.25 c	1.15 c
T ₅ : 600 mg/l thiourea	0.82 d	0.81 d	0.94 d	0.93 d	1.21 d	1.18 d	2.18 d	2.11 d	0.92 d
T ₆ : 800 mg/l thiourea	0.81 e	0.78 e	0.93 e	0.92 e	1.11 e	1.06 e	2.03 e	1.96 e	0.88 e
Means (H×I)	1.13 h	1.17 g	1.19 f	1.24 e	1.30 d	1.34 c	1.73 b	1.79 a	
Means (Irrigation)	1.15 d		1.21 c		1.32 b		1.76 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Stress = 0.003, H×S = 0.003, T = 0.003, H×S×T = 0.008

Table 4.44: Effect of thiourea on turgor potential (MPa) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	1.11 e	1.16 e	0.42 e	0.45 e	0.35 d	0.36 d	0.33 c	0.33 c	0.34 e
T ₃ : 200 mg/l thiourea	1.22 d	1.26 d	0.48 d	0.51 d	0.36 d	0.36 d	0.33 c	0.34 b	0.37 d
T ₄ : 400 mg/l thiourea	1.31 c	1.37 c	0.55 c	0.59 c	0.37 c	0.38 c	0.34 b	0.34 b	0.64 c
T ₅ : 600 mg/l thiourea	1.41 b	1.46 b	0.69 b	0.80 b	0.38 b	0.40 b	0.34 b	0.34 b	0.96 b
T ₆ : 800 mg/l thiourea	1.51 a	1.55 a	0.90 a	1.02 a	0.40 a	0.42 a	0.35 a	0.35 a	1.07 a
Means (H×I)	0.90 a	0.87 b	0.87 b	0.84 c	0.61 d	0.55 e	0.38 f	0.37 e	
Means (Irrigation)	0.89 a		0.86 b		0.58 c		0.37 d		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Stress = 0.002, H×S = 0.004, T = 0.003, H×S×T = 0.009

Osmotic potential (-MPa)

Osmotic stress levels, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for osmotic potential (OP). All the interactions were significant. Osmotic stress decreased the OP in both hybrids. Means for three way interaction show that under -0.8MPa osmotic stress, OP in hybrid 34N43 was -1.96MPa with 800 mg/l TU applied through rooting medium and in hybrid 32F10 it was (-2.03MPa) with same TU treatment and stress level (Table 4.44). However, with distilled water under -0.8MPa osmotic stress, OP in hybrid 34N43 was -2.51MPa and in hybrid 32F10 it was -2.56MPa. Similarly, under control i.e with normal availability of water, in hybrid 34N43 maximum OP (-0.78MPa) was recorded with 800 mg/l TU applied through rooting medium which was decreased to -0.86MPa without TU application. For hybrid 32F10, under control and with 800 mg/l TU applied through rooting medium OP was -0.81MPa and it was reduced to -0.90MPa with distilled water under same moisture level.

Turgor potential (MPa)

Osmotic stress levels, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for turgor potential. All the interactions were significant. More the availability of water more was TP as revealed from data of three-way interaction (Table 4.45). However, TU applied through rooting medium improved TP in well watered conditions as well as under water stress. Maximum TP (1.55MPa) was recorded in hybrid 34N43 with 800 mg/l TU seed treatment under under no stress and it was 0.35MPa with same seed treatment under -0.8MPa osmotic stress. Whereas in hybrid 32F10, TP was 1.51MPa under control with 800 mg/l TU seed treatment and under -0.8MPa osmotic stress, TP was 0.35MPa for same seed treatment.

Table 4.45: Effect of thiourea on relative water contents (%) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	85.76 e	86.41 e	75.45 e	76.52 e	65.93 e	66.84 e	57.16 e	57.76 e	60.86 e
T ₃ : 200 mg/l thiourea	87.21 d	87.80 d	77.45 d	78.19 d	67.60 d	68.72 e	58.94 d	59.27 d	67.27 d
T ₄ : 400 mg/l thiourea	88.83 c	89.21 c	78.62 c	79.35 c	69.85 c	70.29 c	59.86 c	60.55 c	77.32 c
T ₅ : 600 mg/l thiourea	90.46 b	91.35 b	80.63 b	82.59 b	71.13 b	72.32 b	61.72 b	62.17 b	83.58 b
T ₆ : 800 mg/l thiourea	92.05 a	93.43 a	83.68 a	84.22 a	73.73 a	74.85 a	63.53 a	64.17 a	85.42 a
Means (H×I)	80.13 a	79.18 b	79.16 b	78.67 c	74.85 d	73.84 e	67.05 f	66.24 g	
Means (Irrigation)	79.64 a		78.92 b		74.35 c		66.64 d		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD \leq 0.05 for, Stress = 0.03, H×S = 0.04, T = 0.03, H×S×T = 0.02

Table 4.46: Effect of thiourea on proline contents (umol/g FW) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	4.16 e	4.99 e	11.26 e	11.95 e	18.33 e	18.78 e	25.36 e	25.96 e	7.93 e
T ₃ : 200 mg/l thiourea	5.81 d	6.54 d	12.54 d	13.10 d	19.45 d	20.15 d	26.64 d	27.16 d	12.19 d
T ₄ : 400 mg/l thiourea	7.16 c	7.88 c	13.83 c	14.41 c	21.09 c	21.95 c	27.74 c	28.57 c	19.65 c
T ₅ : 600 mg/l thiourea	8.65 b	9.45 b	15.23 b	16.09 b	22.53 b	23.28 b	29.45 b	30.35 b	24.25 b
T ₆ : 800 mg/l thiourea	10.36 a	10.77 a	16.89 a	17.74 a	23.83 a	24.48 a	31.07 a	31.92 a	25.59 a
Means (H×I)	11.29 h	12.00 g	17.10 f	17.78 e	20.44 d	21.11 c	21.14 b	22.22 a	
Means (Irrigation)	11.64 d		17.44 c		20.78 b		21.83 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Stress = 0.01, H×S = 0.01, T = 0.01, H×S×T = 0.04

Relative water contents (%)

Osmotic stress levels, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for relative water contents (RWC). All the interactions were significant. Osmotic stress decreased the RWC in both hybrids. Means for three way interaction show that under -0.8MPa osmotic stress, RWC in hybrid 34N43 were 64.17% with 800 mg/l TU applied through rooting medium and in hybrid 32F10 these were (63.53%) with same TU treatment and stress level (Table 4.46). But with distilled water under -0.8MPa osmotic stress, RWC in hybrid 34N43 were (57.76%) and in hybrid 32F10 these were 57.16%. Similarly, under control i.e with normal availability of water, in hybrid 34N43 maximum RWC were 93.43% was recorded with 800 mg/l TU applied through rooting medium which were decreased to 86.41% without TU application. For hybrid 32F10, under control and with 800 mg/l TU applied through rooting medium RWC were 92.05% and it was reduced to 85.76% with distilled water under same moisture level.

Proline contents (umol/g FW)

Osmotic stress levels, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for proline. All the interactions were significant. There was less synthesis of proline under normal availability of water and increased under osmotic stress as revealed from three way interaction data (Table 4.47). Hybrid 34N43 increased synthesis of proline as compared to 32F10 with 800 mg/l TU applied through rooting medium as well as with distilled water under osmotic stress. Maximum proline contents (31.92umol/g FW) were recorded in hybrid 34N43 under -0.8MPa osmotic stress with 800 mg/l TU whereas in hybrid 32F10, proline contents were 31.07umol/g FW with TU application and stress level. With distilled water under -0.8MPa osmotic stress, proline contents in hybrid 32F10 were 25.36umol/g FW and in hybrid 34N43 proline contents were 25.96umol/g FW.

Table 4.47: Effect of thiourea on MDA contents (umol/g FW) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	7.34 a	6.86 a	12.33 a	11.97 a	18.25 a	17.77 a	24.45 a	23.92 a	21.34 a
T ₃ : 200 mg/l thiourea	6.62 b	6.14 b	11.65 b	10.92 b	17.08 b	16.64 b	23.36 b	22.74 b	17.60 b
T ₄ : 400 mg/l thiourea	5.45 c	4.94 c	10.56 c	10.08 c	16.20 c	15.49 c	22.15 c	21.28 c	11.68 c
T ₅ : 600 mg/l thiourea	4.55 d	4.14 d	9.43 d	8.83 d	14.88 d	14.38 d	20.54 d	20.15 d	8.33 d
T ₆ : 800 mg/l thiourea	3.84 e	3.42 e	8.24 e	7.75 e	13.84 e	13.12 e	19.36 e	18.85 e	7.24 e
Means (H×I)	10.17 h	10.64 g	10.71 f	11.23 e	13.08 d	13.71 c	17.90 b	18.44 a	
Means (Irrigation)	10.40 d		10.97 c		13.40 b		18.17 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD_{0.05} for, Stress = 0.01, H×S = 0.01, T = 0.01, H×S×T = 0.03

Table 4.48: Effect of thiourea on electrolyte leakage (%) of maize hybrids applied through rooting medium

	Stress levels (S)								Means of Thiourea levels (T)
	S ₁ (control)		S ₂ (-0.4MPa)		S ₃ (-0.6MPa)		S ₄ (-0.8MPa)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	32F10	34N43	32F10	34N43	
T ₁ : control (Distilled water)	13.67 a	12.75 a	19.83 a	19.36 a	25.92 a	25.33 a	31.46 a	30.90 a	28.76 a
T ₃ : 200 mg/l thiourea	12.25 b	11.85 b	19.12 b	18.44 b	24.86 b	24.15 b	30.53 b	29.95 b	25.12 b
T ₄ : 400 mg/l thiourea	11.34 c	10.94 c	17.56 c	16.73 c	23.78 c	23.24 c	29.30 c	28.84 c	18.69 c
T ₅ : 600 mg/l thiourea	10.46 d	10.05 d	16.18 d	15.38 d	22.53 d	21.95 d	28.41 d	27.65 d	14.75 d
T ₆ : 800 mg/l thiourea	9.61 e	9.14 e	14.95 e	14.42 e	21.14 e	20.65 e	27.08 e	26.45 e	13.70 e
Means (H×I)	16.86 h	17.29 g	17.45 f	17.89 e	20.25 d	20.88 c	25.24 b	25.78 a	
Means (Irrigation)	17.16 d		17.59 c		20.56 b		25.51 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD_{0.05} for, Stress = 0.009, H×S = 0.01, T = 0.01, H×S×T = 0.03

MDA contents (umol/g FW)

Osmotic stress levels, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for malondialdehyde (MDA). All the interactions were significant. Osmotic stress increased the MDA production in both the hybrids (Table 4.48). However, MDA production was more in hybrid 32F10 as compared to hybrid 34N43. Similarly, MDA production was more with distilled water as compared to TU applied through rooting medium. Maximum MDA (24.45umol/g FW) was recorded in hybrid 32F10 under -0.8MPa with distilled water and in hybrid 34N43 MDA was 23.92umol/g FW. With 800 mg/l TU, MDA in hybrid 32F10 was 19.36umol/g FW and in hybrid 34N43 was 18.85umol/g FW under -0.8MPa osmotic stress.

Electrolyte leakage (%)

Osmotic stress levels, thiourea levels and hybrids differed significantly ($P \leq 0.05$) for electrolyte leakage (EL). All the interactions were significant. The greater the osmotic stress, the greater was electrolyte leakage as revealed from data of three way interaction (Table 4.49). Maximum EL (31.46%) was recorded in hybrid 32F10 with distilled water under -0.8MPa osmotic stress and in hybrid 34N43 it was 30.90% with same treatment and stress level. With 800 mg/l TU applied through rooting medium under -0.8MPa osmotic stress, EL in hybrid 32F10 was 27.08% and in hybrid 34N43 it was 26.45%. However under normal availability of water and with 800 mg/l TU applied through rooting medium EL in hybrid 34N43 was 9.14% and in hybrid 32F10 was 9.61%.

Discussion

A) Growth Parameters

Osmotic stress due to poly ethylene glycol 8000 (PEG) retarded the growth of both maize hybrids 34N43 (Tolerant) and 32F10 (Sensitive). However, growth of hybrid 32F10 was affected more as compared to hybrid 34N43 (Table 4.39-4.42). PEG induced osmotic stress decreased the plant height, plant biomass and leaf area significantly as compared to control i.e normal availability of water. Polyethylene glycol (PEG), a drought-inducing chemical, used frequently to screen out drought tolerant varieties at early stage of seedlings under laboratory conditions. Previous studies revealed that PEG can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water deficit in a relatively controlled manner (Zhu *et al.*, 1997). PEG molecules are inert, non-ionic and virtually impermeable chains that have frequently been used to induce water stress without causing physiological damage and maintain uniform water potential throughout experiment periods (Lu and Neumann, 1998). Molecules of PEG are too small to influence the osmotic potential but large enough not to be absorbed by plant and even not expected to penetrate intact plant tissues rapidly (Carpita *et al.*, 1979). Water is withdrawn from the cell because PEG does not enter into the apoplast therefore PEG solution mimics dry soil more closely (Veslues *et al.*, 1998). Reduction in shoot weight and root weight when treated with PEG solution was attributed to water stress conditions where plants used limited availability of food energy supplied by the seed in an effective way for their establishment and to start photosynthesis to provide energy for growth and development (Rauf *et al.*, 2007). Drought stress is one of the most important abiotic stresses and seriously affects water relations and productivity of a crop (Li Xin *et al.*, 2011). However, thiourea applied through rooting medium improved growth of maize hybrids under osmotic stress. Significantly high plant biomass and leaf area was recorded with TU applied through rooting medium as compared to distilled water. Thiourea has also been reported to suppress the speed of chlorophyll decay (Liu *et al.*, 2002). Sahu *et al.* (1993) reported that foliar spray of thiourea (TU) significantly increased growth and yield of maize, most probably via improvement in canopy photosynthesis under semiarid conditions.

B) Water relations

Osmotic stress significantly decreased water potential, osmotic potential, turgor potential of both maize hybrids. However, water relations were disturbed more in hybrid 32F10 as compared to hybrid 34N43 (Table 4.43-4.45). Medici *et al.* (2003) found that maize hybrid P 6875 showed water potential of -0.78 MPa under control condition and water potential of this hybrid decreased up to -0.96 MPa under water stress condition. Claudio *et al.* (2006) found that osmotic potential of leaves of well watered maize plants increased up to -0.90 MPa 40 DAS, while under water stress it decreased up to -1.20 MPa. Claudio *et al.* (2006) also observed that the leaf turgor potential decreased from 0.54 MPa to 0.180 MPa with the increase of water stress. Relative water contents also significantly reduced under osmotic stress as compared to normal availability of water. One of the early symptoms of water deficiency in plant tissues is the decrease of relative water content (RWC). The reduction of RWC in stressed plants may be associated with the decrease in plant vigour and observed in many plant species (Halder and Burrage 2003; Lopez *et al.*, 2002). Thiourea applied through rooting medium increased water potential, osmotic potential, turgor potential and relative water contents under osmotic stress as compared to distilled water. Thiourea has been reported to significantly improve growth yield and water use efficiency of wheat under arid and semi-arid conditions. (Sahu and Singh, 1995). Cytokinins increase water potential, osmotic potential, turgor potential and relative water contents in maize under water stress (Ali *et al.*, 2011) and TU is known to exhibit cytokinins like activities (Erez, 1978; Vassilev and Mashev, 1974).

C) Proline, MDA and membrane stability

Osmotic stress induced the synthesis of proline in maize hybrids as compared to normal availability of water. However, there was more synthesis of proline in hybrid 34N43 compared to hybrid 32F10 (Table 4.47). Osmotic adjustment is a part of drought avoidance mechanisms. Proline and quaternary ammonium compounds are key osmolytes, which help plants to maintain the cell turgor (Huang *et al.*, 2000). Proline and soluble sugars are the key osmolytes

contributing towards osmotic adjustment (Mundree *et al.*, 2002). They can also improve stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions. Similarly osmotic stress increased MDA production in maize hybrids. However, there was more production of MDA in hybrid 32F10 as compared to hybrid 34N43 (Table 4.48). The higher lipid peroxidation in drought stressed plants was also reported in other studies (Fu and Huang, 2001; Niedzwiedz-Siegen *et al.*, 2004). Osmotic stress disrupted the membrane stability in maize hybrids and as a result, there was more electrolyte leakage (Table 4.49). Under environmental stresses, plant membranes are subjected to changes often associated with the increases in permeability and loss of integrity. Therefore, the ability of cell membranes to control the rate of ion movement in and out of cells is used as a test of damage to a great range of tissues (Blokhina *et al.*, 2003). Thiourea applied through rooting medium suppressed the MDA production and electrolyte leakage under osmotic stress as compared to distilled water i.e without TU application. Better performance of thiourea treated plants in terms of plant growth inspite of experiencing slightly more water deficit could be due to stabilization of lipoprotein structure and less malondialdehyde production compared to control (Mojtaba and Karr, 2001).

Conclusion

TU applied through rooting medium had a positive role particularly under water stress. When present in rooting medium, TU increased drought tolerance by membrane stability, proline synthesis and suppressing MDA synthesis, increasing plant biomass and leaf area. However, effect of TU foliar application is need of day, as foliar application of nutrients and hormones is way to fulfill the deficiencies urgently.

**Experiment 4: Improving drought tolerance in maize (*Zea mays* L.)
by foliar application of thiourea**

Table 4.49: Effect of thiourea foliar application on root length (cm) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	14.08 c	14.80 b	20.76 d	22.96 cd	18.02 d
T ₂ : 500 mg/l thiourea	17.70 ab	17.95 a	23.18 c	23.42 c	20.75 c
T ₃ : 1000 mg/l thiourea	18.93 a	19.36 a	25.49 b	25.55 b	21.94 b
T ₄ : 1500 mg/l thiourea	19.42 a	19.46 a	29.11 a	36.39 a	26.43 a
Means (H×I)	22.57 b	20.56 c	25.70 a	18.30 d	
Means (Irrigation)	19.43 b		24.13 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigations = 0.59, H×I = 0.84, T = 0.84, H x I x T= 1.69

Table 4.50: Effect of foliar application of thiourea on shoot length (cm) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	19.64 d	21.03 cd	14.80 c	15.93 c	16.31 d
T ₂ : 500 mg/l thiourea	21.59 bc	21.84 c	16.61 b	17.80 ab	19.80 c
T ₃ : 1000 mg/l thiourea	22.05 b	23.15 b	17.93 a	17.99 a	20.91 b
T ₄ : 1500 mg/l thiourea	24.75 a	29.60 a	19.19 a	19.24 a	23.75 a
Means (H×I)	21.25 b	17.90 d	23.13 a	18.50 c	
Means (Irrigation)	22.18 a		18.19 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$
LSD ≤ 0.05 for, Irrigation= 0.45, H×I = 0.63, T = 0.63, H x I x T= 1.27

Root length (cm)

Irrigation regimes and thiourea levels differed significantly ($P \leq 0.05$) for root length (RL). All the interactions were significant. Water stress caused increase in RL as revealed from data (Table 4.50). Means for three-way interaction show that maximum RL (36.39cm) was recorded in hybrid 34N43 under 40 % WHC with 1500 mg/l TU foliar application and it was 29.11cm for hybrid 32F10 under same moisture level and TU foliar application.

Shoot length (cm)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for shoot length (SL). All the interactions were significant. Water stress decreased SL in all hybrids. However reduction was decreased with TU foliar application. Maximum SL (29.60cm) was recorded for hybrid 34N43 under 80 % WHC with 1500 mg/l TU foliar application whereas in hybrid 32F10 SL was 24.75cm under same water holding capacity and TU foliar application. However, under 40 % WHC without TU foliar application, hybrid 32F10 had SL 14.80cm and hybrid 34N43 had SL of 15.93cm whereas with 1500 mg/l TU foliar application hybrid 32F10 had SL of 19.64cm and hybrid 34N43 had SL of 21.03cm under same moisture stress.

Table 4.51: Effect of thiourea foliar application on root dry weight (g) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	1.36 d	1.38 bc	0.44 d	0.45 c	0.74 d
T ₂ : 500 mg/l thiourea	1.39 c	1.42 b	0.92 c	1.15 b	1.31 c
T ₃ : 1000 mg/l thiourea	1.68 b	1.94 a	1.16 b	1.26 a	1.55 b
T ₄ : 1500 mg/l thiourea	1.99 a	2.00 a	1.27 a	1.30 a	1.69 a
Means (H×I)	1.50 b	1.06 c	1.63 a	1.10 c	
Means (Irrigation)	1.56 a		1.07 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD \leq 0.05 for, Irrigation= 0.02, H×I = 0.04, T = 0.04, H x I x T = 0.08

Table 4.52: Effect of thiourea foliar application on shoot dry weight (g) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	1.17 d	1.22 d	0.41 b	0.42 b	0.66 d
T ₂ : 500 mg/l Thiourea	1.50 bc	1.51 c	0.90 a	0.91 a	1.25 c
T ₃ : 1000 mg/l thiourea	1.61 b	2.21 b	0.93 a	1.03 a	1.48 b
T ₄ : 1500 mg/l thiourea	2.36 a	3.07 a	1.04 a	1.05 a	1.96 a
Means (H×I)	1.59 b	0.89 c	1.93 a	0.93 c	
Means (Irrigation)	1.75 a		0.91 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation= 0.06, H×I = 0.09, T = 0.09, H x I x T = 0.18

Root dry weight (g)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for root dry weight (RDW). All the interactions were significant. Maximum RDW (2.00g) was recorded in hybrid 34N43 with 1500 mg/l TU foliar application under 80 % WHC and it had RDW (0.91g) with control. Similarly hybrid 32F10 had RDW (1.69g) with 1500 TU foliar application and it had RDW of 0.85g with control under same water holding capacity. Under 40 % WHC, hybrid 32F10 had RDW of 0.44g and hybrid 34N43 had RDW of 0.45g with control but with 1500 mg/l TU foliar application hybrid 32F10 had RDW of 1.36g and hybrid 34N43 had RDW of 1.38g under same water stress.

Shoot dry weight (g)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for shoot dry weight (SDW). All the interactions were significant. Under 80 % WHC, hybrid 34N43 had SDW of 3.07g with 1500 mg/l TU foliar application whereas it had SDW of 0.91g with control. Under 40 % WHC with 1500 mg/l TU foliar application SDW was 1.22g and with control SDW was 0.42g for same hybrid. Hybrid 32F10 under 80 % WHC with 1500 mg/l TU foliar application had SDW of 2.36g and with control it had SDW of 0.90g under same moisture availability. However under 40 % WHC, SDW was 1.17g with 1500 mg/l TU foliar application and with control SDW was 0.41g for the same hybrid.

Table 4.53: Effect of thiourea foliar application on leaf area (cm²) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	479.82	496.82	562.50	533.94	518.27 d
T ₂ : 500 mg/l thiourea	707.76	719.90	474.95	506.50	602.28 c
T ₃ : 1000 mg/l thiourea	759.26	860.72	545.40	579.82	686.30 b
T ₄ : 1500 mg/l thiourea	901.92	938.27	638.58	642.66	780.36 a
Means (H×I)	702.19	480.36	741.43	510.48	
Means (Irrigation)	721.42 a		595.34 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD \leq 0.05 for, Irrigation= 20.92, H×I = NS, T = 29.60, H x I x T= NS

Table 4.54: Effect of Thiourea foliar application on water potential (-MPa) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	0.61 a	0.56 a	1.04 a	0.97 b	0.80 a
T ₂ : 500 mg/l thiourea	0.53 b	0.47 b	0.83 b	0.79 d	0.66 b
T ₃ : 1000 mg/l thiourea	0.43 c	0.42 c	0.75 c	0.71 f	0.58 c
T ₄ : 1500 mg/l thiourea	0.35 d	0.33 d	0.68 d	0.64 h	0.50 d
Means (H×I)	0.48 c	0.83 a	0.44 d	0.78 b	
Means (Irrigation)	0.46 b		0.80 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation= 0.0003, H×I = 0.0004, T = 0.0004, H x I x T= 0.0009

Leaf area (cm²)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for leaf area (LA). Interaction between irrigation and thiourea was significant while no other interaction was significant. Maximum LA (721.42cm²) was recorded under 80 % WHC and it was reduced to 595.34cm² under 40 % WHC. Among TU levels, more LA (780.36cm²) was recorded with 1500 mg/l TU foliar application whereas it was decreased to 518.27cm² without TU foliar application.

Water potential (-MPa)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for water potential (WP). All the interactions were significant. Maximum WP (-0.33MPa) was observed in hybrid 34N43 under 80 % WHC with 1500 mg/l TU foliar application and with control its WP was (-0.56MPa) under same water holding capacity. Hybrid 32F10 had WP (-0.35MPa) with 1500 mg/l TU foliar application and with control it had WP (-0.61MPa) under 80 % WHC. Under 40 % WHC, WP of hybrid 34N43 was -0.64MPa and of hybrid 32F10 WP was -0.68MPa with 1500 mg/l TU foliar application whereas with control hybrid 32F10 had WP of -1.04MPa and hybrid 34N43 had WP of -0.97MPa under same moisture status.

Table 4.55: Effect of thiourea foliar application on osmotic potential (-MPa) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	0.86 a	0.85 a	1.15 a	1.12 a	1.00 a
T ₂ : 500 mg/l thiourea	0.85 b	0.84 b	1.00 b	0.97 b	0.91 b
T ₃ : 1000 mg/l thiourea	0.84 c	0.81 c	0.95 c	0.93 c	0.88 c
T ₄ : 1500 mg/l thiourea	0.81 d	0.80 d	0.88 d	0.88 d	0.84 d
Means (H×I)	0.83 c	1.00 a	0.83 d	0.98 b	
Means (Irrigation)	0.83 b		0.98 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation= 0.0001, H×I = 0.0002, T = 0.0003, H x I x T= 0.0006

Table 4.56: Effect of thiourea foliar application on turgor potential (MPa) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	0.25 d	0.30 d	0.11 d	0.14 d	0.20 d
T ₂ : 500 mg/l thiourea	0.32 c	0.37 c	0.17 c	0.18 c	0.26 c
T ₃ : 1000 mg/l thiourea	0.41 b	0.38 b	0.19 b	0.23 b	0.31 b
T ₄ : 1500 mg/l thiourea	0.46 a	0.47 a	0.20 a	0.24 a	0.34 a
Means (H×I)	0.36 b	0.17 d	0.38 a	0.20 c	
Means (Irrigation)	0.37 a		0.18 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation= 0.0003, H×I = 0.0005, T = 0.0005, H x I x T= 0.001

Osmotic potential (-MPa)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for osmotic potential (OP). All the interactions were significant. Water stress decreased OP of both the hybrids and TU foliar application increased OP. Under 40 % WHC and with 1500 mg/l TU foliar application, OP of hybrid 34N43 was -0.88MPa and with control its OP was -1.12MPa. Similarly, OP of hybrid 32F10 with 1500 mg/l TU foliar application was -0.88MPa and with control its OP was -1.15MPa under 40 % WHC. Under well watered conditions with 1500 mg/l TU foliar application, OP of hybrid 32F10 was -0.81MPa and OP of hybrid 34N43 was -0.80MPa but with control hybrid 34N43 had OP of -0.85MPa and hybrid 32F10 had OP of -0.86MPa under 80 % WHC.

Turgor potential (MPa)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for turgor potential (TP). All the interactions were significant. Maximum TP (0.47MPa) was recorded in hybrid 34N43 under 80 % WHC with 1500 mg/l TU foliar application and with control its TP was 0.30MPa under same water holding capacity. TP of hybrid 32F10 under 80 % WHC with 1500 mg/l TU foliar application was 0.46MPa and with control its TP was 0.25MPa with same moisture level. Under 40 % WHC with 1500 mg/l TU foliar application, TP of hybrid 32F10 was 0.20MPa and TP of hybrid 34N43 was 0.24MPa whereas with control TP of hybrid 32F10 was 0.11MPa and TP of hybrid 34N43 was 0.14MPa under same water level.

Table 4.57: Effect of thiourea foliar application on relative water contents (%) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	81.95	85.63	61.65	66.43	73.91 d
T ₂ : 500 mg/l thiourea	82.33	88.30	62.10	69.00	75.43 c
T ₃ : 1000 mg/l thiourea	85.00	89.53	65.35	70.35	77.56 b
T ₄ : 1500 mg/l thiourea	86.10	89.98	68.53	73.23	79.46 a
Means (H×I)	83.84 b	64.41 d	88.36 a	69.75 c	
Means (Irrigation)	86.10 a		67.06 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD \leq 0.05 for, Irrigation= 0.97, H×I = 1.37, T = 1.37, H x I x T= NS

Table 4.58: Effect of thiourea foliar application on proline contents (umol/g FW) of maize hybrids

	Irrigation levels (L)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	1.76 d	2.62 d	8.66 d	9.36 d	5.60 d
T ₂ : 500 mg/l thiourea	3.86 c	4.46 c	10.52 c	11.78 c	7.66 c
T ₃ : 1000 mg/l thiourea	5.18 b	6.26 b	12.43 b	13.95 b	9.45 b
T ₄ : 1500 mg/l thiourea	6.92 a	7.79 a	14.54 a	15.22 a	11.12 a
Means (H×I)	4.43 d	5.28 c	11.54 b	12.58 a	
Means (Irrigation)	4.85 a		12.06 b		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.02, H×I = 0.03, T = 0.03, H x I x T= 0.06

Relative water contents (%)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for relative water contents (RWC). Three way interaction was non significant but interaction between hybrids and irrigation was significant (Table 4.57). At 80 % WHC, maximum RWC (86.10%) were recorded and under 40 % WHC, RWC were reduced to 67.06%. Among TU foliar applications, maximum RWC 79.46% were observed with 1500 mg/l and with control RWC were 73.91%.

Proline contents (umol/g FW)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for proline contents. Water stress increased the proline synthesis in both the hybrids and foliar application of thiourea improved it additionally. All the interactions were significant (Table 4.59). Under water stress, maximum proline contents (15.22umol/g FW) were recorded in hybrid 34N43 with 1500 mg/l TU foliar application and with control its proline contents were 9.36umol/g FW. Similarly, proline contents of hybrid 32F10 were 14.54umol/g FW with 1500 mg/l TU foliar application and with control its proline contents were 8.66umol/g FW under same water stress.

Table 4.59: Effect of Thiourea foliar application on MDA contents (umol/g FW) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	6.09 a	5.50 a	12.03 a	11.14 a	7.01 a
T ₂ : 500 mg/l thiourea	4.82 b	4.27 b	10.25 b	9.13 b	6.67 b
T ₃ : 1000 mg/l thiourea	3.93 c	3.36 c	8.59 c	7.95 c	6.41 c
T ₄ : 1500 mg/l thiourea	2.75 d	2.14 d	7.31 d	6.63 d	6.38 d
Means (H×I)	4.40 c	3.82 d	9.54 a	8.71 b	
Means (Irrigation)	4.11 b		9.13 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.01, H×I = 0.02, T = 0.02, H x I x T = 0.04

Table 4.60: Effect of thiourea foliar application on electrolyte leakage (%) of maize hybrids

	Irrigation levels (I)				Means of Thiourea levels (T)
	I ₂ (80%WHC)		I ₂ (40%WHC)		
Hybrids (H) Thiourea levels (T)	32F10	34N43	32F10	34N43	
T ₁ : control	17.91 a	16.12 a	38.63 a	35.24 a	26.98 a
T ₂ : 500 mg/l thiourea	14.72 b	13.24 b	33.16 b	31.44 b	23.14 b
T ₃ : 1000 mg/l thiourea	12.85 c	12.45 c	28.97 c	25.66 c	19.98 c
T ₄ : 1500 mg/l thiourea	11.64 d	11.36 d	21.29 d	19.54 d	15.96 d
Means (H×I)	14.28 c	13.29 d	30.51 a	27.97 b	
Means (Irrigation)	13.79 b		29.24 a		

Means not sharing the same letter within a column differ significantly at $P \leq 0.05$

LSD ≤ 0.05 for, Irrigation = 0.01, H×I = 0.02, T = 0.02, H x I x T = 0.04

MDA contents (umol/g FW)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for malondialdehyde contents (MDA). Water stress increased MDA contents in both the hybrids, however extent of increase was different for both the hybrids and TU foliar application decreased MDA contents. All the interactions were significant (Table 4.60). Maximum MDA contents (12.03 umol/gFW) were observed in hybrid 32F10 with control at 40 % WHC whereas with 1500 mg/l TU foliar application, its MDA contents were decreased to 7.31umol/gFW under same water holding capacity. In hybrid 34N43, MDA contents were 6.63umol/g FW with 1500 mg/l TU foliar application and with control, its MDA contents were 11.14umol/g FW under 40 % WHC.

Electrolyte leakage (%)

Irrigation regimes, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for electrolyte leakage (EL). All the TU treatments decreased the electrolyte leakage. However, 1500 mg/l TU foliar application was best treatment in this regard. Hybrid 34N43 improved membrane stability as compared to hybrid 32F10. Under 40 % WHC with control, EL was 35.24% in hybrid 34N43 and in hybrid 32F10 EL was 38.63% whereas with 1500 mg/l TU foliar application EL in hybrid 32F10 was 21.29% and in hybrid 34N43 EL was 19.54% under same moisture level.

Discussion

A) Growth Parameters:

Under normal availability of water i.e 80 % WHC, Thiourea (TU) foliar application improved shoot length, root dry weight, shoot dry weight and leaf area as compared to dry seed sowing. However, increase in growth parameters was more pronounced in hybrid 34N43 (Tolerant hybrid) as compared to 32F10 (Sensitive hybrid) (Tables 4.51-4.54). Water stress i.e 40 % WHC decreased shoot length, root dry weight, shoot dry weight and leaf area in both hybrids. However, decrease was less in hybrid 34N43 as compared to 32F10. Drought causes numerous physiological and biochemical changes in plants like reduced leaf size, stem extension, reduced water use efficiency (Farooq *et al.*, 2009). Foliar application of TU increased shoot length, root dry weight, shoot dry weight and leaf area under water stress and this increase was more significant in hybrid 34N43 as compared to hybrid 32F10. Cytokinins have the strongest effect in retardation of the leaf senescence either applied exogenously or produced endogenously; delay the leaf senescence by scavenging the free radicals involved in the process of senescence and increase the photosynthetically active leaf area (Galuszka *et al.*, 2001) and TU is known to exhibit cytokinin like activities (Erez, 1978; Vassilev and Mashev, 1974). Thiourea has also been reported to suppress the speed of chlorophyll decay (Liu *et al.*, 2002). Sahu *et al.* (1993) reported that foliar spray of thiourea (TU) significantly increased growth and yield of maize, most probably via improvement in canopy photosynthesis. However, root length was increased under water stress and this increase was more in hybrid 34N43 as compared to hybrid 32F10 and shoot length decreased. This is due to the adaptation of the maize plants to cope with drought stress. With the initial effects of drought stress, the maize plants started to divert the assimilates from stem and utilized them for increased root growth in order to increase the water absorption. Hence, the plant height was affected significantly, which is in accordance with the earlier findings (Hamada, 2001; Liu *et al.*, 2004). TU foliar application increased shoot length under water stress. Cytokinins increased plant height in maize under drought (Ali *et al.*, 2011) and TU is known to exhibit cytokinins like activities (Erez, 1978; Vassilev and Mashev, 1974).

B) Water relations

Under 80 % WHC, maximum water potential, osmotic potential, turgor potential and relative water contents were recorded with 1500 mg/l TU foliar application in both the hybrids. Water stress decreased water potential, osmotic potential, turgor potential and relative water contents in two hybrids. RWC were also decreased significantly, possibly because of decreased metabolites and osmotica available to hold the water within the cells. Plant water potential, osmotic potential and turgor potential is reduced under water stress (Farhad *et al.*, 2011). In another study, leaf relative water content (RWC), water potential (Ψ_w), osmotic potential (Ψ_s), turgor potential (P) and osmotic adjustment (OA) were significantly decreased under severe drought stress due to the excessive water loss (Machado and Paulsen, 2001). TU foliar application improved water relations under water stress. However, effect of TU foliar application was more significant in hybrid 34N43 as compared to hybrid 32F10 (Tables 4.55-4.58). Cytokinins increased water potential, osmotic potential, turgor potential and relative water contents in maize under water stress (Ali *et al.*, 2011) and TU is known to exhibit cytokinins like activities (Erez, 1978; Vassilev and Mashev 1974). Seed treatment and foliar application of thiourea improved growth, yield and WUE of pearl millet (*Pennisetum glaucum* L.) under arid and semi-arid conditions (Parihar *et al.*, 1998). Thiourea has been reported to significantly improve growth yield and water use efficiency of wheat under arid and semi-arid conditions (Sahu and Singh, 1995).

C) Proline, MDA and membrane stability

Water stress stimulated the synthesis of proline in maize hybrids as compared to well watered conditions. Hybrid 34N43 being tolerant synthesized more proline. TU foliar application increased the synthesis of proline under water stress. Effect was more in hybrid 34N43 but TU foliar had also good effect on hybrid 32F10 for proline synthesis (Table 4.59). Proline accumulation helps maintaining cell water status, sub-cellular structures and protecting membranes and proteins from denaturing effect of the osmotic stress (Ashraf and Foolad, 2007). Water stress increased the MDA content and electrolyte leakage under stress in maize hybrids (Table 4.60-4.61). There was more electrolyte leakage and MDA content in hybrid 32F10 as compared to hybrid 34N43. Electrolyte leakage and increased MDA

content indicate that membrane stability is disturbed. As cell membranes are the first targets of many plant stresses, ROS may destroy normal metabolism through peroxidation of membrane lipids (Arora *et al.*, 2002). Quan *et al.* (2004) found higher electrolyte leakage in drought stressed maize (*Zea mays* L.) plants than in plants grown under controlled conditions. *Brassica napus* L. exposed to drought stress resulted in 21% reduction in CMS at flowering stage under stress as compared to 83% under well watered conditions due to rise in leaf temperature from 1 to 2°C and damage to the cell membrane structure by the drought stress (Hashem *et al.*, 2008). Lipid peroxidation of biological membranes might lead to structural alterations such as denaturalization of proteins and nucleic acids in drought-stressed plants. Experimental evidence suggest that lipid peroxidation reactions of cellular membranes may play an important role in radical mediated cell injury in view of malondialdehyde (MDA) accumulation (Zhang *et al.*, 2007). Therefore, MDA content may act as efficient determinant criteria in the toxic degree to drought stressed plants (Aslam *et al.*, 2006). However, TU foliar application increased the cell membrane stability thus decreasing electrolyte leakage and MDA content under water stress in both the hybrids. Thiourea (TU) treated plants performed better in terms of plant growth inspite of experiencing slightly more water deficit due to stabilization of lipoprotein structure and less malondialdehyde production compared to control (Mojtaba and Karr, 2001).

Conclusion:

In three green house experiments, role of TU seed treatment, TU applied through rooting medium and foliar application separately for increasing drought tolerance in maize hybrids was studied with promising results. However, combination of TU seed treatment with foliar application under field condition is a new field of study to authenticate practical significane of previous work.

**Field experiment: Improving drought tolerance in maize (*Zea mays* L.)
by exogenous application of thiourea**

Table 4.61: Effect of thiourea under different moisture stress levels on water potential of maize hybrids

Treatments	Water Potential (-MPa)	
	2010	2011
S ₁ H ₁ T ₁	0.71 a	0.71 a
S ₁ H ₁ T ₂	0.55 e	0.56 e
S ₁ H ₁ T ₃	0.62 c	0.63 c
S ₁ H ₁ T ₄	0.44 g	0.45 g
S ₁ H ₂ T ₁	0.66 b	0.67 b
S ₁ H ₂ T ₂	0.48 f	0.49 f
S ₁ H ₂ T ₃	0.59 d	0.60 d
S ₁ H ₂ T ₄	0.38 h	0.40 h
S ₂ H ₁ T ₁	1.06 a	1.07 a
S ₂ H ₁ T ₂	0.88 e	0.88 e
S ₂ H ₁ T ₃	0.97 c	0.98 c
S ₂ H ₁ T ₄	0.79 g	0.79 g
S ₂ H ₂ T ₁	1.00 b	1.02 b
S ₂ H ₂ T ₂	0.83 f	0.84 f
S ₂ H ₂ T ₃	0.93 d	0.94 d
S ₂ H ₂ T ₄	0.75 h	0.76 h
S ₃ H ₁ T ₁	1.32 a	1.33 a
S ₃ H ₁ T ₂	1.21 e	1.22 e
S ₃ H ₁ T ₃	1.26 c	1.27 c
S ₃ H ₁ T ₄	1.15 g	1.16 g
S ₃ H ₂ T ₁	1.28 b	1.29 b
S ₃ H ₂ T ₂	1.18 f	1.19 f
S ₃ H ₂ T ₃	1.23 d	1.24 d
S ₃ H ₂ T ₄	1.10 h	1.11 h
LSD (5%)	0.008	0.008
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	*
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference.

Table 4.62: Effect of thiourea under different moisture stress levels on osmotic potential of maize hybrids

Treatments	Osmotic Potential (-MPa)	
	2010	2011
S ₁ H ₁ T ₁	1.30 a	1.30 a
S ₁ H ₁ T ₂	1.07 e	1.08 e
S ₁ H ₁ T ₃	1.19 c	1.20 c
S ₁ H ₁ T ₄	0.98 g	0.98 g
S ₁ H ₂ T ₁	1.23 b	1.24 b
S ₁ H ₂ T ₂	1.04 f	1.04 f
S ₁ H ₂ T ₃	1.12 d	1.13 d
S ₁ H ₂ T ₄	0.92 h	0.93 h
S ₂ H ₁ T ₁	1.72 a	1.72 a
S ₂ H ₁ T ₂	1.48 e	1.49 e
S ₂ H ₁ T ₃	1.61 c	1.62 c
S ₂ H ₁ T ₄	1.37 g	1.38 g
S ₂ H ₂ T ₁	1.66 b	1.67 b
S ₂ H ₂ T ₂	1.43 f	1.44 f
S ₂ H ₂ T ₃	1.53 d	1.55 d
S ₂ H ₂ T ₄	1.33 h	1.34 h
S ₃ H ₁ T ₁	2.24 a	2.25 a
S ₃ H ₁ T ₂	1.96 e	1.97 e
S ₃ H ₁ T ₃	2.13 c	2.14 c
S ₃ H ₁ T ₄	1.84 g	1.85 g
S ₃ H ₂ T ₁	2.18 b	2.21 b
S ₃ H ₂ T ₂	1.92 f	1.92 f
S ₃ H ₂ T ₃	2.04 d	2.05 d
S ₃ H ₂ T ₄	1.76 h	1.77 h
LSD (5%)	0.01	0.01
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	*
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference.

Water potential (-MPa)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for water potential (WP). Drought stress decreased the water potential in both the hybrids. WP of -1.32MPa and -1.33MPa was recorded in hybrid 32F10 during 2010 and 2011, respectively under stress level of -45KPa. Similarly WP of -1.28MPa and -1.29MPa was observed in hybrid 34N43 in both the years under stress level of -45KPa. Seed treatment with 800 mg/l TU combined with 1500 mg/l foliar application increased the water potential than all other treatments. WP for hybrid 34N43 was increased to -1.10MPa and -1.11MPa during 2010 and 2011 under stress level of -45KPa with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application. For hybrid 32F10 WP was increased to -1.15MPa and -1.16MPa in both years for same TU seed treatment under stress level of -45KPa.

Osmotic potential (-MPa)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for osmotic potential (OP). Drought stress decreased the osmotic potential in both the hybrids. OP of -2.24MPa and -2.25MPa was recorded in hybrid 32F10 during 2010 and 2011, respectively under stress level of -45KPa. Similarly OP of -2.18MPa and -2.21MPa was observed in hybrid 34N43 in both the years under stress level of -45KPa. However, OP for hybrid 34N43 was increased to -1.76MPa and -1.77MPa during 2010 and 2011 under stress level of -45KPa with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application. For hybrid 32F10 OP was increased to -1.84MPa and -1.85MPa in both years for same TU seed treatment under stress level of -45KPa.

Table 4.63: Effect of thiourea under different moisture stress levels on turgor potential of maize hybrids

Treatments	Turgor Potential (MPa)	
	2010	2011
S ₁ H ₁ T ₁	1.15 h	1.12 h
S ₁ H ₁ T ₂	1.34 d	1.32 d
S ₁ H ₁ T ₃	1.21 f	1.21 f
S ₁ H ₁ T ₄	1.40 b	1.38 b
S ₁ H ₂ T ₁	1.18 g	1.16 g
S ₁ H ₂ T ₂	1.37 c	1.35 c
S ₁ H ₂ T ₃	1.26 e	1.26 e
S ₁ H ₂ T ₄	1.44 a	1.42 a
S ₂ H ₁ T ₁	0.83 h	0.82 h
S ₂ H ₁ T ₂	1.00 d	0.99 d
S ₂ H ₁ T ₃	0.92 f	0.92 f
S ₂ H ₁ T ₄	1.07 b	1.05 b
S ₂ H ₂ T ₁	0.88 g	0.86 g
S ₂ H ₂ T ₂	1.04 c	1.02 c
S ₂ H ₂ T ₃	0.95 e	0.95 e
S ₂ H ₂ T ₄	1.10 a	1.08 a
S ₃ H ₁ T ₁	0.55 h	0.54 h
S ₃ H ₁ T ₂	0.69 d	0.67 d
S ₃ H ₁ T ₃	0.62 f	0.61 f
S ₃ H ₁ T ₄	0.77 b	0.76 b
S ₃ H ₂ T ₁	0.59 g	0.58 g
S ₃ H ₂ T ₂	0.73 c	0.72 c
S ₃ H ₂ T ₃	0.66 e	0.64 e
S ₃ H ₂ T ₄	0.80 a	0.79 a
LSD (5%)	0.01	0.01
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	*
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Table 4.64: Effect of thiourea under different moisture stress levels on relative water contents of maize hybrids

Treatments	Relative Water Contents (%)	
	2010	2011
S ₁ H ₁ T ₁	81.07 g	81.17 g
S ₁ H ₁ T ₂	85.10 d	85.77 d
S ₁ H ₁ T ₃	83.10 e	83.66 e
S ₁ H ₁ T ₄	87.78 b	87.65 b
S ₁ H ₂ T ₁	82.24 f	82.42 f
S ₁ H ₂ T ₂	86.54 c	86.92 c
S ₁ H ₂ T ₃	85.08 d	85.34 d
S ₁ H ₂ T ₄	89.43 a	89.86 a
S ₂ H ₁ T ₁	72.11 g	72.37 g
S ₂ H ₁ T ₂	75.35 d	75.57 d
S ₂ H ₁ T ₃	74.35 f	74.45 f
S ₂ H ₁ T ₄	78.18 b	78.26 b
S ₂ H ₂ T ₁	72.14 g	72.73 g
S ₂ H ₂ T ₂	77.28 c	77.47 c
S ₂ H ₂ T ₃	74.76 e	74.96 e
S ₂ H ₂ T ₄	79.35 a	79.87 a
S ₃ H ₁ T ₁	62.30 h	62.51 h
S ₃ H ₁ T ₂	70.05 d	70.07 d
S ₃ H ₁ T ₃	65.99 f	65.87 f
S ₃ H ₁ T ₄	70.31 b	70.78 b
S ₃ H ₂ T ₁	64.35 g	64.57 g
S ₃ H ₂ T ₂	70.15 c	70.27 c
S ₃ H ₂ T ₃	69.44 e	69.84 e
S ₃ H ₂ T ₄	71.75 a	71.66 a
LSD (5%)	0.03	0.01
Block	NS	NS
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	*
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Turgor potential (MPa)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for turgor potential (TP). Maximum TP (1.44MPa) and (1.42MPa) was recorded in hybrid 34N43 during 2010 and 2011, respectively under stress level of -15KPa i.e normal availability of water with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application. Similarly, TP of 1.40MPa and 1.38MPa was recorded in hybrid 32F10 during both the years under same stress level and TU application. Whereas under stress level of -45KPa, with dry seed sowing and distilled water foliar application TP of 0.55MPa and 0.54MPa was recorded in hybrid 32F10 during 2010 and 2011. For hybrid 34N43 TP was 0.59MPa and 0.58MPa recorded during both the years with dry seed sowing and distilled water foliar application.

Relative water contents (%)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for relative water contents (RWC). Maximum RWC (89.43%) and (89.86%) were recorded in hybrid 34N43 during 2010 and 2011, respectively under stress level of -15KPa i.e normal availability of water with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application. Similarly, RWC of 87.78% and 87.65% were recorded in hybrid 32F10 during both the years under same stress level and TU application. Whereas under stress level of -45KPa, with dry seed sowing and distilled water foliar application RWC of 62.30% and 62.51% were recorded in hybrid 32F10 during 2010 and 2011. For hybrid 34N43 RWC were 64.35% and 64.57% during both the years with dry seed sowing and distilled water foliar application.

Table 4.65: Effect of thiourea under different moisture stress levels on proline contents of maize hybrids

Treatments	Proline Contents (umol/g FW)	
	2010	2011
S ₁ H ₁ T ₁	23.40 h	23.50 h
S ₁ H ₁ T ₂	26.28 d	26.66 d
S ₁ H ₁ T ₃	25.10 f	25.17 f
S ₁ H ₁ T ₄	28.12 b	28.38 b
S ₁ H ₂ T ₁	24.33 g	24.45 g
S ₁ H ₂ T ₂	27.37 c	27.78 c
S ₁ H ₂ T ₃	25.41 e	25.45 e
S ₁ H ₂ T ₄	29.19 a	29.52 a
S ₂ H ₁ T ₁	30.31 h	30.28 h
S ₂ H ₁ T ₂	34.53 d	34.45 d
S ₂ H ₁ T ₃	32.28 f	32.75 f
S ₂ H ₁ T ₄	36.53 b	36.94 b
S ₂ H ₂ T ₁	30.38 g	30.65 g
S ₂ H ₂ T ₂	35.19 c	35.57 c
S ₂ H ₂ T ₃	33.20 e	33.37 e
S ₂ H ₂ T ₄	37.06 a	37.12 a
S ₃ H ₁ T ₁	37.41 g	37.65 h
S ₃ H ₁ T ₂	40.03 c	40.10 d
S ₃ H ₁ T ₃	39.23 e	39.73 f
S ₃ H ₁ T ₄	40.20 b	40.84 b
S ₃ H ₂ T ₁	38.62 f	38.77 g
S ₃ H ₂ T ₂	40.08 c	40.36 c
S ₃ H ₂ T ₃	39.91 d	39.93 e
S ₃ H ₂ T ₄	41.37 a	41.54 a
LSD (5%)	0.06	0.03
Block	NS	NS
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	*
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Table 4.66: Effect of thiourea under different moisture stress levels on malondialdehyde (MDA) contents of maize hybrids

Treatments	MDA (umol/g FW)	
	2010	2011
S ₁ H ₁ T ₁	18.92 a	18.80 a
S ₁ H ₁ T ₂	17.32 e	17.41 e
S ₁ H ₁ T ₃	18.31 c	18.37 c
S ₁ H ₁ T ₄	16.91 g	16.84 g
S ₁ H ₂ T ₁	18.63 b	18.54 b
S ₁ H ₂ T ₂	16.95 d	16.97 f
S ₁ H ₂ T ₃	17.73 f	17.57 d
S ₁ H ₂ T ₄	16.28 h	16.32 h
S ₂ H ₁ T ₁	21.76 a	21.67 a
S ₂ H ₁ T ₂	19.77 e	19.46 e
S ₂ H ₁ T ₃	19.41 c	19.68 c
S ₂ H ₁ T ₄	19.22 g	19.19 g
S ₂ H ₂ T ₁	20.21 b	20.17 b
S ₂ H ₂ T ₂	19.55 f	19.29 f
S ₂ H ₂ T ₃	19.23 d	19.61 d
S ₂ H ₂ T ₄	18.98 h	19.03 h
S ₃ H ₁ T ₁	28.36 a	28.34 a
S ₃ H ₁ T ₂	24.93 e	24.93 e
S ₃ H ₁ T ₃	26.20 c	23.26 c
S ₃ H ₁ T ₄	23.32 g	23.32 g
S ₃ H ₂ T ₁	26.52 b	27.70 b
S ₃ H ₂ T ₂	24.40 d	24.40 f
S ₃ H ₂ T ₃	25.27 f	24.52 d
S ₃ H ₂ T ₄	22.10 h	22.19 h
LSD (5%)	0.02	0.02
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	*
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Proline contents (umol/g FW)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for proline contents. Water stress stimulated the proline synthesis and TU seed treatment combined with foliar application further increased it. Maximum proline contents (41.37 umol/g FW and 41.54 umol/g FW during first and second year, respectively) were recorded in hybrid 34N43 at stress level of -0.45KPa + 800 mg/l TU seed treatment and 1500 mg/l foliar application. Proline contents were decreased to 38.62 umol/g FW and 38.77 umol/g FW with dry seed sowing and distilled water foliar application for this hybrid at same stress level. For hybrid 32F10, higher proline contents (40.20 umol/g FW and 40.84 umol/g FW during both years, respectively) were recorded at stress level of -0.45KPa with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application while proline contents decreased to 37.41 umol/g FW and 37.65 umol/g FW during first and second year, respectively, with dry seed sowing and distilled water foliar application for same hybrid and stress level.

MDA (umol/g FW)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for MDA contents. Water stress increased MDA production in both the hybrids. Thiourea seed treatment and foliar application suppressed the MDA synthesis. Maximum MDA contents (28.36 umol/g FW and 28.34 umol/g FW) were recorded in hybrid 32F10 during 2010 and 2011 respectively under stress level of -0.45KPa with dry seed sowing and distilled water foliar application these MDA contents were decreased to 23.32 umol/gFW during both the years with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application for this hybrid under same stress level. For hybrid 34N43 MDA contents were 26.52 umol/g FW and 27.70 umol/g FW during 2010 and 2011, respectively under stress level of -0.45KPa with dry seed sowing and distilled water foliar application and MDA contents were decreased to 22.10 umol/g FW and 22.19 umol/g FW with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application for this hybrid under same stress level.

Table 4.67: Effect of thiourea under different moisture stress levels on electrolyte leakage of maize hybrids

Treatments	Electrolyte Leakage (%)	
	2010	2011
S ₁ H ₁ T ₁	14.33 a	14.35 a
S ₁ H ₁ T ₂	12.86 e	12.96 e
S ₁ H ₁ T ₃	13.89 c	13.79 c
S ₁ H ₁ T ₄	11.28 g	11.62 g
S ₁ H ₂ T ₁	14.22 b	14.28 b
S ₁ H ₂ T ₂	12.31 f	12.49 f
S ₁ H ₂ T ₃	13.49 d	13.55 d
S ₁ H ₂ T ₄	11.13 h	11.15 h
S ₂ H ₁ T ₁	19.21 a	19.23 a
S ₂ H ₁ T ₂	16.93 e	16.91 e
S ₂ H ₁ T ₃	18.01 c	17.95 c
S ₂ H ₁ T ₄	15.64 g	15.86 g
S ₂ H ₂ T ₁	18.38 b	18.20 b
S ₂ H ₂ T ₂	16.82 f	16.74 f
S ₂ H ₂ T ₃	17.32 d	17.51 d
S ₂ H ₂ T ₄	15.40 h	15.26 h
S ₃ H ₁ T ₁	26.64 a	26.84 a
S ₃ H ₁ T ₂	20.93 e	22.77 e
S ₃ H ₁ T ₃	24.28 c	24.45 c
S ₃ H ₁ T ₄	23.32 g	20.82 g
S ₃ H ₂ T ₁	25.27 b	25.37 b
S ₃ H ₂ T ₂	21.32 f	21.63 f
S ₃ H ₂ T ₃	23.73 d	23.55 d
S ₃ H ₂ T ₄	20.15 h	20.26 h
LSD (5%)	0.03	0.01
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	*
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Electrolyte leakage (%)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for electrolyte leakage (EL). Water stress increased electrolyte leakage in both the hybrids. Thiourea seed treatment and foliar application suppressed the membrane injury. Maximum EL (26.64% and 26.84 %) was recorded in hybrid 32F10 during 2010 and 2011 respectively under stress level of -0.45KPa with dry seed sowing and distilled water foliar application and EL was decreased to 23.32% and 20.82 % during both the years with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application for this hybrid under same stress level. For hybrid 34N43 EL of 25.27% and 25.37% was recorded during 2010 and 2011, respectively under stress level of -0.45KPa with dry seed sowing and distilled water foliar application and EL was decreased to 20.15% and 20.26% with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application for this hybrid under same stress level.

Table 4.68: Effect of thiourea under different moisture stress levels on crop growth rate of maize hybrids

Treatments	Crop growth rate (g/m ² /day)	
	2010	2011
S ₁ H ₁ T ₁	20.4 g	22.5 f
S ₁ H ₁ T ₂	21.8 bc	23.9 a
S ₁ H ₁ T ₃	21.3 cde	23.3 abc
S ₁ H ₁ T ₄	22.6 a	24.10 a
S ₁ H ₂ T ₁	20.8 f	22.5 cd
S ₁ H ₂ T ₂	22.2 ab	24.0 a
S ₁ H ₂ T ₃	21.5 cd	23.6 ab
S ₁ H ₂ T ₄	23.1 a	24.4 a
S ₂ H ₁ T ₁	17.4 fg	18.7 efg
S ₂ H ₁ T ₂	19.0 bc	20.96 bc
S ₂ H ₁ T ₃	18.2 de	19.7 de
S ₂ H ₁ T ₄	19.9 a	21.8 a
S ₂ H ₂ T ₁	17.9 ef	19.2 ef
S ₂ H ₂ T ₂	19.5 ab	21.0 b
S ₂ H ₂ T ₃	18.6 cd	20.1 d
S ₂ H ₂ T ₄	20.1 a	22.3 a
S ₃ H ₁ T ₁	11.4 fg	12.3 f
S ₃ H ₁ T ₂	14.7 d	15.2 bc
S ₃ H ₁ T ₃	12.9 f	14.1 de
S ₃ H ₁ T ₄	16.2 b	16.4 a
S ₃ H ₂ T ₁	12.2 g	13.7 e
S ₃ H ₂ T ₂	15.3 c	15.6 ab
S ₃ H ₂ T ₃	13.8 e	14.62 cd
S ₃ H ₂ T ₄	16.9 a	17.0 a
LSD (5%)	0.54	0.78
H (Hybrid)	*	*
Error a	-	-
S (Stress Levels)	**	**
S x H		
Error b	-	-
T (Thiourea)	**	**
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$

. H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Table 4.69: Effect of thiourea under different moisture stress levels on plant height of maize hybrids

Treatments	Plant height (cm)	
	2010	2011
S ₁ H ₁ T ₁	231 def	223 f
S ₁ H ₁ T ₂	247 ab	244 ab
S ₁ H ₁ T ₃	239 bcd	237 bcd
S ₁ H ₁ T ₄	256 a	255 a
S ₁ H ₂ T ₁	235 cde	230 cde
S ₁ H ₂ T ₂	250 a	253 a
S ₁ H ₂ T ₃	242 abc	240 bc
S ₁ H ₂ T ₄	258 a	264 a
S ₂ H ₁ T ₁	190 fg	186 ef
S ₂ H ₁ T ₂	213 bc	207 ab
S ₂ H ₁ T ₃	203 de	197 abcd
S ₂ H ₁ T ₄	225 a	216 a
S ₂ H ₂ T ₁	192 f	190 de
S ₂ H ₂ T ₂	216 b	212 a
S ₂ H ₂ T ₃	211 bcd	204 abc
S ₂ H ₂ T ₄	230 a	222 a
S ₃ H ₁ T ₁	162 efg	154 bcdef
S ₃ H ₁ T ₂	173 abc	164 ab
S ₃ H ₁ T ₃	169 bcde	159 bcd
S ₃ H ₁ T ₄	179 a	177 a
S ₃ H ₂ T ₁	165 cdef	157 bcde
S ₃ H ₂ T ₂	176 ab	174 a
S ₃ H ₂ T ₃	171 bcd	161 bc
S ₃ H ₂ T ₄	186 a	181 a
LSD (5%)	9.95	11.05
H (Hybrid)	**	**
Error a	-	-
S (Stress Levels)	*	*
S x H	*	**
Error b	-	-
T (Thiourea)	*	**
S x T	**	**
H x T	**	*
S x H x T	*	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Crop Growth rate (g/m²/day)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for crop growth rate (CGR). Increasing stress level decreased CGR that was improved with TU application. Maximum CGR 23.1 g/m²/day and 24.4 g/m²/day was recorded in hybrid 34N43 under soil water potential of -15 KPa where seed treatment with 800 mg/l TU was combined with 1500 mg/l TU foliar application during 2010 and 2011 respectively. However hybrid 32F10 had CGR 22.6 g/m²/day and 24.1 g/m²/day in both the years under same soil water potential and TU seed treatment. However, water stress decreased the CGR in both the hybrids. Hybrid 32F10 had CGR 11.4 g/m²/day and 12.3 g/m²/day in both the years under soil water potential of -45 KPa with dry seed sowing and foliar application of distilled water. Similarly hybrid 34N43 had CGR 12.2 g/m²/day and 13.7 g/m²/day in two years under same water potential and with dry seed sowing and distilled water foliar application. But TU seed treatment combined with foliar application increased CGR under water stress in both the hybrids. However, increase was more significant in hybrid 34N43 as compared to hybrid 32F10. Crop growth rate of 16.9 g/m²/day and 17.0 g/m²/day was observed in hybrid 34N43 during years 2010 and 2011 under -45 KPa where 800 mg/l TU seed treatment was combined with 1500 mg/l TU foliar application and hybrid 32F10 had CGR 16.2 g/m²/day and 16.4 g/m²/day during both the years under same moisture stress and TU treatments.

Plant height (cm)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for plant height. Plant height increased with availability of water. Plant height of 235 cm and 230 cm was recorded during 2010 and 2011 respectively for hybrid 34N43 under stress level of -15KPa i.e normal availability of water, with dry seed sowing and foliar application of distilled water. Hybrid 32F10 recorded plant height of 231 cm and 223 cm during 2010 and 2011 respectively under normal availability of water with dry seed sowing and foliar application of distilled water. However, 800 mg/l TU seed treatment combined with 1500 mg/l significantly increased the plant in both the hybrids. Plant height of 255 cm and 256 cm was recorded during 2010 and 2011 respectively for hybrid 32F10 with normal availability of water by 800 mg/l TU seed treatment combined with 1500 mg/l foliar application. Whereas

hybrid 34N43 had plant height of 258 cm and 264 cm was recorded during 2010 and 2011 respectively under same water availability and TU treatments. Similar trend was observed in both the hybrids for other two stress levels with above mentioned TU applications.

Table 4.70: Effect of thiourea under different moisture stress levels on biological yield of maize hybrids

Treatments	Biological yield (t ha ⁻¹)	
	2010	2011
S ₁ H ₁ T ₁	16.2 h	16.2 h
S ₁ H ₁ T ₂	17.7 d	17.9 d
S ₁ H ₁ T ₃	16.9 f	16.8 f
S ₁ H ₁ T ₄	18.5 b	18.6 b
S ₁ H ₂ T ₁	16.3 g	16.5 g
S ₁ H ₂ T ₂	18.2 c	18.3 c
S ₁ H ₂ T ₃	17.3 e	17.5 e
S ₁ H ₂ T ₄	18.8 a	18.9 a
S ₂ H ₁ T ₁	11.5 h	11.6 h
S ₂ H ₁ T ₂	14.1 d	14.3 d
S ₂ H ₁ T ₃	12.3 f	12.4 f
S ₂ H ₁ T ₄	15.3 b	15.2 b
S ₂ H ₂ T ₁	11.9 g	11.8 g
S ₂ H ₂ T ₂	14.9 c	14.8 c
S ₂ H ₂ T ₃	13.5 e	13.7 e
S ₂ H ₂ T ₄	15.7 a	15.6 a
S ₃ H ₁ T ₁	8.1 h	8.2 h
S ₃ H ₁ T ₂	9.8 d	9.9 d
S ₃ H ₁ T ₃	8.9 f	8.9 f
S ₃ H ₁ T ₄	10.8 b	10.8 b
S ₃ H ₂ T ₁	8.6 g	8.6 g
S ₃ H ₂ T ₂	10.4 c	10.5 c
S ₃ H ₂ T ₃	9.2 e	9.3 e
S ₃ H ₂ T ₄	11.1 a	11.1 a
LSD (5%)	0.14	0.14
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	**
S x T	*	**
H x T	**	*
S x H x T	*	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Table 4.71: Effect of thiourea under different moisture stress levels on 1000-grain weight (gm) of maize hybrids

Treatments	1000-grain weight (gm)	
	2010	2011
S ₁ H ₁ T ₁	265.2 h	266.5 h
S ₁ H ₁ T ₂	284.5 d	285.0 d
S ₁ H ₁ T ₃	274.5 f	275.5 f
S ₁ H ₁ T ₄	293.5 b	293.5 b
S ₁ H ₂ T ₁	271.5 g	270.5 g
S ₁ H ₂ T ₂	290.0 c	289.5 c
S ₁ H ₂ T ₃	280.7 e	280.5 e
S ₁ H ₂ T ₄	298.0 a	298.5 a
S ₂ H ₁ T ₁	217.7 h	218.5 h
S ₂ H ₁ T ₂	242.5 d	243.5 d
S ₂ H ₁ T ₃	231.7 f	233.2 f
S ₂ H ₁ T ₄	255.5 b	256.5 b
S ₂ H ₂ T ₁	225.0 g	225.5 g
S ₂ H ₂ T ₂	248.0 c	249.2 c
S ₂ H ₂ T ₃	236.0 e	237.0 e
S ₂ H ₂ T ₄	261.2 a	262.2 a
S ₃ H ₁ T ₁	157.5 h	154.0 h
S ₃ H ₁ T ₂	189.0 d	189.7 d
S ₃ H ₁ T ₃	177.0 f	176.5 f
S ₃ H ₁ T ₄	204.0 b	205.2 b
S ₃ H ₂ T ₁	166.5 g	168.5 g
S ₃ H ₂ T ₂	195.2 c	196.5 c
S ₃ H ₂ T ₃	182.7 e	183.5 e
S ₃ H ₂ T ₄	212.7 a	212.5 a
LSD (5%)	1.19	1.08
S (Stress Levels)	**	*
Error a	-	-
H (Hybrid)	**	**
S x H	NS	*
Error b	-	-
T (Thiourea)	**	**
S x T	*	**
H x T	**	*
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Biological yield (t ha⁻¹)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for biological yield. Maximum biological yield (18.8 t ha⁻¹ and 18.9 t ha⁻¹) during 2010 and 2011 respectively was recorded in hybrid 34N43 under soil water potential of -15 KPa where seed treatment with 800 mg/l TU was combined with 1500 mg/l TU foliar application. However, hybrid 32F10 had biological yield of 18.5 t ha⁻¹ and 18.6 t ha⁻¹ in both the years under same soil water potential and TU seed treatment. However, water stress decreased the biological yield in both the hybrids. Hybrid 32F10 had biological yield of 8.1 t ha⁻¹ and 8.2 t ha⁻¹ in both the years under soil water potential of -45 KPa with dry seed sowing and foliar application of distilled water. Similarly hybrid 34N43 had biological yield of 8.6 t ha⁻¹ and 8.6 t ha⁻¹ in two years under same water potential and with dry seed sowing and distilled water foliar application. But TU seed treatment combined with foliar application increased biological yield under water stress in both the hybrids. However, increase was more significant in hybrid 34N43 as compared to hybrid 32F10. Biological yield of 11.1 and 11.1 t ha⁻¹ was observed in hybrid 34N43 during years 2010 and 2011 under -45 KPa where 800 mg/l TU seed treatment was combined with 1500 mg/l TU foliar application and hybrid 32F10 had biological yield of 10.8 t ha⁻¹ and 10.8 t ha⁻¹ during both the years under same moisture stress and TU treatments.

1000 grain weight (g)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for 1000 grain weight. Water stress significantly decreased 1000 grain weight in both the hybrids. However, extent of decrease was more in hybrid 32F10 as compared to hybrid 34N43. A 1000 grain weight of 157.5 g and 154.0 g during 2010 and 2011, respectively was observed for hybrid 32F10 while for hybrid 34N43, 1000 grain weight was 166.5 g and 168.5 g during 2010 and 2011, respectively under stress level of -45 KPa with dry seed sowing and foliar application of distilled water. But 800 mg/l TU seed treatment combined with 1500 mg/l foliar application significantly increased 1000 grain weight in both hybrids under water stress. Hybrid 34N43 had 1000 grain weight of 212.7 g and 212.5 g during 2010 and 2011, respectively whereas hybrid 32F10 recorded 1000 grain weight of 205.2 g and 204.0

g during 2010 and 2011, respectively by 800 mg/l TU seed treatment combined with 1500 mg/l TU foliar application. Similar trend was observed in both the hybrids for other two stress levels with these TU treatments. Next treatment which gave promising results was when 800 mg/l TU seed treatment was combined with foliar application in all the stress levels.

Table 4.72: Effect of thiourea under different moisture stress levels on grain yield (t ha⁻¹) of maize hybrids

Treatments	Grain yield (t ha ⁻¹)	
	2010	2011
S ₁ H ₁ T ₁	6.08 h	6.12 h
S ₁ H ₁ T ₂	7.18 d	7.25 d
S ₁ H ₁ T ₃	6.58 f	6.52 f
S ₁ H ₁ T ₄	7.74 b	7.71 b
S ₁ H ₂ T ₁	6.35 g	6.31 g
S ₁ H ₂ T ₂	7.45 c	7.47 c
S ₁ H ₂ T ₃	6.84 e	6.77 e
S ₁ H ₂ T ₄	7.87 a	7.92 a
S ₂ H ₁ T ₁	3.85 h	3.88 h
S ₂ H ₁ T ₂	4.97 d	4.92 d
S ₂ H ₁ T ₃	4.37 f	4.42 f
S ₂ H ₁ T ₄	5.54 b	5.61 b
S ₂ H ₂ T ₁	3.97 g	4.07 g
S ₂ H ₂ T ₂	5.24 c	5.28 c
S ₂ H ₂ T ₃	4.64 e	4.68 e
S ₂ H ₂ T ₄	5.79 a	5.83 a
S ₃ H ₁ T ₁	2.44 h	2.43 h
S ₃ H ₁ T ₂	3.07 d	3.12 d
S ₃ H ₁ T ₃	2.72 f	2.75 f
S ₃ H ₁ T ₄	3.48 b	3.53 b
S ₃ H ₂ T ₁	2.57 g	2.61 g
S ₃ H ₂ T ₂	3.30 c	3.35 c
S ₃ H ₂ T ₃	2.86 e	2.91 e
S ₃ H ₂ T ₄	3.66 a	3.72 a
LSD (5%)	0.01	0.02
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H		
Error b	-	-
T (Thiourea)	**	**
S x T	*	**
H x T	**	*
S x H x T	*	**
Error c	-	-

Means within columns sharing different letters vary significantly at P ≤ 0.05.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Table 4.73: Effect of thiourea under different moisture stress levels on Nitrogen uptake of maize hybrids

Treatments	Nitrogen uptake (Kg ha ⁻¹)	
	2010	2011
S ₁ H ₁ T ₁	180.9 h	182.0 h
S ₁ H ₁ T ₂	203.3 d	204.6 d
S ₁ H ₁ T ₃	191.1 f	192.4 f
S ₁ H ₁ T ₄	213.0 b	219.0 b
S ₁ H ₂ T ₁	185.9 g	186.9 g
S ₁ H ₂ T ₂	209.0 c	214.4 c
S ₁ H ₂ T ₃	196.1 e	197.2 e
S ₁ H ₂ T ₄	219.0 a	226.1 a
S ₂ H ₁ T ₁	116.1 h	118.0 h
S ₂ H ₁ T ₂	149.5 d	153.9 d
S ₂ H ₁ T ₃	134.2 f	136.9 f
S ₂ H ₁ T ₄	166.9 b	168.1 b
S ₂ H ₂ T ₁	128.6 g	126.4 g
S ₂ H ₂ T ₂	161.5 c	162.0 c
S ₂ H ₂ T ₃	139.3 e	143.1 e
S ₂ H ₂ T ₄	172.8 a	174.8 a
S ₃ H ₁ T ₁	32.2 h	36.13 h
S ₃ H ₁ T ₂	73.0 d	74.70 d
S ₃ H ₁ T ₃	50.0 f	55.40 f
S ₃ H ₁ T ₄	95.4 b	92.43 b
S ₃ H ₂ T ₁	42.7 g	46.78 g
S ₃ H ₂ T ₂	78.3 c	81.74 c
S ₃ H ₂ T ₃	62.3 e	63.79 e
S ₃ H ₂ T ₄	104.3 a	107.56 a
LSD (5%)	1.16	1.13
S (Stress Levels)	*	**
Error a	-	-
H (Hybrid)	*	*
S x H	**	*
Error b	-	-
T (Thiourea)	**	**
S x T	**	*
H x T	*	**
S x H x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Grain yield (t ha⁻¹)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for grain yield. Maximum grain yield (7.87 t ha⁻¹ and 7.92 t ha⁻¹) during 2010 and 2011 respectively was recorded in hybrid 34N43 under soil water potential of -15 KPa where seed treatment with 800 mg/l TU was combined with 1500 mg/l TU foliar application. However, hybrid 32F10 had grain yield of 7.74 t ha⁻¹ and 7.71 t ha⁻¹ in both the years under same soil water potential and TU seed treatment. However, water stress decreased the grain yield in both the hybrids. Hybrid 32F10 had grain yield of 2.44 t ha⁻¹ and 2.43 t ha⁻¹ in both the years under soil water potential of -45 KPa with dry seed sowing and foliar application of distilled water. Similarly hybrid 34N43 had grain yield 2.57 t ha⁻¹ and 2.61 t ha⁻¹ in two years under same water potential and with dry seed sowing and distilled water foliar application. However, TU seed treatment combined with foliar application increased grain yield under water stress in both the hybrids. However, increase was more pronounced in hybrid 34N43 as compared to hybrid 32F10. Grain yield of 3.66 t ha⁻¹ and 3.72 t ha⁻¹ was observed in hybrid 34N43 during years 2010 and 2011 under -45 KPa where 800 mg/l TU seed treatment was combined with 1500 mg/l TU foliar application and hybrid 32F10 had grain yield of 3.48 t ha⁻¹ and 3.53 t ha⁻¹ during both the years under same moisture stress and TU treatments.

Nitrogen uptake (kg ha⁻¹)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for nitrogen uptake. Maximum N uptake (219.0 kg ha⁻¹ and 226.1 kg ha⁻¹) was recorded in hybrid 34N43 under soil water potential of -15 KPa where seed treatment with 800 mg/l TU was combined with 1500 mg/l TU foliar application. However hybrid 32F10 had N uptake of 213.0 kg ha⁻¹ and 219.0 kg ha⁻¹ in both the years under same soil water potential and TU seed treatment. However, water stress decreased the N uptake in both the hybrids. Hybrid 32F10 had N uptake of 32.2 kg ha⁻¹ and 36.1 kg ha⁻¹ in both the years under soil water potential of -45 KPa with dry seed sowing and foliar application of distilled water. Similarly, hybrid 34N43 had N uptake of 42.7 kg ha⁻¹ and 46.7 kg ha⁻¹ in two years under same water potential and with dry seed sowing and distilled water foliar application. But TU seed treatment combined with foliar application increased N uptake under water

stress in both the hybrids. However increase was more significant in hybrid 34N43 as compared to hybrid 32F10. N uptake of 104.3 kg ha⁻¹ and 107.5 kg ha⁻¹ was observed in hybrid 34N43 during years 2010 and 2011 under -45 KPa where 800 mg/l TU seed treatment was combined with 1500 mg/l TU foliar application and hybrid 32F10 had N uptake of 95.4 kg ha⁻¹ and 92.4 kg ha⁻¹ during both the years under same moisture stress and TU treatments.

Table 4.74: Effect of thiourea under different moisture stress levels on phosphorous uptake of maize hybrids

Treatments	Phosphorus (kg ha ⁻¹)	
	2010	2011
S ₁ H ₁ T ₁	106.7 h	107.0 h
S ₁ H ₁ T ₂	118.9 d	119.9 d
S ₁ H ₁ T ₃	112.9 f	114.0 f
S ₁ H ₁ T ₄	126.0 b	126.9 b
S ₁ H ₂ T ₁	110.5 g	111.0 g
S ₁ H ₂ T ₂	122.8 c	123.9 c
S ₁ H ₂ T ₃	115.7 e	116.9 e
S ₁ H ₂ T ₄	129.0 a	130.1 a
S ₂ H ₁ T ₁	77.0 h	77.8 h
S ₂ H ₁ T ₂	90.8 d	92.0 d
S ₂ H ₁ T ₃	83.9 f	84.8 f
S ₂ H ₁ T ₄	99.0 b	99.1 b
S ₂ H ₂ T ₁	81.0 g	82.0 g
S ₂ H ₂ T ₂	95.5 c	96.8 c
S ₂ H ₂ T ₃	87.6 e	88.7 e
S ₂ H ₂ T ₄	101.0 a	102.9 a
S ₃ H ₁ T ₁	39.4 h	40.0 h
S ₃ H ₁ T ₂	58.9 d	59.9 d
S ₃ H ₁ T ₃	49.6 f	50.7 f
S ₃ H ₁ T ₄	67.9 b	69.0 b
S ₃ H ₂ T ₁	45.2 g	46.1 g
S ₃ H ₂ T ₂	63.8 c	64.9 c
S ₃ H ₂ T ₃	56.0 e	57.1 e
S ₃ H ₂ T ₄	72.8 a	73.8 a
LSD (5%)	0.70	0.70
H (Hybrid)	*	*
Error a	-	-
S (Stress Levels)	*	**
H x S	**	*
Error b	-	-
T (Thiourea)	*	**
H x T	**	*
S x T	*	**
H x S x T	**	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Table 4.75: Effect of thiourea under different moisture stress levels on potash uptake of maize hybrids

Treatments	Potash uptake (kg ha ⁻¹)	
	2010	2011
S ₁ H ₁ T ₁	144.9 h	146.0 h
S ₁ H ₁ T ₂	171.0 d	172.0 d
S ₁ H ₁ T ₃	157.0 f	157.9 f
S ₁ H ₁ T ₄	182.8 b	183.8 b
S ₁ H ₂ T ₁	151.4 g	152.9 g
S ₁ H ₂ T ₂	176.7 c	177.8 c
S ₁ H ₂ T ₃	163.9 e	165.0 e
S ₁ H ₂ T ₄	188.9 a	190.0 a
S ₂ H ₁ T ₁	95.9 h	96.9 h
S ₂ H ₁ T ₂	118.9 d	120.9 d
S ₂ H ₁ T ₃	108.7 f	109.8 f
S ₂ H ₁ T ₄	131.0 b	132.4 b
S ₂ H ₂ T ₁	101.9 g	103.0 g
S ₂ H ₂ T ₂	124.8 c	125.9 c
S ₂ H ₂ T ₃	113.1 e	114.1 e
S ₂ H ₂ T ₄	140.9 a	141.8 a
S ₃ H ₁ T ₁	45.8 h	46.9 h
S ₃ H ₁ T ₂	69.9 d	70.8 d
S ₃ H ₁ T ₃	56.0 f	57.0 f
S ₃ H ₁ T ₄	85.5 b	85.8 b
S ₃ H ₂ T ₁	52.2 g	53.2 g
S ₃ H ₂ T ₂	76.7 c	77.8 c
S ₃ H ₂ T ₃	62.0 e	63.0 e
S ₃ H ₂ T ₄	89.8 a	90.8 a
LSD (5%)	0.88	1.03
S (Stress Levels)	**	*
Error a	-	-
H (Hybrid)	*	*
S x H	**	**
Error b	-	-
T (Thiourea)	**	*
S x T	**	*
H x T	*	**
S x H x T	*	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Phosphorus uptake (kg ha⁻¹)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for phosphorus uptake. Maximum P uptake 129.0 kg ha⁻¹ and 130.1 kg ha⁻¹ was recorded in hybrid 34N43 under soil water potential of -15 KPa where seed treatment with 800 mg/l TU was combined with 1500 mg/l TU foliar application during 2010 and 2011 respectively. However, hybrid 32F10 had P uptake 126.0 kg ha⁻¹ and 126.9 kg ha⁻¹ in both the years under same soil water potential and TU seed treatment. However, water stress decreased the P uptake in both the hybrids. Hybrid 32F10 had P uptake 39.4 kg ha⁻¹ and 40.0 kg ha⁻¹ in both the years under soil water potential of -45 KPa with dry seed sowing and foliar application of distilled water. Similarly, hybrid 34N43 had P uptake 45.2 kg ha⁻¹ and 46.1 kg ha⁻¹ in two years under same water potential and with dry seed sowing and distilled water foliar application. But TU seed treatment combined with foliar application increased P uptake under water stress in both the hybrids. However, increase was more significant in hybrid 34N43 as compared to hybrid 32F10. P uptake 72.8 kg ha⁻¹ and 73.8 kg ha⁻¹ was observed in hybrid 34N43 during years 2010 and 2011 under -45 KPa where 800 mg/l TU seed treatment was combined with 1500 mg/l TU foliar application and hybrid 32F10 had P uptake 67.9 kg ha⁻¹ and 69.0 kg ha⁻¹ during both the years under same moisture stress and TU treatments.

Potash uptake (kg ha⁻¹)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for potash uptake. Maximum K uptake (188.9 kg ha⁻¹ and 190.0 kg ha⁻¹) was recorded in hybrid 34N43 under soil water potential of -15 KPa where seed treatment with 800 mg/l TU was combined with 1500 mg/l TU foliar application. However, hybrid 32F10 had (182.8 kg ha⁻¹ and 183.8 kg ha⁻¹) K uptake in both the years under same soil water potential and TU seed treatment. However, water stress decreased the K uptake in both the hybrids. Hybrid 32F10 had (45.8 kg ha⁻¹ and 46.9 kg ha⁻¹) K uptake in both the years under soil water potential of -45 KPa with dry seed sowing and foliar application of distilled water. Similarly hybrid 34N43 had (52.2 kg ha⁻¹ and 53.2 kg ha⁻¹) K uptake in two years under same water potential and with dry seed sowing and distilled water foliar application. But TU seed treatment combined with foliar application increased K uptake under water stress in both the hybrids.

However increase was more significant in hybrid 34N43 as compared to hybrid 32F10. Potassium uptake of 89.8 kg ha⁻¹ and 90.8 kg ha⁻¹ was observed in hybrid 34N43 during years 2010 and 2011 under -45 KPa where 800 mg/l TU seed treatment was combined with 1500 mg/l TU foliar application and hybrid 32F10 had K uptake of 85.52 kg ha⁻¹ and 85.87 kg ha⁻¹ during both the years under same moisture stress and TU treatments.

Table 4.76: Effect of thiourea under different moisture stress levels on protein contents (%) of maize hybrids

Treatments	Protein (%)	
	2010	2011
S ₁ H ₁ T ₁	10.32 h	10.37 h
S ₁ H ₁ T ₂	11.87 d	11.92 d
S ₁ H ₁ T ₃	11.07 f	11.16 f
S ₁ H ₁ T ₄	12.82 b	12.86 b
S ₁ H ₂ T ₁	10.64 g	10.76 g
S ₁ H ₂ T ₂	12.36 c	12.49 c
S ₁ H ₂ T ₃	11.58 e	11.67 e
S ₁ H ₂ T ₄	13.12 a	13.18 a
S ₂ H ₁ T ₁	6.91 h	6.96 h
S ₂ H ₁ T ₂	8.37 d	8.43 d
S ₂ H ₁ T ₃	7.59 f	7.64 f
S ₂ H ₁ T ₄	9.22 b	9.25 b
S ₂ H ₂ T ₁	7.26 g	7.32 g
S ₂ H ₂ T ₂	8.87 c	8.92 c
S ₂ H ₂ T ₃	7.98 e	8.11 e
S ₂ H ₂ T ₄	9.78 a	9.85 a
S ₃ H ₁ T ₁	3.95 h	3.89 h
S ₃ H ₁ T ₂	5.47 d	5.51 d
S ₃ H ₁ T ₃	4.56 f	4.60 f
S ₃ H ₁ T ₄	6.16 b	6.19 b
S ₃ H ₂ T ₁	4.27 g	4.29 g
S ₃ H ₂ T ₂	5.87 c	5.93 c
S ₃ H ₂ T ₃	4.96 e	5.03 e
S ₃ H ₂ T ₄	6.37 a	6.42 a
LSD (5%)	0.02	1.05
S (Stress Levels)	**	**
Error a	-	-
H (Hybrid)	*	*
S x H	**	*
Error b	-	-
T (Thiourea)	**	*
S x T	**	*
H x T	*	**
S x H x T	*	**
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Table 4.77: Effect of thiourea under different moisture stress levels on oil contents (%) of maize hybrids

Treatment	Oil contents (%)	
	2010	2011
S ₁ H ₁ T ₁	4.28 h	4.32 h
S ₁ H ₁ T ₂	4.95 d	4.97 d
S ₁ H ₁ T ₃	4.58 f	4.63 f
S ₁ H ₁ T ₄	5.28 b	5.26 b
S ₁ H ₂ T ₁	4.45 g	4.48 g
S ₁ H ₂ T ₂	5.13 c	5.14 c
S ₁ H ₂ T ₃	4.73 e	4.82 e
S ₁ H ₂ T ₄	5.44 a	5.53 a
S ₂ H ₁ T ₁	3.06 h	3.12 h
S ₂ H ₁ T ₂	3.57 d	3.62 d
S ₂ H ₁ T ₃	3.30 f	3.38 f
S ₂ H ₁ T ₄	3.90 b	3.84 b
S ₂ H ₂ T ₁	3.17 g	3.23 g
S ₂ H ₂ T ₂	3.73 c	3.76 c
S ₂ H ₂ T ₃	3.45 e	3.52 e
S ₂ H ₂ T ₄	4.07 a	4.15 a
S ₃ H ₁ T ₁	2.04 h	2.07 h
S ₃ H ₁ T ₂	2.55 d	2.57 d
S ₃ H ₁ T ₃	2.34 f	2.31 f
S ₃ H ₁ T ₄	2.78 b	2.83 b
S ₃ H ₂ T ₁	2.21 g	2.19 g
S ₃ H ₂ T ₂	2.66 c	2.71 c
S ₃ H ₂ T ₃	2.43 e	2.45 e
S ₃ H ₂ T ₄	2.91 a	2.94 a
LSD (5%)	0.01	0.01
S (Stress Levels)	*	*
Error a	-	-
H (Hybrid)	*	*
S x H	*	*
Error b	-	-
T (Thiourea)	*	*
S x T	**	*
H x T	*	*
S x H x T	*	*
Error c	-	-

Means within columns sharing different letters vary significantly at $P \leq 0.05$.

H₁= Drought sensitive hybrid (32F10), H₂=, Drought tolerant (34N43), S₁ = -15 KPa, S₂ = -30 KPa, S₃ = -45 KPa, T₁ = Dry seed + Distilled water foliar application, T₂ = 800 mg/l (seed priming) + Distilled water foliar application, T₃ = Dry seed + 1500 mg/l. Thiourea (foliar application), T₄ = 800 mg/l (seed priming) + 1500 mg/l. Thiourea (foliar application) NS = Non- significant, LSD = Least significance difference

Protein contents (%)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for protein contents. Maximum protein (13.12 % and 13.18 %) was recorded in hybrid 34N43 under soil water potential of -15 KPa where seed treatment with 800 mg/l TU was combined with 1500 mg/l TU foliar application. However, hybrid 32F10 had (12.82 % and 12.86 %) protein in both the years under same soil water potential and TU seed treatment. However, water stress decreased the protein percentage in both the hybrids. Hybrid 32F10 had (3.95 % and 3.89 %) protein in both the years under soil water potential of -45 KPa with dry seed sowing and foliar application of distilled water. Similarly, hybrid 34N43 had (4.27 % and 4.29 %) protein in two years under same water potential and with dry seed sowing and distilled water foliar application. But TU seed treatment combined with foliar application increased protein contents under water stress in both the hybrids. However, increase was more significant in hybrid 34N43 as compared to hybrid 32F10. Protein contents of 6.37 % and 6.42 % were observed in hybrid 34N43 during years 2010 and 2011 under -45 KPa where 800 mg/l TU seed treatment was combined with 1500 mg/l TU foliar application and hybrid 32F10 had protein 6.16 % and 6.19 % during both the years under same moisture stress and TU treatments.

Oil contents (%)

Stress levels, hybrids as well as thiourea levels, differed significantly ($P \leq 0.05$) for oil contents. More oil contents were recorded under normal availability of water and with combination of TU seed treatment and foliar application. Oil contents of 5.28 % and 5.26% were recorded in hybrid 32F10 under soil water potential of -15 KPa with combination of 800 mg/l TU seed treatment and 1500 mg/l foliar application in 2010 and 2011 respectively. Whereas oil contents 5.44 % and 5.54% were recorded in hybrid 34N43 during 2010 and 2011 under same soil water potential and TU applications.

Discussion:

A) Water relations

Water stress decreased the water potential of maize hybrids. Water potential (ψ_w) is considered a reliable parameter for measuring plant water stress response. It varies greatly, depending on the type of plant and on environmental conditions. Decrease in water potential causes decrease in grain number, size and yield (Saini and Westgate, 2000). However, this reduction was more prominent in hybrid 32F10 as compared to hybrid 34N43. Different genotypes respond differently to availability of water and water stress so that some are more sensitive to water stress and some are relatively tolerant. (Farhad *et al.*, 2011). Relative water contents, leaf water potential, rate of transpiration are important characteristics that influence plant water relations (Siddique *et al.*, 2001). Mohammady and Hasannejad (2006) screened out maize hybrids on the basis of leaf water content. The results showed that the relationship of leaf water content was significant with osmotic or turgor potential under drought. Claudio *et al.* (2006) found that osmotic potential of leaves of well watered plants increased up to -0.90 MPa 40 DAS, while under water stress it decreased up to -1.20 MPa. Application of TU through seed treatment and foliar application increased water potential, osmotic potential, turgor potential and relative water contents under water stress. In view of a large number of studies it is evident that foliar application of those nutrients that become deficient in plants under stress, could maintain the optimum level of these nutrients in plants that lead to osmotic adjustment and finally play a role in growth and yield improvement (Irshad *et al.*, 2002; Akram *et al.*, 2007; Ashraf *et al.*, 2007). Seed treatment and foliar application of thiourea improved growth, yield and WUE of pearl millet under arid and semi arid conditions (Parihar *et al.*, 1998). Thiourea has been reported to significantly improve growth yield and water use efficiency of wheat under arid and semi-arid conditions. (Sahu and Singh, 1995).

B) Proline, MDA, membrane stability

Water stress stimulated the proline synthesis and TU application further increased it as compared to well watered conditions. Osmotic adjustment (OA) is a part of drought avoidance mechanisms to counteract the loss of turgor by increasing

and maintaining higher amount of intracellular compatible solutes in cytosol and vacuole and has been proved to be particularly significant among all the stress adaptation mechanisms (Cushman, 2001). It has been widely reported that plants accumulate a variety of compatible solutes such as proline and betaine, as an adaptive mechanism of tolerance to salinity and drought (Hasegawa *et al.*, 2000; Ashraf and Harris, 2004). Similarly more electrolyte leakage and MDA production was recorded due to membrane injury. It has been reported that membranes are subject to damage rapidly with increasing water stress. This leakiness of membranes is caused by an uncontrolled increase in free radicals, which cause lipid peroxidation. Further damage to fatty acids could then produce small hydrocarbon fragments including malondialdehyde (MDA) (Alscher *et al.*, 2002). Thiourea application decreased the electrolyte leakage by increasing membrane stability. Better performance of thiourea treated plants in terms of plant growth inspite of experiencing slightly more water deficit could be due to stabilization of lipoprotein structure and less malondialdehyde production compared to control (Mojtaba and Karr, 2001).

C) Growth parameters

Water stress significantly reduced the growth of maize crop. But this reduction was different for two hybrids. Hybrid 34N43 showing tolerance to water stress recorded more growth as compared to hybrid 32F10. More crop growth rate and plant height was recorded in hybrid 34N43 as compared to hybrid 32F10. TU 800 mg/l seed treatment combined with 1500 mg/l foliar application increased growth of both maize hybrids under water stress as well as normal availability of water. With limited water supply, TU seed treatment combined with significantly increased the growth as compared to control. Sahu *et al.* (1993) reported that foliar spray of thiourea (TU) significantly increased growth and yield of maize, most probably via improvement in canopy photosynthesis. Thiourea has been reported to significantly improve growth yield and water use efficiency of wheat under arid and semi-arid conditions (Sahu and Singh, 1995). In cluster bean (*Cyamopsis tetragonoloba* L.), thiourea increased seed yield under rainfed conditions due to enhanced photosynthetic efficiency and more efficient N metabolism (Garg *et al.*, 2006). Seed treatment and foliar application of thiourea improved growth, yield and

WUE of pearl millet (*Pennisetum glaucum* L.) under arid and semi arid conditions (Parihar *et al.*, 1998). Combined application of thiourea and phosphorus significantly improved plant growth and seed yield of clusterbean under water stress (Burman *et al.*, 2003).

Yield parameters

Water stress decreased the yield in both maize hybrids. Zhang *et al.*, (2008) reported that final yield of all maize inbred lines decreased due to water stress. Maize yield was significantly increased with 800 mg/l TU seed treatment and 1500 mg/l foliar application under stress as well as normal availability of water as compared with control. Next more yield was recorded where 800 mg/l TU seed treatment was combined with foliar application of distilled water in both the hybrids under stress and well watered conditions. However, hybrid 34N43 had more improvement in biological yield, 1000 grain weight and grain yield under all water levels as compared to sensitive hybrid 32F10. Thiourea may play an important role in the active accumulation of starch and phloem transport of soluble sugars (Giaquinta, 1976). Sahu *et al.* (1993) reported that foliar spray of thiourea (TU) significantly increased growth and yield of maize, most probably via improvement in canopy photosynthesis. Thiourea has been reported to significantly improve growth yield and water use efficiency of wheat under arid and semi-arid conditions (Sahu and Singh 1995). In cluster bean, thiourea increased seed yield under rainfed conditions due to enhanced photosynthetic efficiency and more efficient N metabolism (Garg *et al.*, 2006). Seed treatment and foliar application of thiourea improved growth, yield and WUE of pearl millet under arid and semi arid conditions (Parihar *et al.*, 1998). Combined application of thiourea and phosphorus also significantly improved plant growth and seed yield of clusterbean under water stress (Burman *et al.*, 2003).

NPK Contents

Maximum nitrogen, phosphorus and potash uptake was recorded under well watered conditions in both hybrids. However, under water stress uptake of nutrients was significantly decreased. The availability of nutrients in the soil decreases under water deficit conditions due to their less solubility as well as precipitation of salts that result in altered physiological processes including low absorption and uptake of nutrients in plants grown under such conditions (Garg, 2003; Fageria *et al.*, 2002).

Farooq *et al.* (2009) reported that prime effect of water stress is that nutrient mobility and uptake is impaired in plants. TU seed treatment and foliar application increased uptake of these nutrients under water stress and well watered conditions. Protein contents also increased as nitrogen percentage increased in maize hybrids. Under conditions of water stress or high temperature, external use of thiourea can increase K⁺ uptake by chickpea and reduce ABA biosynthesis (Aldasoro *et al.*, 1981).

Similarly, oil contents were increased in both the hybrids under normal availability of water where TU seed treatment was combined with foliar application. But under water stress oil contents were decreased. Ali *et al.* (2011) reported that under drought stress oil yield and quality is severely reduced in maize.

Chapter 5

SUMMARY

The experiments were conducted in the laboratory, and Research Area, Department of Agronomy, University of Agriculture Faisalabad from 2009-2011 while analytical work was done in the Analytical laboratory, Department of Agronomy and Seed Physiology Laboratory, Department of Crop Physiology, University of Agriculture, Faisalabad. The green house experiments were laid out in completely randomized design (CRD) with four replications and one field trial was laid out in randomized complete block design (RCBD) split-split plot arrangement.

The first experiment was screening trial. Seven maize hybrids (32F10, 32B33, 33H25, 3335, 34N43, 6142, 6525) were used as experimental material in screening trial. These hybrids were screened against three water holding capacities (80% WHC, 60% WHC, 40% WHC). Data regarding emergence parameters, growth and water relations were recorded. Hybrid 34N43 recorded maximum emergence percentage, emergence index, uniformity of emergence, emergence energy and minimum mean emergence time and time for 50 % emergence as compared to all other hybrids under water stress and normal availability of water. Similarly more plant biomass, plant height, leaf area and maximum water potential, osmotic potential, turgor potential, relative water contents were observed under all irrigation regimes. Performance of hybrid 32F10 was poor with respect to emergence parameters, water relations, plant growth as compared to all other hybrids with normal availability of water and particularly under water stress. From screening trial, it was concluded that hybrid 32F10 is sensitive to water stress and hybrid 34N43 is drought tolerant hybrid. Later on three green house experiments were conducted.

In second experiment effects of seed priming with thiourea in improving the water stress tolerance in maize hybrids (32F10, 34N43) were studied. Four levels of thiourea (200mg/l, 400mg/l, 600mg/l, 800mg/l) were applied through seed treatment along with hydro priming and dry seed sowing as control under two water regimes (80% WHC, 40% WHC). Seed priming with 800 mg/l TU was most successful to maintain tissue water contents, reduce electrolyte leakage and E_{50} , mean emergence time, emergence index and improved superoxide dismutase, catalase and ascorbate peroxidase activities, shoot and root length, seedling dry weight, as compared with

control under both stress and optimal conditions. Nevertheless, activities of antioxidant enzymes were better from seed treatment with 600 mg/l thiourea under water stress. Similarly TU seed treatment decreased electrolyte leakage, MDA content and increased proline synthesis. However, effects of seed priming was more significant in hybrid 34N43 as compared to hybrid 32F10 under water stress and well watered conditions.

In third experiment, four levels of thiourea (200mg/l, 400mg/l, 600mg/l, 800mg/l) were applied through rooting medium using distilled water as control treatment against four osmotic stress levels (Control, -0.4 MPa, -0.6 MPa, -0.8 MPa) for two hybrids i.e 32F10, sensitive to water stress and 34N43, tolerant to water stress. TU translocated through roots facilitated plants for osmotic adjustment by preserving more water through enhancing turgor potential by increasing proline content and reducing electrolyte leakage. Thus growth was improved under osmotic stress with application of thiourea through rooting medium.

For fourth experiment three levels of thiourea (500mg/l, 1000mg/l, 1500mg/l) were applied through foliar application along with control under two water regimes (80% WHC, 40% WHC) for same hybrid. Foliar application of TU increased plant biomass, leaf area, plant water relations. Similarly, proline content were increased thus enhancing drought tolerance in maize hybrids. MDA content and electrolyte leakage were reduced so that stabilizing cell membrane. Foliar application of 1500 mg/l was best treatment in this regard. However, effects of foliar application of TU were more significant in hybrid 34N43 as compared to hybrid 32F10.

In field experiment seeds were treated with 800 mg/l TU combined with 1500 mg/l foliar application because these two treatments gave best results in green house trials and dry seed sowing with foliar application of distilled water was used as control treatment. Three soil water potentials (-15KPa, -30KPa, -45KPa) were used for hybrids 32F10 and 34N43. Water stress decreased crop growth, yield and nutrients uptake, protein contents and oil contents, water potential, osmotic potential, turgor potential and relative contents. More electrolyte leakage and MDA contents were observed under water stress. All these effects were more significant in hybrid 32F10 as compared to hybrid 34N43. However, seed treatment with TU combined with foliar application increased growth, improved water relation, enhanced nutrients uptake and increased yield of maize hybrids under water stress. There was

adequate increase in proline synthesis while electrolyte leakage and MDA production was suppressed significantly.

The present study suggests that seed priming with TU and its foliar application not only improved water relations, increased growth, nutrients uptake and yield of maize crop under water stress but also improved performance of maize under normal availability of water.

Future Prospects:

The ultimate goal of all agricultural research is to increase the yield of crops. In this regard, following research areas need to be addressed:

- To select plants/cultivars on the basis of drought tolerance and use of TU for improving drought tolerance in other field crops
- Formation of maize hybrid groups (tall, medium, dwarf) for comparative study against drought tolerance
- Effect of TU in combination with other inorganic salts and hormones
- Role of TU in regulating the phenolics, heat shock proteins and tocopherols under water stress

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