Influence of Thiourea Application on Biochemical Changes in Chickpea (*Cicer arietinum* L.) Genotypes Under Water Stress at Flowering Stage

जल तनाव परिस्थितियों में चने (*साइसर एरीटिनम* एल.) की चयनित प्रजातियों में पुष्पन अवस्था पर थायोयूरिया अनुप्रयोग के जैव रासायनिक प्रभाव

Shanker Lal Choudhary

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In the

Faculty of Agriculture

(Biochemistry)

by

Shanker Lal Choudhary

2016

Sri Karan Narendra Agriculture University, Jobner

S.K.N. College of Agriculture, Jobner

CERTIFICATE - I

Date : ____2016

This is to certify that **Mr. Shanker Lal Choudhary** has successfully completed the comprehensive examination held on 05-05-2016 as required under the regulation for **Master's degree.**

Head
Department of Biochemistry

Sri Karan Narendra Agriculture University, Jobner

S.K.N. College of Agriculture, Jobner

CERTIFICATE - II

Date : ____2016

This is to certify that this thesis entitled **"Influence of Thiourea Application on Biochemical Changes in Chickpea (*Cicer arietinum* L.) Genotypes Under Water Stress at Flowering Stage"** submitted for the degree of Master of Science in the subject of Biochemistry embodies bonafide research work carried out by **Mr. Shanker Lal Choudhary** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on _____

Head
Department of Biochemistry

(V.K. Yadav)
Major Advisor

(R.C. Kumawat)
Dean
S.K.N. College of Agriculture,
Jobner

Sri Karan Narendra Agriculture University, Jobner

S.K.N. College of Agriculture, Jobner

CERTIFICATE - III

Date : ____2016

This is to certify that this thesis entitled **"Influence of Thiourea Application on Biochemical Changes in Chickpea (*Cicer arietinum* L.) Genotypes Under Water Stress at Flowering Stage",** submitted by **Mr. Shanker Lal Choudhary** to the Sri Karan Narendra Agriculture University, Jobner in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Biochemistry,** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory. We therefore, recommend that the thesis be approved.

(V.K. Yadav)
Major Advisor

(G.K. Mittal)
Advisor

(Neelam Yadav)
Advisor

(M.L. Jakhar)
Director Education Nominee

Head
Department of Biochemistry

(R.C. Kumawat)
Dean
S.K.N. College of Agriculture, Jobner

Approved

Director Education
Sri Karan Narendra Agriculture University, Jobner

Sri Karan Narendra Agriculture University, Jobner

S.K.N. College of Agriculture, Jobner

CERTIFICATE - IV

Date : ____2016

This is to certify that **Mr. Shanker Lal Choudhary** of the **Department of Biochemistry,** S.K.N. College of Agriculture, Jobner has made all corrections /modifications in the thesis entitled **"Influence of Thiourea Application on Biochemical Changes in Chickpea (*Cicer arietinum* L.) Genotypes Under Water Stress at Flowering Stage"** which were suggested by the external examiner and the advisory committee in the oral examination held on _____. The final copies of the thesis duly bound and corrected were submitted on _____, are enclosed herewith for approval.

(V.K. Yadav)
Major Advisor

Head
Department of Biochemistry

(R.C. Kumawat)
Dean
S.K.N. College of Agriculture,
Jobner

Approved

Director Education
Sri Karan Narendra Agriculture University, Jobner

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Place: Jobner
Dated: / /2016 ***(Shanker Lal Choudhary)***

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ACRONYMS

@	:	At the rate of
%	:	Per cent
µl	:	Microlitre
^{0}C	:	Degree Celsius
$*O_2^-$	:	Superoxide anion
AICRP	:	All India Co-ordinated Research Project
CD	:	Critical difference
Cm	:	Centimetre
DAS	:	Days after sowing
DAIS	:	Days after imposing stress
Df	:	Degree of freedom
EC	:	Electrical conductivity
EC	:	Enzyme commission
Fig.	:	Figure
G	:	Gramme
Ha	:	Hectare
hrs	:	Hours
i.e.	:	That is
K	:	Potassium
kg/ha	:	Kilogram per hectare
M	:	Metre
m^2	:	Square metre
mg/g	:	Milli gram per gram
Ml	:	Milli litre
Mm	:	Milli metre
MT	:	Matric Tonne
N	:	Nitrogen
No.	:	Number
NS	:	Non-significant
OD	:	Optical density
P	:	Phosphorus
RBD	:	Randomized block design
Rpm	:	Revolution per minute
S	:	Sulphur
SEm±	:	Standard error of mean
TBARS	:	Thiobarbituric acid reducing substances
viz.,	:	Which are

Chapter – 1
INTRODUCTION

Pulses, generally known as food legumes, belonging to the family leguminoseae are important group of staple crops, next only to cereals for human diet, especially for the vegetarian population across the world. Apart from human and animal nutrition they also play an important role in sustaining soil fertility with their unique ability to fix atmospheric nitrogen through symbiosis with *Rhizobium species.* These crops have been considered the best option for diversification and intensification of agriculture across the globe because of their intrinsic values such as nitrogen fixing ability, higher protein content and ability to thrive well in the less endowed environments. However, per capita availability of pulses in India has been continuously decreasing, which at present is 32.5 g against the requirement of 80 g (Anonymous, 2009), thus enhancement and sustainability of production of chickpea is of paramount importance for meeting future needs. Chickpea is maximally used pulse and therefore increase in its production is very important.

Drought is commonly defined as a period without significant rainfall. It is one of the most universal and significant environmental stress affecting plant growth and productivity worldwide. There are significant differences in the tolerance of plants to drought stress depending upon intensity and duration of stress, plant species and the stage of development (Singh *et al*., 2012). Drought is the major limiting factor to crop production especially chickpea. Drought may affect the crop at any time during growth of plants. Terminal drought alone has been contributing to more than 50% yield losses in chickpea. Therefore research strategies should focus to enhance the maximum utilization of available depleting soil moisture; through

breeding efforts to focus on improving root traits that enhance the efficient extraction of soil moisture.

Water stress is a common phenomenon and it severely reduces yields of field crops grown under rainfed conditions (Jangpromma *et al*., 2010a). Drought is the major cause of yield reduction in crop plants, since water is a major limiting factor for plant growth and development mainly in arid and semiarid regions. However, the response of agricultural crops to drought stress has not yet been extensively studied. It is well known that drought stress brings about numerous metabolic, biochemical and physiological changes in plants like growth (Ashraf and Iram, 2005; Benjamin and Nielsen, 2006), water status, membrane stability (Bai *et al*., 2006), pigment content and photosynthetic activity (Ekmekc *et al.,* 2005). The first response of plants to acute water deficit is the closure of their stomata to prevent the transpirational water loss. This has been attributed to the decrease in both photosynthetic rate and internal CO_2 concentration.

Chickpea flour is used as ‘besan’ for preparation of sweets and ‘namkeen’, consumed in the form of whole grain as salad and roasted for eating. Chickpea is the premier pulse crop of India in terms of both area and production. India is the largest producer of chickpea in the world with total area of 11.55 m ha and production of 10.46 m t. About 70% of this area is under rainfed cultivation. Area under chickpea in Rajasthan accounted 12.56 lakh hectares and production 9.11 lakh tones (Directorate of Agriculture Govt. of Rajasthan, Jaipur, 2014-15). Apart from Rajasthan, other chickpea producing states in India are Madhya Pradesh, Andhra Pradesh, Maharashtra and Uttar Pradesh (Pulse Market Report, May, 2011).

Seeds of chickpea (*Cicer arietinum* L.) contain about 20 per cent protein, 5 per cent fat and 55 per cent carbohydrate. Furthermore, the

seed protein contain essential amino acids like lysine, methionine, threonine, valine, isolucine and leucine. Besides providing the essential components of human dietary and health requirements, they fix atmospheric nitrogen and enrich the soil fertility. In spite of its economic importance and its role in human health, over the last five decades, neither the area under cultivation nor productivity has increased to meet the current demands. The low production of chickpea is due to several abiotic and biotic stresses that have been challenging the crops. The ever-increasing population further aggravates growing demands of food grains include chickpea and conventional breeding efforts need to be supplemented by genomics-assisted breeding (Varshney *et al*., 2005, 2007).

Chickpea, though an important pulses crop in India, it is largely grown on stored soil moisture facing terminal drought stress, a primary limiting factor to productivity (Sharma *et al.,* 2007). The drought accounts for about 20-30 per cent loss in chickpea production depending on the severity of the stress. In recent years, rising global temperature has also become a major concern worldwide as it is affecting adversely the productivity of a number of crops including chickpea.

Water stress tolerance is a complex character and can be associated with thickness of cuticle, opening and closing of stomata, root depth and extension, hormone composition, osmotic adjustment and antioxidant production (Szegletes *et al.,* 2000). Water stress is a serious problem in many parts of world that influences the overall productivity of agricultural system. The retardation of plant growth under water stress is attributed to reduced accumulation of dry biomass due to inhibition of physiological processes (Singh *et al.,* 2000).

A major constraint to improve tolerance to abiotic stress is the lack of understanding of complex genetic and biochemical basis of stress tolerance. The development of water deficit leads to a wide range of changes in plant processes (Turner and Begg, 1981). It has been observed that, instead of normal flowering, plants show early flowering and pod development but low yield due to poor pod filling under water stress. Internal water deficit, particularly at flowering, has been found to be associated with low grain yield under drought (Pantuman *et al*., 2001). The moisture stress affects almost all biochemical processes, crop growth and the final yield. Despite many decades of research, drought continues to be a major challenge to agricultural scientists because of unpredictability of its occurrence, severity, timing and duration, coupled with other abiotic stresses, particularly high temperature.

In view of the impact of water stress on productivity it is important to develop crop plants which are tolerant to water stress. Such development can be attained by exploiting natural or induced genetic variability along with a physiological / biochemical study of the underlying mechanism. Plant resistance to water stress is a result of many complex and interrelated mechanisms. Biochemical studies therefore, can be helpful to the breeders to identify specific characteristics to be modified while planning selection and breeding.

Water stress at flowering stage significantly reduces yield while stress at pod filling stage causes greater yield reduction in green gram and pea. Reduction in the content of chlorophyll and carotenoids in plants subjected to water stress has widely been demonstrated (Baisak *et al*., 1994). Water defict influences both storage and remobilization of water soluble carbohydrates of stem. Plants under water stress often store less

amount of water soluble carbohydrates than non- stressed plants (Davidson, 1992).

Many stress alleviating molecules including thiols are crucial for enhancing the crop productivity. Thiols are well-known to maintain the redox state (-SH/-S-S-ratio) of the cell and its proper functioning under stress condition (Sahu *et al.,* 2005; Nathawat *et al*., 2007; Srivastava *et al.,* 2009; Anjum *et al.,* 2011; Perveen *et al.,* 2013). Since, thiourea has been identified as an effective bioregulator imparting resistance to crop plants against abiotic stress, it is quite possible that seed treatment with external thiols in the form of thiourea might result in up-regulation of antioxidant defense system. Improvement in plant growth and development under different stresses due to application of thiourea has been observed in crops like maize (Sahu *et al*., 1993), wheat (Sahu and Singh, 1995; Sahu *et al.,* 2006), pearl millet (Parihar *et al.,* 1998), culsterbean (Garg *et al.,* 2006). There is little information available on the influence of exogenously applied thiourea on membrane stability and antioxidant defence system of chickpea under water stress. Hence, the present study is proposed to investigate the effects of water stress and application of thiourea on some biochemicals / enzymes activity in chickpea.

With this background, the proposed research study has been under taken to understand the physiological and biochemical changes in contrasting chickpea genotypes under normal and water stress conditions. It is hypothesized that the information will help the molecular biologists and plant breeders in developing drought tolerant chickpea genotype in near future. The objectives of this investigation were.

(1) To study stress related metabolites under water stress in chickpea genotypes.

(2) To study stress tolerant enzymes under water stress in chickpea genotypes.

Chapter – 2
REVIEW OF LITERATURE

2.1 Antioxidant defence system in plants:-

As mechanisms of responses to drought stress varies with genotypes and growth stages of individual plants (Ashraf, 2004), it would be much more valuable if biochemical indicators could be specified for individual crop species. Knowledge of interrelationships among various physiological responses to dehydration can offer insight for developing useful strategies to improve drought stress tolerance in chickpea.

Pulses are important dietary constituent in human and animal diets. Abiotic stresses (drought, salt, temperature, UV radiations) alone are responsible for more than 50% yield reduction of major crops including chickpea (Kahraman *et al.,* 2015). Deepti *et al.,* (2007) reported that water deficit or dehydration is the most crucial environmental factor that limits crop productivity and influences geographical distribution of many crop plants.

Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel *et al*., 2008).

When plant cells are exposed to severe environment conditions, they generate reactive oxygen species, (ROS) that causes oxidative damage. ROS are produced in sufficient quantities in various subcellular compartments or organelles and each organelle has mechanism for scavenging/quenching these harmful ROS. Protective antioxidant defence exists in plants, both enzymic and non-enzymic. Examples of enzymic antioxidant defence includes ascorbate peroxidase (APX) and glutathione reductase (GR), having a role in scavenging of H_2O_2 efficiently. The

superoxide dismutase (SOD) scavenge superoxide anion ($^{*}O_2^{-}$).The catalase (CAT) and peroxidase (POX) are also active in removing excess of H_2O_2. The combined action of CAT/POX and SOD enzymes convert the potentially dangerous superoxide anion ($^{*}O_2^{-}$) and H_2O_2 to water (H_2O) and molecular oxygen. They ensure that formation of the next species such as partial reduction of dioxygen series, the dihydroxyl ($^{*}OH$) radical are formed less. These dihydroxyl radicals can react indiscriminately with all macromolecules. It is also believed that SOD partakes in the scavenging of yet another species, the singlet oxygen ($^{1}O_2$) (Matheson *et al*; 1975; Scandalios, 1993).

$$2\,{}^{*}O^{-}_{2} + 2H^{+} \xrightarrow[\text{Spontanious}]{\text{SOD or}} H_2O_2 + O_2$$

$$H_2O_2 + AH_2 \xrightarrow[\text{Electron doner}]{\text{POX}} 2H_2O + A \quad (\text{Electron acceptor})$$

$$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2$$

Examples of antioxidants molecules specialized in scavenging the free radicals by regenerating the oxidized form of antioxidants are L-ascorbic acid (ASC) and glutathione (GSH, reduced form). The GR enzyme and the mono- and dehydro-ascorbic reductase (MDHAR and DHAR) belong to this mechanism of protection. ASC can also react non-enzymatically with superoxide and singlet oxygen species. ASC and GSH are present in high concentration in the chloroplast of higher plants (Winston, 1990; Asada, 1992a & 1992b).

2.2 Water stress and role of plant antioxidant system

Drought stress is one of the crucial factors affecting the global plant growth and crop productivity. Many crop plants have been studied for their responses to the generation of ROS when water stress is imposed on these plants. Further, tolerant species have been reported to accumulate

certain metabolites that regulate osmotic changes (osmoregulants) and display antioxidant activity through an elevation in the contents of molecular antioxidants and the activities of scavenging enzymes (Larson, 1988; Price and Hendry, 1989; Badiani *et al*; 1990; Smirnoff, 1993; Alscher and Donahue, 1997). The critical roles of proline and glycine-betaine, as well as the role of abscisic acid (ABA), under drought stress conditions have been actively researched to understand the tolerance of plants to dehydration. In addition, drought stress-induced generation of active oxygen species is well recognized at the cellular level and is tightly controlled at both the production and consumption levels in vivo, through increased antioxidative systems.

There is no option except to produce more food and other commodities under conditions of diminished *per capita* arable land and irrigation water resources. Harnessing the best out of frontier technologies and their integration with traditional wisdom in farming as well as environmental protection is an important action to meet the growing food demand (Swaminathan, 2006). One prominent area of research is study on drought tolerance in crop plants for sustainable agriculture. Drought or water stress is especially important in countries where agricultural crops are essentially rainfed. While irrigation is the method of choice in averting the drought conditions in many areas of the world, alternative low-input approaches are being explored. Biotechnology offers a promising array of tools that may be useful in achieving drought tolerance in plants. Certain plants have devised mechanisms to survive under low water conditions often classified as tolerance, avoidance or escape through accumulation of an array of large and small molecular weight compounds conferring drought tolerance (Zidenga, 2006).

The identification of biochemical responses that have adaptive value in conferring tolerance is of basic interest to plant biochemists. Their intensive research over decades reveal occurrence of a molecular cascade of events; beginning with stress perception and signaling, followed by a

change, either directly in the membrane components or by way of gene expression (Bray *et al,* 2000). Moreover, interacting signal transduction processes and cross-talk between signaling systems may have important consequences for the plant's subsequent responses to other biological and environmental assaults, and thus its survival and fitness (Cosgrove *et al;* 2000).

In a study Rut, (2015) compared capacity of antioxidant enzymes between a drought-tolerant, and a drought-sensitive sugarcane variety. Young sugarcane plants were grown under controlled conditions. Water stress was imposed at 4 leaf stage by withholding water supply for 4, 6 and 8 days as compared to control that received adequate water supply. First fully expanded leaves were harvested and proteins were extracted for assaying the activities of ascorbate peroxidase (APX), peroxidase (POX) and catalase (CAT). APX was a major antioxidant system in leaves of both the sugarcane varieties as it accounted for 65 % in tolerant variety and 69 % in susceptible variety in scavenging H_2O_2. POX accounted for 27 % and 23 % respectively in the removal of H_2O_2. Negligible scavenging activities of CAT were observed in both sugarcane varieties. APX activities in leaves of tolerant variety were induced and maintained during progressive water stress and were 15 % higher than in sensitive variety. POX activities in tolerant variety were 30 % greater than that in susceptible variety. Thus, drought tolerance in tolerant variety was, at least partially, due to the greater activities of APX and POX under water deficit stress as compared to those in susceptible variety.

Burman *et al.* (2004) subjected clusterbean (*Cyamopsis tetragonoloba* Taub.) plants to water stress by withholding irrigation at pre- and post-flowering stages in pot culture and reported that water stress significantly decreased shoot water potential, relative water content of leaves, net photosynthetic rate, contents of total chlorophyll, starch and soluble proteins as well as nitrate reductase activity at both the growth stages. Application of phosphorus and thiourea seperatly or combined

application increased most of these parameters. These results also revealed synergistic effects of P and thiourea in enhancing net photosynthesis, leaf area, chlorophyll content and nitrogen metabolism leading to significant improvement in plant growth and seed yield under water stress condition. Akladious, (2014) studied two levels of thiourea (10 and 20 mM), applied before sowing (seed treatment). The results indicated that the plants exposed to temperature stress exhibited a significant decline in growth parameters, chlorophylls, relative leaf water content, oil content, leaf nutrient status, and nitrate reductase activity. Treatment with thiourea, especially when applied at 10 mM, improved the above parameters and induced non-enzymatic and enzymatic antioxidants responsible for antioxidation.

2.3 Water stress in relation to physiological and biochemical parameters

Gupta *et al.,* (2000) studied the physiological mechanism of drought tolerance in chickpea. It was observed that tolerant genotypes had lower membrane injury, retain imbibitions growth, have osmotic adjustment and higher water use efficiency. Behboudian *et al.,* (2001) studied the effect of water stress, imposed after podding on the accumulation of amino acids and soluble sugars that were reduced in chickpea seeds.

Deshmukh *et al*., (2002) suggested the possibility of using simple traits like relative water content (RWC) and membrane injury index (MII) for screening chickpea genotypes for drought tolerance. The relative water content and membrane injury index of a genotype measured during early phase was found to provide an indication of its relative membrane injury index during reproductive stage.

Reddy *et al.* (2004) reported that drought stress progressively decreases CO2 assimilation rates due to reduced stomatal conductance. Drought stress also reduced the contents and activities of enzymes, ribulose-1,5-bisphosphate carboxylase/oxygenase.

Kumar *et al.* (2003) reported genotype differences in chickpea for chlorophyll content and relative water content (RWC) at 50% flowering and podding stages. Relative water content (RWC) was higher in drought-tolerant genotypes than susceptible genotypes and chlorophyll was reduced in both drought-tolerant and susceptible genotypes under water stress.

Kushwaha *et al.* (2003) reported that genotypes which possessed high initial water content (IWC) along with high relative water content had less damage to the assimilatory system that resulted in production of relatively higher biomass. Water stress reduced the osmotic potential of tissue in some plants. This helps the plant in maintenance of turgor pressure for normal metabolic activities. The significant correlation between drought susceptibility index (DSI) and RWC suggest that these parameters may be used as a selection criteria for chickpea genotypes in drought – stressed environment. (Gunes *et al*., 2008 and Sairam *et al.,* 2000).

Rozrokh *et al.,* (2012) studied twenty genotypes of chickpea which included drought resistant genotypes and drought susceptible genotypes for amounts of potassium, sodium and calcium, proline, soluble proteins, soluble carbohydrate in grains. In comparison of rain-fed and irrigated conditions, proline concentration in the grains increased, while potassium, sodium and calcium decreased. Akitha *et al*. (2015) studied that soybean varieties for the changes in their antioxidant enzyme activity, total isoflavone content and also the influence on its growth under drought stress. They reported that mild water deficit stress for 5 days during late vegetative stage increased total superoxide dismutase activity. Water deficit stress also triggered the activities of catalase, peroxidase, ascorbate peroxidase and glutathione reductase enzymes in all the genotypes. High accumulation of proline and lower level of lipid peroxidation were also reported.

Nayyar *et al.,* (Gosavi *et al*. (2014) studied contents of proline, glycine betaine, and the activity of Delta ^{1}-pyrroline-5-carboxylate

synthetase (P5CS) along with soluble protein in the leaves of these genotypes by imposing -0.5MPa stress, created by PEG-6000. They reported that crude protein and tryptophan contant were higher in susceptible genotypes as compared to tolerant ones. Drought tolerant genotype, showed comparatively higher potential to accumulate proline and glycine betaine as compared to susceptible genotypes.

Nayyer *et al*., (2004) reported that Chickpea (*Cicer arietinum* L.) yields are drastically reduced by water stress that occurs frequently in northern region of India. The effects of the stress were investigated at the metabolic level by examining the endogenous status of polyamines, active oxygen species and antioxidants which are reduced. Biochemical analysis showed that the activity of antioxidant enzymes increases under water stress conditions in chickpea. Application of 20 g/ha selenium increased enzyme activity of Catalase, Glutathion peroxidase and Superoxide dismutase (Mohammadi *et al.,* 2011). Terminal drought causes major yield loss in chickpea, so it is important to identify genotypes with the best suited adaptive traits to secure yield in terminal drought-prone environment (Krithika *et al*., 2015).

Nayyar *et al.,* (2005) reported that soyabean seedlings experienced significantly more stress injury (as electrolyte leakage) than chickpea at different water stress levels. In chickpea, the root water content was higher than in soyabean during stress. In both the crops, drought stress coincided with a decrease in relative water content (RWC) that was less severe in the upper leaves than elsewhere in the plant. The drought also increased proline levels in both the crop plants.

Kumar *et al*., (2006) reported that chickpea genotypes were more sensitive to moisture stress at early vegetative growth stage and the maximum reduction in yield and yield components were observed when moisture stress treatment was given between 40-60 DAS. Basu *et al.,* (2007) reported that Chickpea is cultivated predominantly over 75-80% in water-limiting environment. The crop usually faces terminal drought during

pod filling stage leading to significant reduction in the grain yield. Among various traits, osmotic adjustment (OA) is considered as an important physiological trait for adaptation to drought.

Najaphy *et al.,* (2010) reported that the water deficit stress increased concentration of soluble proteins in the leaves up to 43% in comparison with normal watering in chickpea. It is suggested that dehydration-responsive changes in expression of proteins may lead to cellular adaptation against water deficit conditions. Neeru *et al.,* (2011) reported that Chickpea is a heat sensitive crop hence its potential yield is considerably reduced under high temperatures exceeding 35°C. The oxidative damage measured as malondialdehyde and hydrogen peroxide content that increased manifolds in water stressed plants coupled with inhibition in the activities of several enzymes such as Superoxide dismutase, Catalase, Ascorbate peroxidase, Glutathione reductase and increased levels of non-enzymatic antioxidants (ascorbic acid, glutathione, proline). Mehrjerdi *et al.,* (2013) reported that with increasing drought stress, the photosynthetic rate, evapotranspiration, water use efficiency, chlorophyll a, chlorophyll b, total phenols and membrane stability index were significantly reduced in chickpea.

Cevik *et al*., (2014) reported that Cyclitol derivatives significantly decreased leaf water potential, lipid peroxidation and H_2O_2 levels of wild and cultivated species of chickpea under water stress. Cyclitol treatments affected antioxidant enzyme activities differently in both species of chickpea under water stress. Siamak *et al.,* (2014) reported that seed yield, plant biomass, plant height and water use efficiency decreased whereas protein content and soluble carbohydrates increased as a result of drought stress in chickpea. Their results also showed that application of ascorbic acid increased water use efficiency significantly as compared to control plants under water stress.

Merlo *et al.,* (2014) reported that the growth inhibition and reduction of ribonuclease activity induced by ABA were counteracted or reversed by

polyamines in chickpea. In a study, nutritional (starch, total sugars, proteins, iron, zinc and copper) and antinutritional (tannins, phytic acid and trypsin inhibitor activity) components in the seeds of chickpea genotypes were analyzed and the results showed that higher contents of iron and starch along with lower phytic acid content could be the characteristics of chickpea genotypes exhibiting tolerance towards both salinity stress and water stress (Kaur *et al.,* 2015).

Application of thiourea through seed treatment and foliar application has been shown to mitigate the effect of water stress in mung bean (Mathur *et al*., 2006); clusterbean (Garg and Burman., 2006) and mothbean (Kumawat *et al*., 2012). Foliar spray (1000 ppm) of thiourea at pre-flowering stage showed increase in net photosynthesis, total chlorophyll, starch, reducing sugar, soluble protein, nitrate reductase activity and seed yield. In another experiment, the induced water stress created by withholding water supply from flowering stage causes significant decline in the level of chlorophyll with an increased amount of carotenoids in cowpea (Patel and Rao., 2007).

Mafakheri *et al.* (2010) studied the effect of drought stress on proline content, chlorophyll content, photosynthesis, transpiration, stomatal conductance and yield characteristics in three varieties of chickpea (drought tolerant Bivaniej and ILC482 and drought sensitive Pirouz). They reported that drought stress imposed during vegetative growth or anthesis significantly decreased chlorophyll a and chlorophyll b, induced proline accumulation, photosynthesis, transpiration, stomatal conductance and yield.

Nazarali *et al.* (2011) reported that drought stress significantly increased carotenoid content, soluble sugar and proline content in the leaves of sunflower. Shahid *et al.* (2012) showed that salt stress in pea reduced the growth, plant fresh/dry biomass and number of leaves, photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll contents and cell membrane stability index (MSI) while elevated antioxidant

enzymes, i.e. superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) as well as organic solutes proline, glycinebetaine and total free amino acids, lipid peroxidation (LPO), hydrogen peroxide (H_2O_2) and leaf abscisic acid (ABA).

Ulemale *et al*. (2013) evaluated fourteen chickpea genotypes under moisture stress and non-stress condition to study the physiological indices for drought tolerance. Significant differences were exhibited amongst the genotypes for phenology, vegetative growth, sink capacity and physiological parameters and yield due to moisture stress. They also reported that RLWC, membrane injury index, chlorophyll content, chlorophyll stability index were decreased and proline accumulation and nitrate reductase activity were increased. These can be used as parameters for selecting genotypes for drought tolerance.

Kumar *et al*. (2012) studied five contrasting genotype of chickpea, ICCV-4958, HC-5, RSG-931 and CSJ-379 having wide adaptability to drought under two conditions irrigated and rainfed and found that under rainfed condition genotype ICCV-4958 and HC-5, had high dry weight, root and shoot ratio, photosynthesis and transpiration rates, photochemical efficiency and better plant water status but lower stomatal conductance than other genotypes. These traits are directly associated with maximum seed yield per plant, i.e. 15.6 g and 14.7 g per plant, respectively, in these genotypes. Therefore, both the genotypes in future can be used in crop improvement programme of chickpea breeding for drought tolerance.

Sabaghpour *et al*., (2006) reported that yield losses due to terminal drought range from 35 to 50% in chickpea. It seems that effects of drought stress in legume crops are more complex than that of other plants; because the plant establishment and legume-Rhizobium symbiosis are susceptible to drought stress (Asghari *et al*., 2010).

Chapter – 3
MATERIALS AND METHODS

3.1 Plant materials

The present investigation was carried out on chickpea (*Cicer arietinum* L.). The seeds of three genotypes (RSG-888, RSG-896, RSG-974) were obtained from Incharge, AICRP on chickpea, RARI, Rajasthan Agriculture Research Institute, Durgapura, Jaipur. A field experiment was carried out using 3 genotypes at Agronomy farm, S.K.N. Collage of Agriculture, Jobner during *Rabi* season 2015-2016 to investigate "Influnce of Thiourea Application on Biochemical Changes in Chickpea (*Cicer arietinum* L.) Genotypes Under Water Stress at Flowering Stage". The proposed investigation was conducted in randomized block design. In order to achieve the objectives of present investigation the experiment was planned and executed as described below

3.2 Chemical and glassware

All the chemicals used for analysis and biochemical work were of Analytical grade. All glasswares used were of either corning or borosil.

3.3 Preparation of samples

Chickpea genotypes were raised/sown in 2m x 3m plot, keeping row to row distance (30 cm x 30 cm) and plant to plant distance (10 cm x 10 cm). Sowing was done on 4 November 2015 using dibbler. Thirum treated seeds were placed at a depth of 6-8 cm. The recommended dose of fertilizer (20 kg N, 30 kg P_2O_5, 30 kg K_2O) were given to the crop. The sowing was done in randomised block design with three replications of three genotypes in 4 sets as follows :-

1. Control (normal irrigation)
2. Control + Thiourea application

3. Water stress
4. Water stress + Thiourea application

3.4 Application of stress

Stress was created in replication sets by withholding irrigation at 70 days after sowing (DAS). Thiourea (1000 ppm) was applied through foliar spray at the same time DAS. The observations were recorded after 77 DAS (7 days stress), 84 DAS (14 days stress), 91 DAS (21 days stress) and 98 DAS (28 days stress). Leaf samples from both control and stressed plants were used for analysis. Meteorological data were recorded during experiment.

3.5 Analysis

All physiological and biochemical parameters were analyzed at four stages viz. 77 DAS, 84 DAS, 91 DAS and 98 DAS and the yield was recorded at maturity (118 DAS).

Second and third (from terminal) fresh leaves were collected for the analysis at all the stages. Analysis was done in triplicate for following parameters:-

3.5.1 Membrane stability index

3.5.2 Relative water content

3.5.3 Proline Content

3.5.4 Total amino acids

3.5.5 Glutathione reduced

3.5.6 Peroxidase

3.5.7 Carotenoids content

3.5.8 Malondialdehyde

3.5.9 Phenols

3.5.10 Soluble proteins

3.5.11 Seed yield

3.6 Procedure and Techniques

3.6.1 Membrane stability index (Sairam,1994)

The procedure described by Premchandra *et al.* (1990) modified by Sairam (1994) was used for membrane stability index. Leaf sample (0.5 g) was placed in distilled water (50 ml). One set was kept at 40^{o}C for 30 minutes and its conductivity (C_1, for electrolytic leakage) was recorded using conductivity meter. The second set was kept in boiling water bath (100 ^{o}C) for 10 minutes and its conductivity (C_2) was recorded after cooling at room temperature. The MSI was calculated according to the formulae:

$$MSI\% = (1-C_1/C_2) \times 100$$

3.6.2 Relative water content

100 mg fresh weight of the leaves were kept in distilled water for 4 hours (Barrs and Weatherly, 1992) to obtained turgid weight.

The turgid weight was recorded after blotting the excess water on the surface of the sample. Dry weight was obtained after drying the samples in oven at 60˚ C till constant weight occurred. The relative water content (RWC) was calculated by the formula given by Slavik (1994).

$$RWC\% = \frac{\text{Fresh weight – dry weight}}{\text{Turgid weight – dry weight}} \times 100$$

3.6.3 L-Proline content (Bates *et al.* 1973)

Fresh leaf sample (0.2 g) were extracted in 5.0 ml sulphosalicylic acid (SSA) 3% [Appendix-III A (a)], using mortar-pestle at room temperature. The homogenate were centrifuged at 8000 rpm for 10 minutes. The clear supernatants were collected in clear test tubes separately. To 1.0 ml of supernatant was added 2 ml of glacial acetic acid and mixed throughly, followed by 2.0 ml acid ninhydrin reagent [Appendix-III A (b)] was added and mixed well. The test tubes containing assay mixtures were incubated in a boiling water bath for an hour and then cooled

to room temperature. Four ml of toluene solvent was added to each tube and mix well using vortex mixture. The pink colour of L-Proline as extracted in SSA and taken up by the solvent after incubation was separated using a separating funnel. Toluene fractions were collected and intensity of pink colour read at 520 nm on a spectrophotometer. A standard curve was prepared using L-Proline (0.1 mg/ ml).

3.6.4 Total free amino acids (Mertze ET, Mishra PS and Jambunathan R, 1974)

Amino acids were extracts with hot 80% ethanol from 100 mg leaves in 2ml distilled water. Pipetted out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard solution and 0.2 ml of the sample extract in different numbered test tubes. Volume was made up to 1ml with distilled water in each test tube. In other tube take 1ml distilled water in a separate tube acted as control. Added 1ml of ninhydrin reagent to each tube, mixed and kept the test tube in boiling water bath for 15 minutes. At this stage, the colour is developed. The tubes were brought to room temperature and 3 ml diluents were added to each tube. OD was taken at 570 nm in spectrophotometer, adjusting zero OD with control using standard curve. Quantity of amino acids was calculates and results were expressed as mg amino acid per g of the sample.

3.6.5 Glutathione Reduced (GSH) (Bailey, 1998).

Fresh leaf samples (0.25 g) were extracted in mortar-pestle using 5.0 ml of 0.1 M phosphate- buffer, pH 7.8 [Appendix-III B (a)] containing 1mM EDTA [Appendix-III B (c)] prepared by dissoluing 50 mg EDTA disodium salt in 10 ml of 0.1 M phosphate buffer, (pH 7.8) and centrifuged at 8,000 rpm for 10 minutes. Supernatant collected after centrifugation was made up to 5 ml with 0.1 M phosphate buffer and used for assay. To 1.0 ml of aliquot, 2.8 ml of 0.1 M phosphate buffer (pH 7.8) was added followed by 0.2 ml of 5-5 Dithiobis Nitro Benzoic Acid (DTNB) [Appendix-III B (b)]. After mixing, the reaction mixture was incubated for 30 minutes at room temperature after which 4 ml distilled water was added. The intensity of

yellow colour was measured at 410 nm on spectrophotometer (Systronic) against reagent blank. A standard curve was prepared using GSH (0.1 mg/ml).

3.6.6 Peroxidase: EC. 1.11.1.7 (Costa *et al.* 2002).

200 mg fresh leaves were homogenized in a pre chilled mortar pestle kept under ice cold condition using 2 ml extraction buffer, 0.1M sodium phosphate buffer, pH 7.2[Appendix-III C (a)] containing 1 mM β-mercaptoethanol and 1% (w/v) polyvinyl pyrolidone (PVP). The homogenates were centrifuged at 10,000 rpm for 20 minutes. The supernatants were used for the enzyme assay. POX activity was determined in the supernatant of centrifuged homogenates by measuring the increase in absorption at 470 nm in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.05 ml enzyme extract, and 10 mM H_2O_2.

3.6.7 Carotenoid content (Wellburn, 1994).

Total chlorophyll in leaves was determined by DMSO (dimethylsulphoxide) method. Finely chopped 50 mg chickpea leaves were weighed and taken in graduated test tubes. 10 ml of DMSO was added to each tube and incubated at 65^{o}C for 3 hrs. After incubation, the tubes were allowed to cool at room temperature and the volume was made up to a total of 10 ml by adding DMSO. The optical density (OD) was recorded at 663 and 645 nm by taking DMSO as blank .The amount of chlorophyll present in the sample was calculated using standard formulae:-

Total chlorophyll (mg/g) = 22.2 (O.D. at 663) + 8.02 (O.D. at 645)

The procedure was same for carotenoids as total chlorophyll content. For estimation of carotenoids absorbance was recorded at 480 nm and the content was calculated using the formula:-

Carotenoid µg /ml = (1000 A_{480} - 2.14 Ca- 70.16 Cb / 220)

C_a (mg/litre) = (12.7 A663- 2.69 A645)

C_b (mg/Litre) = (22.9 A645 - 4.68 A663)

3.6.8 Malondialdehyde (Heath and Packer, 1968).

Fresh leaf samples (0.2 g) were extracted in 5.0 ml of 6% trichloroacetic acid (TCA) [Appendix-III D (a)] and the extract was centrifugation at 8,000 rpm for 10 minute. Supernatant were collected in separate tubes. To 1 ml of the supernatant taken in a clean, dry test tube, was added 2.0 ml of Thio-Barbituric Acid (TBA) reagent [Appendix-III D (b)], mixed and incubated for half an hour in a boiling water bath. The tubes were than cooled to room temperature. The assay mixture was then centrifuged at 5,000 rpm for 10 min. and clear supernatant bearing yellow to light orange colour was read on spectrophotometer at two wavelengths *viz*. 532 nm (major for MDA) and 600 nm (minor for interfaring substance) and the concentration of MDA was calculated as follows:-

MDA (mM) = (O.D.532 –O.D. 600) x 155 (extinction coefficient)

3.6.9 Total phenol (Mahadevan and Sridhar, 1974)

Total phenol content (mg/g) in leaves was estimated by folin-ciocalteu method in which 500 mg leaves were grinded in 5-6 ml hot 80% alcohol [Appendix-III E (b)] and the homogenate was centrifuged at 5000 rpm for 10 minutes. The clear supernatant was taken and volume of this supernatant was made up to 10 ml with 80% alcohol. 0.5 ml of the phenol extract is taken out in another test tubes and 1 ml volume was made with distilled water. Folin reagent 0.5 ml, 1ml 20% sodium carbonate solution [Appendix-III E (a)] was added and final volume was made to 10 ml with distilled water and place the tubes in boiling bath for 1 min. after cooling the tubes takes OD at 650nm. Total phenol content was worked out from the readings with the help of standard curve made from concetration of catechol.

3.6.10 Soluble Protein (Lowry *et al*., 1951)

For determination of soluble protein (mg g^{-1} f.wt) 100 mg of leaf sample was homogenized in phosphate buffers (0.1 M, pH 7.5). The

homogenate was centrifuged at 5000 rpm for 10. The supernatant was collected and final volume was made to 5 ml with buffer. The proteins in the extract were estimated using the method of Lowry *et al*. (1951). An extract volume of 0.2 ml was taken in test tube and at the same time 0.2, 0.4, 0.6, 0.8, 1.0 ml of standard protein solution (BSA 0.1 mg/ml) were taken in separate numbered test tubes. In each test tube, the volume was made to 1.0 ml with dH_2O that served as control.

After adding 5.0 ml of alkaline copper solution in each test tube, the mixture was kept at room temperature for 10 min. This was followed by addition of 0.5 ml of diluted (1:1) folin ciocatteau's reagent. The mixture was incubated at room temperature for 30 min under dark and the absorbance of the blue colour was recorded at 760 nm using spectrophotometer. The quantity of the protein in leaf sample was calculated using the standard curve.

Reagent preparation

Reagent A = 2% Na2 CO3 in 0.1 N NaOH

Reagent B = 0.5% CuSO4 (copper sulphate) in 1% potassium sodium tartrate

Reagent C = Alkaline copper solution was prepared by mixing reagent A and B in the ratio of 50:1at the time of use

Reagent D = the commercial folin- ciocatteau's reagent was Diluted with equal volume of water

Statistical analysis

The experiments were conducted in Randomised Block Design (RBD). All the observations were taken in three replications for each genotype and treatment. The data were statistically analysed using Factorial Randomized Block Design (FRBD) according to Panse and Sukhatme, 1985.

Chapter-4
EXPERIMENTAL RESULTS

The results of the experiment entitled **"Influence of thiourea application on biochemical changes in chickpea (*Cicer arietinum* L.) genotypes under water stress at flowering stage"** conducted during the *rabi* season 2015-2016 under field condition in agronomy farm, S.K.N. College of Agriculture, Jobner are presented and described in this chapter. This experiment was condected in three replications of each variety. The water stress was created by withholding irrigation at 70 days after sowing and the same time thiourea application was given to both the sets of the plants, control (Normal irrigation) and water stress. The observations of different parameters were recorded at 7, 14, 21, and 28 days after imposing stress (DAIS).

4.1 Membrane stability index (%)

Table 4.1 shows the effect of water stress on membrane stability index (%) in three chickpea varieties at four developmental stages. Data presented in the table showed that membrane stability index varied significantly among varieties and treatments at flowering stage. The membrane stability index increased in all the three varieties with the growth stages. The membrane stability index increased from 67.42 to 86.77 percent in variety RSG-888, from 68.50 to 84.14 percent on RSG-896 and 60.66 to 79.99 percent in RSG-974 under control condition (normal irrigation).

The membrane stability index decreased due to water stress in all three varieties. The decreased in membrane stability index due to water

stress in variety RSG-888 was 12.53%, 1.95%, 3.48% and 3.34% respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. Similarly, the decrease in MSI due to water stress as compared to control was 16.90%, 1.70%, 1.96% and 2.76% in variety RSG-896 at the four stages 7, 14, 21 and 28 days after imposing stress (DAIS), respectively. The decrease in MSI due to water stress in RSG-974 was 12.52%, 1.68%, 3.96% and 1.27% respectively at 7, 14, 21 and 28 DAIS. The result thus shows that the decrease in MSI due to water stress was maximum at 7 DAIS in all the three varieties. Among three varieties the maximum decrease in MSI due to water stress was observed in RSG-896 followed by RSG-888 and RSG-974. The later two varieties had almost the same magnitude of reduction in MSI due to water stress at 7 DAIS.

The application of thiourea (1000 ppm) through spray to the plants at 70 days after sowing (DAS) showed increase in MSI under control (Normal irrigation) as well as under water stress. The increase in MSI due to application of thiourea under water stress was maximum at 7 DAIS in all the three varieties was 19.06% in RSG-888, 11.54% in RSG-896 and 4.65% in RSG-974 as compared to water stress. Thus, the response of RSG-888 to thiourea in reversing the effect of water stress was maximum followed by RSG-896 and RSG-974. The effect of thiourea application was negligeable at later stages in all the three varieties.

Table : 4.1 Effect of thiourea on membrane stability index in chickpea under water stress

Treatments / Varieties	Membrane stability index (%)			
	7 DAIS	14 DAIS	21 DAIS	28 DAIS
RSG-888				
Control	67.42	75.03	85.05	86.77
Control + thiourea	72.75	75.83	86.16	88.22
Water stress (WS)	58.97 (-12.53)	73.56 (-1.95)	82.09 (-3.48)	83.87 (-3.34)
WS + Th	70.21 (19.06)	76.31 (3.73)	82.53 (0.53)	84.65 (0.93)
RSG-896				
Control	68.50	72.89	81.26	84.14
Control + thiourea	65.07	74.03	83.61	86.06
Water stress (WS)	56.92 (-16.90)	71.65 (-1.70)	79.66 (-1.96)	81.81 (-2.76)
WS + Th	63.49 (11.54)	72.78 (1.57)	80.21 (0.69)	82.76 (1.16)
RSG-974				
Control	60.66	71.42	77.42	79.99
Control + thiourea	65.46	73.12	78.26	83.07
Water stress (WS)	53.06 (-12.52)	70.22 (-1.68)	74.56 (-3.69)	78.97 (-1.27)
WS + Th	55.53 (4.65)	70.96 (1.05)	77.25 (3.60)	81.70 (3.45)
SEm±	**3.55**	**3.91**	**4.35**	**4.48**
CD (P=0.05)	**10.40**	**11.45**	**12.75**	**13.14**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent decrease due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

4.2 Relative water content (%)

Table 4.2 shows the effect of water stress on relative water content (%) in three chickpea varieties at four developmental stages. Data presented in the table showed that relative water content varied significantly among varieties and treatments at flowering stage. The relative water content decreased in all the three varieties with the growth stages. The relative water content decreased from 82.82 to 76.61 percent in variety RSG-888, from 76.69 to 71.41 percent in RSG-896 and 74.75 to 68.15 percent in RSG-974 under control condition (normal irrigation).

The relative water content decreased due to water stress in all three varieties. The decrease in relative water content due to water stress in variety RSG-888 was 4.68%, 2.96%, 5.33% and 5.48% respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. The maximum decrease due to water stress was observed at 28 DAIS. Similar, decrease in relative water content due to water stress as compared to control was observed in other two varieties which were 2.08%, 6.16%, 6.87% and 4.78% in variety RSG-896 and 3.45%, 3.91%, 4.30% and 6.95% in RSG-974 respectively at 7, 14, 21 and 28 DAIS. The result shows that the decrease in relative water content due to water stress was maximum at 28 DAIS in all the three varieties. Among three varieties the maximum decreased in relative water content due to water stress was observed in RSG-974 followed by RSG-896 and RSG-888. The later two varieties almost the same magnitude of reduction in relative water content due to water stress at 28 DAIS.

The application of thiourea (1000 ppm) through spray to the plants at 70 days after stress (DAS) showed decrease in relative water content under control (Normal irrigation) as well as under water stress. The decrease in relative water content due to application of thiourea under water stress was maximum in all the three varieties which was 3.89% in RSG-896, 3.34% in RSG-888 and 2.95% in RSG-974 as compared to water stress. Thus, the effect of thiourea in the effect of water stress was maximum in RSG-896 followed by RSG-888 and RSG-974. The effect of thiourea application was negligeable at later stages in all the three varieties.

Table : 4.2 Effect of thiourea on relative water content in chickpea under water stress

Treatments / Varieties	Relative water content (%)			
	7 DAIS	14 DAIS	21 DAIS	28 DAIS
RSG-888				
Control	82.82	81.41	78.70	76.61
Control + thiourea	79.12	78.22	75.92	74.50
Water stress (WS)	78.94 (-4.68)	79.00 (-2.96)	74.50 (-5.33)	72.41 (-5.48)
WS + Th	78.60 (-0.43)	76.36 (-3.34)	72.28 (-2.97)	70.94 (-2.03)
RSG-896				
Control	76.69	77.91	74.77	71.41
Control + thiourea	75.32	74.93	71.98	69.13
Water stress (WS)	75.09 (-2.08)	73.11 (-6.16)	69.63 (-6.87)	67.99(-4.78)
WS + Th	72.79 (-3.06)	70.99 (-2.89)	67.97 (-2.38)	65.34 (-3.89)
RSG-974				
Control	74.75	73.06	69.00	68.15
Control + thiourea	72.92	70.28	67.04	65.01
Water stress (WS)	72.17 (-3.45)	70.20 (-3.91)	66.03 (-4.30)	63.41 (-6.95)
WS + Th	70.04 (-2.95)	68.53 (-2.37)	64.18 (-2.80)	63.10 (-0.48)
SEm±	**4.05**	**4.00**	**3.84**	**3.73**
CD (P=0.05)	**11.87**	**11.74**	**11.27**	**10.94**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent decrease due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent decrease due to application of thiourea as compare to water stress.

4.3 Proline (mg g^{-1})

Table 4.3 shows the effect of water stress on proline content in three chickpea varieties at four **developmental** stages. Data in the table shows that proline varied among varieties and treatments at flowering stage. Proline content increased in all the three varieties with the growth stages. Proline increased from 1.97 to 2.77 mg g^{-1} in variety RSG-888, from 1.98 to 2.83 mg g^{-1} in RSG-896 and 2.13 to 2.92 mg g^{-1} in RSG-974 under control condition (normal irrigation).

Proline content increased due to water stress in all three varieties. The increase in proline due to water stress in variety RSG-888 was 13.70%, 11.76%, 10.56% and 10.10% respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. Similarly, the increase in proline due to water stress as compared to control was 5.05%, 9.41%, 7.22% and 2.12% in variety RSG-896 at the four stages 7, 14, 21 and 28 days after imposing stress (DAIS) respectively. The increase in proline due to water stress in RSG-974 was 9.85%, 8.46%, 2.94% and 3.76% respectively at 7, 14, 21 and 28 DAIS. The result thus shows that the increase in proline content due to water stress was maximum at 7 DAIS in all the three varieties. Among three varieties the maximum increase in proline due to water stress was observed in RSG-888 followed by RSG-974 and RSG-896.

The application of thiourea (1000 ppm) through spray to the plants at 70 days after stress (DAS) showed increase in proline content under control (Normal irrigation) as well as under water stress. The increase in proline content due to application of thiourea under water stress was maximum at 14 DAIS in RSG- 888, at 7 DAIS in RSG-896 and at 28 DAIS in RSG-974 which was 2.83%, 13.46% and 10.23% respectively as compared to water stress. Thus, the response of RSG-896 to thiourea under water stress was maximum followed by RSG-974 and RSG-888.

Table : 4.3 Effect of thiourea on proline content in chickpea under water stress

Treatments / Varieties	Proline content (mg g^{-1})			
	7 DAIS	**14 DAIS**	**21 DAIS**	**28 DAIS**
RSG-888				
Control	1.97	2.21	2.46	2.77
Control + thiourea	2.16	2.35	2.50	2.81
Water stress (WS)	2.24 (13.70)	2.47 (11.76)	2.72 (10.56)	3.05 (10.10)
WS + Th	2.26 (0.89)	2.54 (2.83)	2.76 (1.47)	3.12 (2.29)
RSG-896				
Control	1.98	2.23	2.49	2.83
Control + thiourea	2.18	2.42	2.54	2.86
Water stress (WS)	2.08 (5.05)	2.44 (9.41)	2.67 (7.22)	2.89 (2.12)
WS + Th	2.36 (13.46)	2.74 (12.29)	2.96 (10.86)	3.17 (9.68)
RSG-974				
Control	2.13	2.48	2.72	2.92
Control + thiourea	2.28	2.65	2.74	3.08
Water stress (WS)	2.34 (9.85)	2.69 (8.46)	2.80 (2.94)	3.03 (3.76)
WS + Th	2.43 (3.84)	2.78 (3.34)	3.04 (8.57)	3.34 (10.23)
SEm±	**0.12**	**0.13**	**0.14**	**0.16**
CD (P=0.05)	**0.34**	**0.38**	**0.42**	**0.46**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent increase due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

4.4 Total free amino acids (mg g^{-1})

Amino acid content varied significantly among varieties and treatments during flowering stage as shown in table 4.4. The amino acids increased in all the three varieties with the growth stages under control (normal irrigation). The amino acid increased from 0.006 to 0.127 mg g^{-1} in variety RSG-888, from 0.010 to 0.132 mg g^{-1} in RSG-896 and 0.012 to 0.133 mg g^{-1} in RSG-974 under control condition (normal irrigation).

Content of amino acids increased due to water stress in all three varieties. The increase in amino acid content due to water stress in variety RSG-888 was 40.00%, 33.33%, 21.81% and 13.60% respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. Similarly, the increase in amino acid contents due to water stress as compared to control was 37.50%, 26.92%, 22.41% and 10.20% in variety RSG-896 at the four stages 7, 14, 21 and 28 days after imposing stress (DAIS), respectively. The increase in amino acid due to water stress in RSG-974 was 33.33%, 32.25%, 25.42% and 17.90% respectively at 7, 14, 21 and 28 DAIS. The result thus shows that the increase in amino acid due to water stress was maximum at 7 DAIS in all the three varieties. Among three varieties, the maximum increase in amino acid due to water stress was observed in RSG-888 followed by RSG-896 and RSG-974 at most of the growth period studies.

The application of thiourea (1000 ppm) through spray to the plants at 70 days after stress (DAS) showed increase in amino acids under control (Normal irrigation) as well as under water stress. The increase in amino acid due to application of thiourea under water stress was maximum at 14 DAIS in RSG- 888 and RSG-896 and 7 DAIS in RSG-974 varieties which was 33.33% in RSG-888, 19.23% in RSG-896 and 22.22% in RSG-974 as compared to water stress. Thus, the effect of thiourea under water stress was maximum in RSG-888 followed by RSG-974 and RSG-896.

Table : 4.4 Effect of thiourea on total amino acid in chickpea under water stress

Treatments/ Varieties	Total amino acid (mg/g)			
	7 DAIS	14 DAIS	21 DAIS	28 DAIS
RSG-888				
Control	0.006	0.012	0.043	0.127
Control + thiourea	0.008	0.014	0.047	0.136
Water stress (WS)	0.010 (40.00)	0.018 (33.33)	0.055 (21.81)	0.147 (13.60)
WS + Th	0.012 (20.00)	0.024 (33.33)	0.059 (7.27)	0.161 (9.52)
RSG-896				
Control	0.010	0.019	0.045	0.132
Control + thiourea	0.012	0.023	0.049	0.141
Water stress (WS)	0.016 (37.50)	0.026 (26.92)	0.058 (22.41)	0.147 (10.20)
WS + Th	0.018 (12.50)	0.031 (19.23)	0.062 (6.89)	0.159 (8.16)
RSG-974				
Control	0.012	0.021	0.044	0.133
Control + thiourea	0.014	0.026	0.049	0.146
Water stress (WS)	0.018 (33.33)	0.031 (32.25)	0.059 (25.42)	0.162 (17.90)
WS + Th	0.022 (22.22)	0.034 (9.67)	0.065 (10.16)	0.167 (3.08)
SEm±	**0.001**	**0.001**	**0.003**	**0.008**
CD (P=0.05)	**0.002**	**0.003**	**0.008**	**0.022**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent increase due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

4.5 Glutathione Reduced (GSH) (mg g^{-1})

Table 4.5 shows the effect of water stress on glutathione reduced content in three chickpea varieties at four developmental stages. The result showed that glutathione reduced varied significantly among varieties and treatments at flowering stage. The glutathione reduced increased in all the three varieties with the growth stages. The glutathione reduced increased from 1.66 to 2.81 mg g^{-1} in variety RSG-888, from 1.65 to 2.62 mg g^{-1} on RSG-896 and 1.44 to 2.58 mg g^{-1} in RSG-974 under control condition (normal irrigation).

The glutathione reduced decreased due to water stress in all three varieties. The decrease in glutathione reduced due to water stress in variety RSG-888 was 3.01%, 6.80%, 3.57% and 4.98% respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. Similarly, the decrease in glutathione reduced due to water stress as compared to control was 10.30%, 8.60%, 5.85% and 2.29% in variety RSG-896 at the four stages 7, 14, 21 and 28 days after imposing stress (DAIS) respectively. The decrease in glutathione reduced due to water stress in RSG-974 was 6.94%, 8.72%, 4.97% and 7.36% respectively at 7, 14, 21 and 28 DAIS. The result thus shows that the decrease in glutathione reduced due to water stress was maximum at 14 DAIS in all the three varieties. Among three varieties the maximum decrease in glutathione reduced due to water stress was observed in RSG-896 followed by RSG-974 and RSG-888.

The application of thiourea (1000 ppm) through spray to the plants at 70 days after stress (DAS) showed increase in glutathione reduced under control (Normal irrigation) as well as under water stress. The increase in glutathione reduced due to application of thiourea under water stress was maximum at 14 DAIS in all the three varieties which was 3.82% in RSG-974, 2.94% in RSG-896 and 2.24% in RSG-888 as compared to water stress. Thus, the response of RSG-974 to thiourea effect of water stress was maximum followed by RSG-896 and RSG-888.

Table : 4.5 Effect of thiourea on glutathione reduced (GSH) in chickpea under water stress

Treatments/ Varieties	Glutathione reduced (mg g^{-1})			
	7 DAIS	**14 DAIS**	**21 DAIS**	**28 DAIS**
RSG-888				
Control	1.66	1.91	2.24	2.81
Control + thiourea	1.69	1.95	2.30	2.89
Water stress (WS)	1.61 (-3.01)	1.78 (-6.80)	2.16 (-3.59)	2.67 (-4.98)
WS + Th	1.62 (0.62)	1.82 (3.24)	2.20 (1.85)	2.69 (0.74)
RSG-896				
Control	1.65	1.86	2.22	2.62
Control + thiourea	1.56	1.79	2.12	2.67
Water stress (WS)	1.48 (-10.30)	1.70 (-8.60)	2.09 (-5.85)	2.56 (-2.29)
WS + Th	1.52 (2.70)	1.75 (2.94)	2.15 (2.87)	2.59 (1.17)
RSG-974				
Control	1.44	1.72	2.01	2.58
Control + thiourea	1.41	1.68	2.05	2.51
Water stress (WS)	1.34 (-6.94)	1.57 (-8.72)	1.91 (-4.97)	2.39 (-7.36)
WS + Th	1.38 (2.98)	1.63 (3.82)	1.95 (2.09)	2.42 (1.25)
SEm±	**0.08**	**0.10**	**0.12**	**0.14**
CD (P=0.05)	**0.25**	**0.28**	**0.34**	**0.42**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent decrease due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

4.6 Peroxidase (EC. 1.11.1.7)

Proxidase was assayed in chickpea lraves following the method of costa *et al* (2002) and the result are shown in table 4.6. The result showed that peroxidase varied significantly among varieties and treatments at flowering stage. Peroxidase activity increased in all the three varieties with the growth period. The peroxidase activity increased from 0.017 to 0.073 in variety RSG-888, from 0.015 to 0.076 in RSG-896 and 0.021 to 0.085 in RSG-974 under control condition (normal irrigation).

Application of water stress resulted in increased peroxidase activity in all three varieties. The increase in peroxidase due to water stress in variety RSG-888 was 23.52%, 23.07%, 12.50% and 9.58% respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. Variety RSG-896 also showed increase in peroxidase activity due to water stress as compared to control which was 73.33%, 53.57%, 33.33% and 14.47% respectively at the four stages. The increase in peroxidase due to water stress in RSG-974 was 42.85%, 28.94%, 16.94% and 8.23%, respectively at 7, 14, 21 and 28 DAIS. The result thus shows that the increase in peroxidase activity due to water stress was maximum at 7 DAIS in all the three varieties. Among three varieties, the maximum increase in peroxidase activity due to water stress was observed in RSG-896 followed by RSG-974 and RSG-888.

The application of thiourea (1000 ppm) through spray of the plants at 70 days after stress (DAS) showed increase in peroxidase activity under control (Normal irrigation) as well as under water stress. The increase in peroxidase activity due to application of thiourea under water stress was maximum at 7 DAIS in RSG- 888, at 14 DAIS in RSG-896 as well as in RSG-974 varieties which was 9.52%, 11.62% and 10.20% respectivily as compared to water stress. The effect of thiourea under water stress was maximum in RSG-896 followed by RSG-974 and RSG-888.

Table : 4.6 Effect of thiourea on peroxidase (POX) in chickpea under water stress

Treatments / Varieties	Peroxidase (OD unit per minute/100 mg)			
	7 DAIS	**14 DAIS**	**21 DAIS**	**28 DAIS**
RSG-888				
Control	0.017	0.026	0.048	0.073
Control + thiourea	0.020	0.029	0.051	0.075
Water stress (WS)	0.021 (23.52)	0.032 (23.07)	0.054 (12.50)	0.080 (9.58)
WS + Th	0.023 (9.52)	0.034 (6.25)	0.056 (3.70)	0.083 (3.75)
RSG-896				
Control	0.015	0.028	0.048	0.076
Control + thiourea	0.019	0.031	0.052	0.078
Water stress (WS)	0.026 (73.33)	0.043 (53.57)	0.064 (33.33)	0.087 (14.47)
WS + Th	0.028 (7.69)	0.048 (11.62)	0.068 (6.25)	0.092 (5.74)
RSG-974				
Control	0.021	0.038	0.059	0.085
Control + thiourea	0.024	0.044	0.065	0.090
Water stress (WS)	0.030 (42.85)	0.049 (28.94)	0.069 (16.94)	0.092 (8.23)
WS + Th	0.033 (10.00)	0.054 (10.20)	0.074 (7.24)	0.097 (5.43)
SEm±	**0.001**	**0.002**	**0.003**	**0.004**
CD (P=0.05)	**0.003**	**0.006**	**0.009**	**0.013**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent increase due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

4.7 Total Carotenoids (mg g^{-1})

The effect of water stress on carotenoids in three chickpea varieties at four developmental stages is shown in table 4.7. Data presented in the table shows that carotenoids varied significantly among varieties and with the treatments at flowering stage. The carotenoids content increased in all the three varieties with the growth period. The carotenoids increased from 1.22 to 3.82 mg g^{-1} in variety RSG-888, from 1.16 to 3.70 mg g^{-1} in RSG-896 and 0.92 to 3.74 mg g^{-1} in RSG-974 under control condition (normal irrigation).

Content of carotenoids decreased due to water stress in all three varieties. The decrease in carotenoids due to water stress in variety RSG-888 was 36.06%, 10.98%, 5.73% and 4.45%, respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. The decrease in carotenoids due to water stress as compared to control was 60.34%, 19.84%, 17.41% and 18.10% in variety RSG-896 at the four stages 7, 14, 21 and 28 days after imposing stress (DAIS), respectively. Variety RSG-974 showed 39.13%, 7.72%, 6.72% and 21.39% decrease in carotenoids due to water stress respectively at 7, 14, 21 and 28 DAIS. The results show that the decrease in carotenoids due to water stress was maximum at 7 DAIS in all the three varieties. Among three varieties, the maximum decrease in carotenoids due to water stress was observed in RSG-896 followed by RSG-974 and RSG-888.

The application of thiourea (1000 ppm) through spray of the plants at 70 days after stress (DAS) showed increase in carotenoids under control (Normal irrigation) as well as under water stress. The increase in carotenoids content due to application of thiourea under water stress was maximum at 7 DAIS in all the three varieties which was 15.38% in RSG-888, 34.78% in RSG-896 and 35.71% in RSG-974 as compared to water stress. The results showed that the response of RSG-974 to thiourea under water stress was maximum followed by RSG-896 and RSG-888.

Table : 4.7 Effect of thiourea on carotenoids content in chickpea under water stress

Treatments /Varieties	Carotenoids content (mg g^{-1})			
	7 DAIS	**14 DAIS**	**21 DAIS**	**28 DAIS**
RSG-888				
Control	1.22	2.73	3.49	3.82
Control + thiourea	1.54	2.84	3.54	3.93
Water stress (WS)	0.78 (-36.06)	2.43 (-10.98)	3.29 (-5.73)	3.65 (-4.45)
WS + Th	0.90 (15.38)	2.52 (3.70)	3.33 (1.21)	3.77 (3.28)
RSG-896				
Control	1.16	2.62	3.10	3.70
Control + thiourea	0.68	2.53	3.27	3.78
Water stress (WS)	0.46 (-60.34)	2.10 (-19.84)	2.56 (-17.41)	3.03 (-18.10)
WS + Th	0.62 (34.78)	2.28 (8.57)	2.61 (1.95)	3.09 (1.98)
RSG-974				
Control	0.92	2.20	2.38	3.74
Control + thiourea	0.96	2.39	2.42	3.81
Water stress (WS)	0.56 (-39.13)	2.03 (-7.72)	2.22 (-6.72)	2.94 (-21.39)
WS + Th	0.76 (35.71)	2.13 (4.92)	2.35 (5.85)	3.03 (3.06)
SEm±	**0.06**	**0.13**	**0.16**	**0.19**
CD (P=0.05)	**0.18**	**0.39**	**0.48**	**0.56**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent decrease due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

4.8 Malondialdehyde (m moles g^{-1})

Table 4.8 shows the effect of thiourea on malondialdehyde in three chickpea varieties at four **developmental** stages. Data presented in the table showed that malondialdehyde content varied among varieties as well as with the treatments during flowering stage. The malondialdehyde content increased in all the three varieties with the growth period. The malondialdehyde increased from 16.49 to 28.55 in variety RSG-888, from 17.30 to 34.26 in RSG-896 and 21.03 to 35.89 in RSG-974 under control condition (normal irrigation).

Application of water stress increased malondialdehyde in all three varieties. The increase in malondialdehyde due to water stress in variety RSG-888 was 15.76%, 21.09%, 17.44% and 11.27% respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. Variety RSG-896 also witnessed increase in malondialdehyde due to water stress as compared to control which was 10.86%, 7.80%, 26.71% and 7.09% at the four stages. The increase in malondialdehyde due to water stress in RSG-974 was 11.03%, 10.81%, 20.95% and 11.28% respectively at 7, 14, 21 and 28 DAIS. The results thus show that the increase in malondialdehyde due to water stress was maximum at 21 DAIS in all the three varieties. Among the varieties, the maximum increase in malondialdehyde due to water stress was observed in RSG-896 followed by RSG-888 and RSG-974.

Content of malondialdehyde increased due to application of thiourea (1000 ppm) through spray of the plants at 70 days after stress (DAS) under control (Normal irrigation) as well as under water stress. The increase in malondialdehyde due to application of thiourea under water stress was maximum at 21 DAIS in RSG- 888, at 14 DAIS in RSG-896 and RSG-974 varieties which was 8.56%, 21.10% and 9.26% respectivily as compared to water stress. Among varities, the effect of thiourea under water stress was maximum in RSG-896 followed by RSG-974 and RSG-888.

Table : 4.8 Effect of thiourea on Malondialdehyde (MDA) in chickpea under water stress

Treatments / Varieties	Malondialdehyde (m moles g^{-1})			
	7 DAIS	**14 DAIS**	**21 DAIS**	**28 DAIS**
RSG-888				
Control	16.49	21.52	18.69	28.55
Control + thiourea	18.31	23.84	19.27	31.59
Water stress (WS)	19.09 (15.76)	26.06 (21.09)	21.95 (17.44)	31.77 (11.27)
WS + Th	20.02 (4.87)	27.15 (4.18)	23.83 (8.56)	33.16 (4.37)
RSG-896				
Control	17.30	22.42	19.39	34.26
Control + thiourea	19.21	24.84	22.12	35.16
Water stress (WS)	19.18 (10.86)	24.17 (7.80)	24.57 (26.71)	36.69 (7.09)
WS + Th	22.19 (15.69)	29.27 (21.10)	26.88 (9.40)	39.79 (8.44)
RSG-974				
Control	21.03	25.90	23.17	35.89
Control + thiourea	21.29	26.78	24.57	38.13
Water stress (WS)	23.35 (11.03)	28.70 (10.81)	27.98 (20.75)	39.94 (11.28)
WS + Th	24.34 (4.23)	31.36 (9.26)	28.34 (1.28)	41.68 (4.35)
SEm±	**1.04**	**1.36**	**1.19**	**1.86**
CD (P=0.05)	**3.06**	**3.98**	**3.49**	**5.46**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent increase due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

4.9 Phenol content (mg/g)

Effect of thiourea on phenol content in three chickpea varieties at four growth periods is shown in table 4.9. Results showed that phenol content varied among varieties and treatments at flowering stage. The phenol content increased in all the three varieties with the growth period from 2.07 to 2.57 in variety RSG-888, from 2.11 to 2.59 in RSG-896 and 2.22 to 2.61 in RSG-974 under control condition (normal irrigation).

Content of phenol increased due to water stress in all three varieties. The increase in phenol due to water stress in variety RSG-888 was 8.21%, 5.06%, 2.10% and 5.83% respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. Similar trend was observed in that two varieties RSG-896 witnessed 10.42%, 9.04%, 11.81% and 8.88% increase in phenol content due to water stress as compared to control at the four stages 7, 14, 21 and 28 days after imposing stress (DAIS) respectively. RSG-974 showed 5.85%, 7.04%, 10.28% and 10.72% increase in phenol due to water stress respectively at 7, 14, 21 and 28 DAIS. The results, thus shows that the increase in phenol content due to water stress was maximum at 7 DAIS in RSG-888, at 21 DAIS in RSG-896 and at 28 DAIS in RSG-974. Among three varieties the maximum increase in phenol due to water stress was observed in RSG-896 followed by RSG-974 and RSG-888.

Content of phenols decreased due to application of thiourea (1000 ppm) through spray of the plants at 70 days after stress (DAS) under control (Normal irrigation) as well as under water stress. The decrease in phenol content due to application of thiourea under water stress was maximum at 7 DAIS in RSG- 888, RSG-896 and RSG-974 varieties which was 1.78%, 5.57% and 8.51% respectively as compared to water stress. Thus, the response of RSG-974 to thiourea under water stress was maximum followed by RSG-896 and RSG-888.

Table : 4.9 Effect of thiourea on phenol content in chickpea under water stress

Treatments / Varieties	Phenol content (mg/g)			
	7 DAIS	14 DAIS	21 DAIS	28 DAIS
RSG-888				
Control	2.07	2.17	2.38	2.57
Control + thiourea	2.00	2.10	2.34	2.56
Water stress (WS)	2.24 (8.21)	2.28 (5.06)	2.43 (2.10)	2.72 (5.83)
WS + Th	2.20 (-1.78)	2.24 (-1.75)	2.41 (-0.82)	2.68 (-1.47)
RSG-896				
Control	2.11	2.21	2.37	2.59
Control + thiourea	2.04	2.14	2.35	2.57
Water stress (WS)	2.33 (10.42)	2.41 (9.04)	2.65 (11.81)	2.82 (8.88)
WS + Th	2.20 (-5.57)	2.34 (-2.90)	2.61 (-1.50)	2.67 (-5.31)
RSG-974				
Control	2.22	2.27	2.43	2.61
Control + thiourea	2.13	2.25	2.39	2.57
Water stress (WS)	2.35 (5.85)	2.43 (7.04)	2.68 (10.28)	2.89 (10.72)
WS + Th	2.15 (-8.51)	2.36 (-2.88)	2.64 (-1.49)	2.83 (-2.07)
SEm±	**0.11**	**0.12**	**0.13**	**0.14**
CD (P=0.05)	**0.33**	**0.35**	**0.38**	**0.41**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent increase due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent decrease due to application of thiourea as compare to water stress.

4.10 Protein content (mg g^{-1})

Estimation of protein by Lowrys method (1951) in three chickpea varieties at four growth periods showed that their content varied among varieties and treatments at flowering stage as shown in table 4.10. The protein content decreased in all the three varieties with the growth stages from 80.44 to 73.51 mg g^{-1} in variety RSG-888, from 77.14 to 71.39 mg g^{-1} in RSG-896 and 75.98 to 71.02 mg g^{-1} in RSG-974 under control condition (normal irrigation).

Application of water stress led to decrease in protein content in all three varieties. The decrease in protein content due to water stress in variety RSG-888 was 2.38%, 0.92%, 6.03% and 2.78%, respectively at 7, 14, 21 and 28 days after imposing stress (DAIS) as compared to control. Protein content also decreased in RSG-896 due to water stress as compared to control which was 2.59%, 3.97%, 3.21% and 8.57% at the four stages 7, 14, 21 and 28 days after imposing stress (DAIS), respectively. The decrease in protein content due to water stress in RSG-974 was 2.60%, 4.97%, 6.87% and 4.98%, respectively at 7, 14, 21 and 28 DAIS. The result, thus showed that the decrease in protein due to water stress was maximum at 21 DAIS in RSG-888, at 28 DAIS in RSG-896 and 21 DAIS in RSG-974. Among three varieties, the maximum decrease in protein content due to water stress was observed in RSG-896 followed by RSG-974 and RSG-888.

The application of thiourea (1000 ppm) through spray of the plants at 70 days after stress (DAS) showed increase in protein content under control (Normal irrigation) as well as under water stress. The increase in proteins due to application of thiourea under water stress was maximum at 21 DAIS in RSG-888 and at 7 DAIS in other two varieties was 1.92% in RSG-888, 2.67% in RSG-896 and 2.43% in RSG-974 as compared to water stress. Thus, the response of RSG-896 to effect of thiourea under water stress was maximum followed by RSG-974 and RSG-888.

Table : 4.10 Effect of thiourea on protein content in chickpea under water stress

Treatments/ Varieties	Protein content (mg g^{-1})			
	7 DAIS	**14 DAIS**	**21 DAIS**	**28 DAIS**
RSG-888				
Control	80.44	78.58	74.21	73.51
Control + thiourea	80.62	79.06	74.68	73.95
Water stress (WS)	78.52 (-2.38)	77.85 (-0.92)	69.73 (-6.03)	71.46 (-2.78)
WS + Th	79.21 (0.87)	77.90 (0.06)	71.07 (1.92)	72.08 (0.86)
RSG-896				
Control	77.14	76.21	73.44	71.39
Control + thiourea	79.03	75.58	74.14	72.11
Water stress (WS)	75.14 (-2.59)	73.18 (-3.97)	71.08 (-3.21)	65.27 (-8.57)
WS + Th	77.15 (2.67)	74.13 (1.29)	71.69 (0.85)	66.13 (1.31)
RSG-974				
Control	75.98	76.59	73.87	71.02
Control + thiourea	76.97	76.97	72.91	71.39
Water stress (WS)	74.00 (-2.60)	72.78 (-4.97)	68.79 (-6.87)	67.48 (-4.98)
WS + Th	75.80 (2.43)	73.34 (0.76)	69.37 (0.84)	68.71 (1.82)
SEm±	**4.14**	**4.06**	**3.87**	**3.76**
CD (P=0.05)	**12.15**	**11.91**	**11.35**	**11.01**

DAIS = Days after imposing stress, + = Increase, – = Decrease

The values in parenthesis against water stress are percent decrease due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

4.11 Seed yield (kg/plot)

Table 4.11 shows the effect of water stress on seed yield in three chickpea varieties. Data presented in the table showed that seed yield varied among varieties and treatments at flowering stage. The seed yield increased due to application of thiourea (1000 ppm) in all the three varieties under control condition (normal irrigation) as well as under water stress. The maximum increase in yield due to thiourea application under normal irrigation was observed in RSG-888 (26.54%), followed by RSG-974 (18.96%) and RSG-896 (12.17%).

Decrease in seed yield was observed due to water stress in all three varieties. The decrease in seed yield due to water stress was 30.59% in RSG-888, 56.53% in RSG-896 and 50.45% in RSG-974 as compared to control. The result thus shows that the decrease in seed yield due to water stress was maximum in variety RSG-896 followed by RSG-974 and variety RSG-888.

The application of thiourea (1000 ppm) through spray of the plants at 70 days after sowing (DAS) resulted in increased seed yield under control as well as under water stress. The increase in seed yield due to application of thiourea under water stress was 22.42% in RSG-888 103.4% in RSG-896 and 78.30% in RSG-974 as compared to water stress. Thus, the effect of thiourea under water stress was maximum in RSG-896 followed by RSG-974 and RSG-888. The effect of thiourea application in chickpea variety RSG-896 was effective than other two varieties.

Table : 4.11 Effect of thiourea on yield in chickpea under water stress

Treatments/ Varieties	Yield (Kg/Plot)
RSG-888	
Control	0.938
Control + thiourea	1.187 (26.54)
Water stress (WS)	0.651 (-30.59)
WS + Th	0.797 (22.42)
RSG-896	
Control	0.994
Control + thiourea	1.115 (12.17)
Water stress (WS)	0.432 (-56.53)
WS + Th	0.879 (103.47)
RSG-974	
Control	0.775
Control + thiourea	0.922 (18.96)
Water stress (WS)	0.384 (-50.45)
WS + Th	0.685 (78.38)
SEm±	**0.052**
CD (P=0.05)	**0.152**

Plot size = 3x2 M = 6 M^2 + = Increase, – = Decrease

The values in parenthesis against water stress are percent decrease due to water stress as compare to control.

The values in parenthesis against water stress+thiourea are percent increase due to application of thiourea as compare to water stress.

Chapter-5
DISCUSSION

Study on **"Influence of thiourea application on biochemical changes in chickpea (*Cicer arietinum* L.) genotypes under water stress at flowering stage"** was conducted in the Department of Biochemistry, S.K.N. College of Agriculture, Jobner during November 2015 to April 2016. Three varieties of chickpea viz. RSG-888, RSG-896 and RSG-974 were used in this study. The optimum chickpea sowing time is from mid October to first forthnight of November. The investigation was carried out with the objectives to study the effect of thiourea on physiological, biochemical parameters and seed yield of chickpea under water stress which was creates by withholding irrigation at 70 DAS. Application of thiourea (1000 ppm) was given through spray of the plants at the same time ie 70 days after sowing (DAS) to all the three chickpea varieties grown under normal and water stress. Chickpea crop responds to water stress in the form of changes in various physiological, biochemical and molecular processes. Research on water stress tolerance mechanisms in chickpea has gained momentum in many laboratories around the world. In the present study, with three genotypes varying in field performance in response to water stress, physiological indices (membrane stability index and relative water content) have been monitored at four stages i.e., 77 DAS, 84 DAS, 91 DAS and 98 DAS. Besides, biochemical parameters were also measured (*viz.,* contents of lipid peroxidation product – Malondialdehyde (MDA), molecular antioxidant Glutathion reduced (GSH),

antioxidative enzyme Peroxidase (POX) along with osmolytes – Proline and Amino acid content, photosynthetic pigments- carotenoids and Phenols and Soluble proteins. These parameters can be used in assessing tolerant versus susceptible genotypes to water stress at different stages of development.

Physiologically, drought is a complex process, in which many molecules such as (DNA, RNA), proteins, carbohydrates, lipids, hormones, ions, free radicals, mineral elements and others are involved (Wang *et al*., 2003). The reactions of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stages of developments (Chaves *et al*., 2003). Water stress causes a wide array of biochemical and physiological changes, starting with a decrease in osmotic potential at the cellular level (Bajji *et al*., 2001).

Membrane stability index may be used as parameter to estimate the cellular injury caused to membrane due to peroxidation of fatty acids of the membrane and the levels of membrane lipid peroxidation can be measured by Thiobarbituric acid substance called Malondialdehyde content. In present study, the increased levels of MDA in stress condition indicate the membrane sensitivity/ membrane damage due to water stress. Lower rate of increase of MDA in genotypes indicate better membrane strength. In this study, the MDA was highest in genotype RSG-896 followed by RSG-888 and RSG-974 under water stress condition. Application of thiourea increased, MDA content in all the three varieties. In the present study, the MSI was found lowest in variety RSG-974, thus indicating this to be tolerant to water stress at both the stages. Maximum reduction in MSI values was

observed in genotype RSG-896 and RSG-888 at both stages, indicating their high susceptibility to water stress. The results are supported by the earlier findings of Deshmukh *et al*. (2002).

Spray of thiourea could eliminate the effect of water stress on MSI to some extent in all the three varieties. Effect of thiourea (1000 ppm) was observed in all the varieties, however, the highest MSI was noticed in RSG-888 followed by RSG-896 and RSG-974. Foliar spray of thiourea (it has imino and thiol functional groups) provides a ready sources of nitrogen and thiol which have great role in alleviating damage due to oxidative stress damage in physiologically important leaf tissues. Decrease in MSI due to water stress could result in more fluid lipid bilayer of biological membrane by either denaturation of proteins or an increase in unsaturated fatty acids (Savchenko *et al,* 2002). Such alteration enhances permeability of membrane and results in loss of electrolytes.

The present study showed decrease in relative water content (RWC) in all the three varieties with the increasing growth period. RWC also decreased in all the three genotypes due to water stress. Spray of thiourea (1000 ppm) under water stress further reduces RWC in all the three genotypes, the highest decline being in RSG-896 at 28 DAIS. In similar studies, it was observed that soyabean seedlings experienced significantly more stress injury (as electrolyte leakage) than chickpea at different water stress levels. In chickpea, the root water content was higher than in soyabean during stress. In both the crops, drought stress coincided with a decrease in relative water content (RWC) that was less severe in the upper leaves than elsewhere in the plant.. (Nayyar *et al.,* 2005; Kumar *et al.,* 2003; Kushwaha *et al.,* 2003).

In the present study, higher levels of L-proline and total free amino acid were observed in all the three varieties, subjected to water stress as compared to control. Hence, the results of present investigation showing higher accumulation of L-proline and total free amino acid at 77 DAS that at 84, 91 and 98 days after sowing (DAS) in the water stressed plants of all the three varieties indicate that early growth stage is more responsive stage in terms of cellular osmotic adjustment than later stages. Comparing performance of all the three varieties with respect to proline content under water stress as compared to control, RSG-888 was found to be tolerant more to water stress followed by RSG-974and RSG-896. The present study showed that content of proline increased with the growth period under control and water stress in all the three genotypes. The highest percent increase of proline was noticed in RSG-888 at 77 DAS under water stress as compared to control. Spray of thiourea further increased the proline content in all the three genotypes under control and water stress. Maximum proline increase was observed with thiourea (1000 ppm) in RSG-896 genotype, followed by RSG-974 and RSG-888. Effect of thiourea application, thus, also shows that variety RSG-888 is more tolerant to water stress as compared to RSG-896 and RSG-974. Gosavi *et al*. (2014) studied contents of proline, glycine betaine, and the activity of Delta ^{1}-pyrroline-5-carboxylate synthetase (P5CS) along with soluble proteins in the leaves of chickpea by imposing -0.5MPa water stress, created by PEG-6000. They reported that crude protein and tryptophan contant were higher in susceptible genotypes as compared to tolerant ones. Drought tolerant genotypes, showed comparatively higher potential to accumulate proline and glycine betaine as compared to susceptible genotypes.

Quantitative analysis of total free amino acids showed that their content increased with the growth period under control and water stress in all the three genotypes. The highest percent increase in amino acids due to water stress was noticed in RSG-888 at 77 DAS followed by RSG-896 and RSG-974. Spray of thiourea further increased content of total amino acids to some extent in all the three varieties under control and water stress. Maximum increase was observed with thiourea 1000 ppm in RSG-888 followed by RSG-974 and RSG-896 under water stress.

Under drought stress, a variety of reactive oxygen species (ROS) are produced, which result in oxidative damage of cell membranes. Shahid *et al.,* (2012) showed that salt stress in pea reduced the growth, plant fresh/dry biomass and number of leaves, photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll contents and cell membrane stability index (MSI) while elevated antioxidant enzymes, i.e. superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) as well as organic solutes proline, glycinebetaine and total free amino acids, lipid peroxidation (LPO), hydrogen peroxide (H_2O_2) and leaf abscisic acid (ABA).

In the present study, decrease of GSH contents during water stress in all the three varieties, the magnitude being higher at 84 DAS (14 DAIS) reveals that the chickpea plants have detoxified ROS intermediates ($^{\cdot}O_2^-$ $\rightarrow$ H_2O_2) at this stage quickly thus indicating the operation of ASC-GSH cycle during water stress. Decrease in GSH due to water stress was highest in RSG-896 (10.30%) followed by RSG-974 and RSG-888. Spray of thiourea increased GSH to some extent in all the three varieties under control and water stress. Maximum increase was observed with 1000 ppm thiourea in all the three varieties at 21 DAIS, however, highest GSH was

noticed in RSG-974 followed by RSG-896 and RSG-888 under water stress.

Akitha *et al*., (2015) studied that soybean varieties for the changes in their antioxidant enzyme activity, total isoflavone content and also the influence on its growth under drought stress. It has been, reported that mild water deficit stress for 5 days during late vegetative stage increased total superoxide dismutase activity, triggered the activities of catalase, peroxidase, ascorbate peroxidase and glutathione reductase enzymes in soybean. High accumulation of proline and lower level of lipid peroxidation were also reported. Peroxidases are involved in scavenging of reactive oxygen species (ROS) which are highly reactive and capable of causing oxidative to cells. They use hydrogen peroxide as electron acceptor to catalyze a number of oxidative reactions.

The study showed that activity of peroxidase (POX) increased with increasing growth period under control and water stress in all the three varieties. Peroxidase activity increased due to water stress in all the three chickpea varieties. The highest increase was noticed in RSG-896 followed by RSG-974 and RSG-888 at 77 DAS under water stress as compared to control. Spray of thiourea increased POX activity to some extent in all the three genotypes under control and water stress. Maximum increase due to application of 1000 ppm thiourea was observed at 84 DAS in all the three varieties. Among varieties, the highest (POX) activity was noticed in RSG-896 under thiourea application followed by RSG-974 and then RSG-888. Increased activity of peroxidase due to thiourea suggests that there is need to scavange free redicals.

In a study Rut (2015) compared capacity of antioxidant enzymes between a drought-tolerant, and a drought-sensitive sugarcane variety. Young sugarcane plants were grown under controlled conditions. Water stress was imposed at 4 leaf stage by withholding water supply for 4, 6 and 8 days. First fully expanded leaves were harvested and proteins were extracted for assaying the activities of ascorbate peroxidase (APX), peroxidase (POX) and catalase (CAT). APX was a major antioxidant system in leaves of both the sugarcane varieties as it accounted for 65 % in tolerant variety and 69 % in susceptible variety in scavenging H_2O_2. POX accounted for 27 % and 23 % respectively in the removal of H_2O_2. APX activities in leaves of tolerant variety were induced and maintained during progressive water stress and were 15 % higher than in sensitive variety. POX activities in tolerant variety were 30 % greater than that in susceptible variety. Thus, drought tolerance in tolerant variety was, at least partially, due to the greater activities of APX and POX under water deficit stress as compared to those in susceptible variety. These finding support results of present investigation that showed increased peroxidase activity under water stress.

Reactive oxygen species have an adverse impact on photosynthesic apparatus of the cell by damaging chlorophyll and carotenoids (Mittova *et al*., 2000). Results of present study showed that total carotenoid content decreased significantly in the three chickpea varieties at all the growth stages due to water stress. Low level decrease in carotenoid indicates that their photosynthetic apparatus is able to resist adverse condition due to water stress. On the other hand, accumulation of higher carotenoid content at 77 DAS compared to later stages 84, 91 and 98 DAS may be due to

sugar synthesized via photosynthesis and its breaks down during respiration by plants. The results are supported by the findings of Abdouli *et al*., (2012) and Aggrawal *et al*., (2013).

Carotenoid content decreased under water stress in all the three genotypes. Highest percent decrease in RSG-896 was noticed at 77 DAS under water stress as compared to control followed by RSG-974 and RSG-888. Spray of thiourea increased carotenoid to some extent in all the three genotypes under control and water stress. Maximum increase was observed with spray of 1000 ppm thiourea at 77 DAS in all the three varieties. Among the varieties, the maximum increase in carotenoid content was noticed in RSG-974 under thiourea application compared to water stress, followed by RSG-896 and RSG-888.

Similar results have been reported in other crops by many researchers. Application of thiourea through seed treatment and foliar application has been shown to mitigate the effect of water stress in mung bean (Mathur *et al*., 2006); clusterbean (Garg and Burman., 2006) and mothbean (Kumawat *et al*., 2012). Foliar spray (1000 ppm) of thiourea at pre-flowering stage showed increase in net photosynthesis, total chlorophyll, starch, reducing sugar, soluble protein, nitrate reductase activity and seed yield. In another experiment, the induced water stress created by withholding water supply at flowering stage causes significant decline in the level of chlorophyll with an increased amount of carotenoids in cowpea (Patel and Rao., 2007).

There was increase in phenol content at different stages after sowing in three varieties under water stress as compared to control. The maximum increase of phenol was in genotype RSG-896 (11.81%) at 91

DAS followed by RSG-974 (10.72%) at 99 DAS and RSG-888 (8.21%) at 77 DAS under water stress condition. Spray of thiourea (1000 ppm) decreased phenol content in all the three varieties under control as well as water stress. Phenol content decreased due to thiourea application under water stress was highest in RSG-974. However Mehrjerdi *et al.,* (2013) have reported decrease in phenol content with increasing drought stress along with decrease in photosynthetic rate, evapotranspiration, water use efficiency, chlorophyll a, chlorophyll b and membrane stability index in chickpea.

Recent studies have further shown that sucrose transport in plants requires special proteins known as “sucrose transport protein” which require the involvement of –SH group for their activity in phloem transport. Thus, those plants having –SH group activity are known to display higher transport of sucrose. Consequently the process of grain formation and grain filling is faster and more effective in such plants. This probably explains the fact that thiourea application as foliar spray in drought affected plants leads to higher transport of assimilates and better grain filling leading to higher yield. Improvement in plants growth and development due to application of thiourea has been observed in several crops like maize (Sahu *et al.*, 1993), wheat (Sahu and Singh., 1995), pearl millet (Parihar *et al.*, 1998) and mustard (Khafi *et al.*, 1997).

There was slight decrease in protein content with the increasing growth duration in RSG-888, RSG-896 and RSG-974 under water stress as well as control. Present study showed that protein content under water stress was lower at 14 DAIS as compared to other stages in all the three varieties. Spray of thiourea increased the protein content in the three varieties under water stress. The highest protein content due to thiourea

application was observed in RSG-896 followed by RSG-974 and RSG-888 varieties. Thus thiourea was able to effect of water stress on protein in there chickpea genotypes.

Drought susceptibility of a genotype is often measured as a function of the reduction in yield under water stress (Blum, 1988). Yield is the most important parameter for a crop to judge its tolerance to any stress including water stress. Although, the yield contributing parameters are different in cereals, pulses, oilseed and seed spices crops effect of water stress on yield may be similar. In case of chickpea, we have measured yield under control, water stress and thiourea application under control and water stress conditions. The results have shown reduction in yield due to water stress in all the three varieties. The decrease in yield due to water stress was maximum in genotype RSG-896 and the minimum in RSG-888. Present study thus showed that genotypes RSG-974 and RSG-888 are less affected by water stress. Based on yield data, variety RSG-888 seems to be tolerant to water stress while variety RSG-896 seems to be susceptible to water stress. Spray of thiourea (1000 ppm) increased the yield in all three genotypes under control as well as water stress condition.

Sabaghpour *et al*., (2006) reported that yield losses due to terminal drought range from 35 to 50% in chickpea. It seems that effects of drought stress in legume crops are more complex than that of other plants; because the plant establishment and legume-Rhizobium symbiosis are susceptible to drought stress (Asghari *et al*., 2010).

Ulemale *et al*., (2013) evaluated fourteen chickpea genotypes under moisture stress and non-stress condition to study the physiological indices for drought tolerance. Significant differences were exhibited amongst the genotypes for phenology, vegetative growth, sink capacity and

physiological parameters and yield due to moisture stress. They also reported that membrane injury index, chlorophyll content, chlorophyll stability index were decreased and proline accumulation and nitrate reductase activity were increased. These can be used as parameters for selecting genotypes for drought tolerance.

Chapter-6
SUMMARY AND CONCLUSION

A study on **"Influnce of thiourea application on biochemical changes in chickpea (*Cicer arietinum* L.) genotypes under water stress at flowering stage"** was conducted in the Department of Biochemistry and Agronomy Farm, S.K.N. College of Agriculture, Jobner during Nov to April 2015-16. The study was conducted using three varieties of chickpea RSG-888, RSG-896 and RSG-974. Water stress was created by withholding water supply at 70 DAS. At the same time, thiourea (1000 ppm) was sprayed on two sets of plants-control (normal irrigation) and water stress. The physiological and biochemical parameters studied in the present investigation were Membrane stability index (MSI), Relative water content (RWC), Proline content, Total amino acids, Glutathione reduced (GSH), Peroxidase, Carotenoids content, Malondialdehyde (MDA), Phenols, Soluble proteins and Seed yield were investigated to analyze the effect of thioruea treatment under water stress.

Fully expanded upper leaves were used for recording observations of different physiological, biochemical parameters at 7, 14, 21 and 28 days after imposing stress (DAIS) and yield was recorded at harvest. The study was conducted with the follows objectives:

- To study stress related metabolites under water stress in chickpea genotypes
- To study stress tolerant enzymes under water stress in chickpea genotypes.

Summary of results of the present study is as follows:

1 Membrane stability index decreased with the growth stages and also due to water stress in all the three varieties. Spray of thiourea could restore MSI to some extent in all the three genotypes under normal as well as water stress. Maximum decrease in MSI was noticed in RSG-896 variety under water stress.

2 Relative water content decreased due to water stress in all the three varieties. Among three varieties the maximum decrease in relative water content due to water stress was observed in RSG-974 followed by RSG-896 and RSG-888. Treatment of thiourea (1000 ppm) was ineffective in restoring RWC under water stress.

3 There was increase in proline content in all the three varieties with the growth period. Water stress also increased proline content and the maximum increase was observed in variety RSG-888 (13.70%), followed by RSG-974 (9.85%) and RSG-896 (9.41%). Spray of thioruea (1000 ppm) further increased proline content in all the three varieties under water stress. The maximum increase due to thiourea (1000 ppm) application was noticed in variety RSG-896 followed by RSG-974 and RSG-888.

4 Amino acids increased due to water stress in all the three varieties RSG-888, RSG-896 and RSG-974. The maximum increase under water stress was observed at 7 DAIS. The variety RSG-888 showed the highest amino acid content under water stress, while variety RSG-974 showed minimum. Application of thiourea (1000 ppm) showed increase in proline content under control (normal irrigation) as well as under water stress. The effect of

thiourea under water stress was maximum in RSG-888 followed by RSG-974 and RSG-896.

5 **The antioxidant response was studied by measuring changes in content of molecular antioxidants *viz.* glutathione reduced (GSH) that revealed a decreasing trend in all the genotypes during water stress. The decrease in glutathione due to water stress was maximum at 14 DAIS in all the three varieties. Among the varieties, the maximum decrease in glutathione due to water stress was observed in RSG-896 followed by RSG-974 and RSG-888. Application of thiourea increased GSH content and the response was maximum in RSG-974 under water stress followed by RSG-896 and RSG-888.**

6 **The increase in activities of antioxidative enzyme, *viz.* POX during water stress in all genotypes suggest an efficient ROS scavenging mechanism under operation. The highest activities of the enzymes POX due to water stress was found in variety RSG-896 at 7 DAIS. The increase in peroxidase activity due to application of thiourea under water stress was maximum at 14 DAIS in RSG- 896, followed by RSG-974 and 7 DAIS in RSG-888 varieties.**

7 Carotenoids content decreased due to water stress in all the three varieties, and the maximum decrease was observed in RSG-896 followed by RSG-974 and RSG-888. The spray of thiourea (1000 ppm) showed increase in carotenoids content under control as well as water stress. The maximum increase in carotenoids content due to thiourea (1000 ppm) application was

observed in variety RSG-974 followed by RSG- 896 and RSG-888.

8 There was increase in malondialdehyde content in all the three varieties with the growth period and also due to water stress. Maximum increase in malondialdehyde content due to water stress was observed in variety RSG-896 (26.71%), followed by RSG-888 (21.09%) and RSG-974 (20.95%). Content of malondialdehyde also increased due to application of thiourea (1000 ppm) under control (Normal irrigation) as well as under water stress.

9 Content of phenol increased due to water stress in all the three varieties, and the increase was maximum in RSG-896 followed by RSG-974 and RSG-888. The decrease in phenol content due to application of thiourea under water stress was maximum at 7 DAIS in all the three varieties.

10 The protein content decreased in all the three varieties with the growth period. Application of water stress also led to decrease in protein content in all three varieties, and the decrease was maximum in RSG-896 followed by RSG-974 and RSG-888. The effect of thiourea under water stress was maximum in RSG-896 followed by RSG-974 and RSG-888.

11 There was reduction in yield due to water stress in all three genotypes, and the decrease was maximum in genotype RSG-896. Spray of thiourea increased the yield to some extent under water stress. The effect of thiourea in reversing the effect of water stress was maximum in RSG-896 followed by RSG-974 and RSG-888.

Conclusion

The study thus revealed that physiological parameters of relative water content (RWC) and membrane stability index and biochemical traits like carotenoids, MDA, peroxidase, total phenol, total free amino acids, proline, GSH and protein are important characters for studying effect of water stress in plants. Performance of chickpea genotypes may be improved by treatment with thiourea (1000 ppm) at 70 DAS under water stress conditions. Based on yield data, variety RSG-888 seems to be tolerant to water stress while variety RSG-896 seems to be susceptible to water stress and were associated with increase peroxidase activity, total amino acids, protein, GSH, proline, MDA contain and decreased content of carotenoid, RWC and MSI under water stress. Spray of thiourea (1000 ppm) increased the yield in all three genotypes under control as well as water stress condition.

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"Influence of thiourea application on biochemical changes in chickpea (*Cicer arietinum* L.) genotypes under water stress at flowering stage"

Shanker Lal Choudhary* (Research Scholar)

Dr. V.K. Yadav** Major Advisor

ABSTRACT

The study entitled "Influence of thiourea application on biochemical changes in chickpea (*Cicer arietinum* L.) genotypes under water stress at flowering stage" was conducted at Department of Biochemistry in field grown crop at Agronomy farm, S.K.N. College of Agriculture, Jobner during the Rabi season of 2015-2016, with the objectives of analysis of stress related metabolites and enzymes under water stress in chickpea genotypes.

Three chickpea varieties viz., RSG-888 and RSG-974 (drought tolerant) and RSG-896 (drought susceptible) were grown in the field. Water stress was created by withholding normal irrigation at 70 DAS. At the same time, treatment of thioure (1000 ppm) was given through spray on field grown two sets of plants under normal irrigation and water stress. The non-stressed plants were irrigated as frequently as needed and observations were recorded at flowering stage. Physiological indices like RWC, MSI; biochemical parameters *viz.,* contents of lipid peroxidation product –MDA, molecular antioxidant GSH, antioxidative enzyme–POX, osmolytes – Proline, Total amino acids; photosynthetic pigments- Carotenoids; Phenols and Proteins were measured in fully expanded leaves. Sampling was done at 7, 14, 21 and 28 days after inducing stress.

Thiourea increased membrane stability index, proline content, total amino acid, glutathione reduced, peroxidase, carotenoids content, malondialdehyde, protein content and yield in both cultivars under normal and water stress conditions. Genotypes RSG-974 was found to be the most drought sensitive among all genotypes on the basis of maximum decrease in relative water content (RWC). The yield in water stress tolerant variety RSG-888 was more as compared to other genotypes. Results signify the role of thiourea in regulating the water stress response of chickpea; hence it could be used as a potential growth regulator under water stress condition.

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* Post graduate student, Department of Biochemistry, SKN College of Agriculture, SKN Agriculture University, Jobner-303 329

** Thesis submitted in partial fulfillment of the requirement for M. Sc. (Ag.) degree in Biochemistry under the supervision of Dr. V.K. Yadav, Professor, SKN College of Agriculture, SKN Agriculture University, Jobner-303 329

जल तनाव परिस्थितियों में चने (*साइसर एरीटिनम* एल.) की चयनित प्रजातियों में पुष्पन अवस्था पर थायोयूरिया अनुप्रयोग के जैव रासायनिक प्रभाव

शंकर लाल चौधरी* (शोधकर्ता)

डॉ. वी.के. यादव** (मुख्य सलाहकार)

सारांश

जल तनाव परिस्थितियों में चने (साइसर एरीटिनम एल.) की चयनित प्रजातियों में पुष्पन अवस्था पर थायोयूरिया अनुप्रयोग के जैव रासायनिक प्रभाव का अध्ययन जैव रासायनिक विभाग, श्री कर्ण नरेन्द्र कृषि महाविद्यालय के सस्य विज्ञान विभाग के फार्म पर रबी ऋतु 2015–16 के दौरान तनाव से संबंधित चयापचयों तथा तनाव सहिष्णु एंजाइमों के विश्लेषण के उद्देश्यों के साथ आयोजित किया गया था।

चने की तीन प्रजातिया जैसे – आर.एस. जी.–888, आर.एस.जी.–974 (सुखा सहिष्णु) और आर.एस.जी.–896 (सुखा अति संवेदनशील) को खेत में उगायी गई। बुवाई के 70 दिन बाद सामान्य सिंचाई देने के साथ जल तनाव उत्पन्न किया गया। उसी समय, थायोयूरिया (1000 पी.पी.एम.) का छिड़काव सामान्य सिंचाई व जल तनाव दोनों स्थितियों में किया गया। प्रायः बिना जल तनाव वाले पौधों को सिंचाई उसकी आवश्यकता के अनुसार दी तथा पुष्पन अवस्था पर प्रेक्षण किया गया। शारीरिक सूचकांक, जैसे – सापेक्ष जल मात्रा, झिल्ली स्थिरता सूचकांक, जैव रासायनिक मापदण्डों जैसे – चर्बी परोक्सीडेस उत्पाद की मात्रा– मेलोन्डीएलडिहाइड, आणविक एंटीऑक्सीडेंट – ग्लुटाथियॉन, अनॉक्सीकृत एन्जाइम की क्रियाये – परॉक्सीडेस, ऑस्मोलाइट– प्रोलीन, कुल अमीनों अम्ल, प्रकाश संश्लेषक वर्णक – कैरोटीनॉइड्स, फिनॉल और प्रोटीन को पूर्ण विकसित पत्तियों में आकलन किया गया। तनाव उत्पन्न करने के 7, 14, 21 और 28 दिन बाद नमूने लिये गये।

थायोयूरिया अनुप्रयोग की दोनों पस्थितियों सामान्य सिंचाई व जल तनाव में झिल्ली स्थिरता सूचकांक, प्रोलीन, कुल अमीनों अम्ल, ग्लुटाथियॉन, परोक्सीडेंस, कैरोटिनॉइड, मेलान्डीएलडिहाइड, प्रोटीन और उपज को बढ़ाता है। प्रजाति आर.एस.जी.–974 सबसे कम जल सामर्थ्य (सापेक्ष जल मात्रा) के आधार पर मुख्य रूप से सुखा सहिष्णु प्रजाति पायी गयी। जल तनाव सहिष्णु प्रजाति आर.एस.जी.– 888 की उपज अन्य प्रजातियों की अपेक्षा अधिक दर्ज की गयी। परिणाम चना के जल तनाव प्रतिक्रया के विनियमन में थायोयूरिया की भूमिका को दर्शाता हैं, जल तनाव स्थिति में एक संभावित वृद्धि नियामक के रूप में इस्तेमाल किया जा सकता है।

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* स्नातकोत्तर छात्र, जैव रसायन विभाग, श्री कर्ण नरेन्द्र कृषि महाविद्यालय, जोबनेर–303 329

** आचार्य, जैव रसायन विभाग, श्री कर्ण नरेन्द्र कृषि विश्वविद्यालय, जोबनेर के निर्देशन में आंशिक पूर्ति हेतु स्नातकोत्तर (कृषि) जैव रसायन विभाग की उपाधि के सन्दर्भ में प्रस्तुत किया गया शोध ग्रन्थ।

Appendix – I

Analysis of variance for thioura on membrane stability index in chickpea under water stress at 77 days after sowing

Source of variance	d.f.	Mean sum of square									
		MSI	**RWC**	**Total amino acid**	**Proline content**	**GSH**	**POX**	**Carotenoids**	**MDA**	**Phenol content**	**Protein content**
Stressed	2	25.9697	35.3491	0.0001	0.0270	0.0152	0.0000	0.0036	2.1776	0.0286	36.5711
Genotypes	11	114.6221	38.6529	0.0001	0.0631	0.0424	0.0001	0.2858	16.8808	0.0351	13.1552
Error	22	34.8857	48.4242	0.0001	0.0363	0.0215	0.0000	0.0056	2.8877	0.0383	50.3052
SEm±		3.4101	4.0176	0.0007	0.1100	0.0846	0.0012	0.0433	0.9811	0.1129	4.0949
CD (p = 0.05)		9.9999	11.7815	0.0020	0.3227	0.2480	0.0034	0.1270	2.8770	0.3312	12.0082
CV		9.3500	9.1840	8.8861	8.6636	9.5751	8.8496	8.5240	8.4333	9.0138	9.1518

** and * significantly at 1% and 5%, respectively

Appendix – II

Analysis of variance for thioura on membrane stability index in chickpea under water stress at 84 days after sowing

Source of variance	d.f.	Mean sum of square									
		MSI	**RWC**	**Total amino acid**	**Proline content**	**GSH**	**POX**	**Carotenoids**	**MDA**	**Phenol content**	**Protein content**
Stressed	2	32.8799	34.6944	0.0001	0.0350	0.0205	0.0000	0.0358	3.6206	0.0315	34.9785
Genotypes	11	10.9169	45.7565	0.0001	0.1016	0.0379	0.0003	0.1983	24.8099	0.0325	16.2395
Error	22	45.4227	47.5496	0.0001	0.0473	0.0284	0.0000	0.0486	4.7676	0.0423	47.9863
SEm±		3.8911	3.9812	0.0012	0.1255	0.0972	0.0019	0.1273	1.2606	0.1188	3.9994
CD (p = 0.05)		11.4106	11.6747	0.0035	0.3681	0.2851	0.0055	0.3732	3.6967	0.3484	11.7282
CV		9.2134	9.2559	8.8198	8.6972	9.5494	8.5873	9.1851	8.3980	9.0656	9.1751

** and * significantly at 1% and 5%, respectively

Appendix – III

Analysis of variance for thioura on membrane stability index in chickpea under water stress at 91 days after sowing

Source of variance	d.f.	Mean sum of square									
		MSI	**RWC**	**Total amino acid**	**Proline content**	**GSH**	**POX**	**Carotenoids**	**MDA**	**Phenol content**	**Protein content**
Stressed	2	41.6805	31.9281	0.0001	0.0424	0.0290	0.0001	0.0553	1.8086	0.0335	33.8751
Genotypes	11	35.5459	52.8583	0.0002	0.0992	0.0429	0.0002	0.7387	25.8944	0.0424	12.8561
Error	22	57.6793	43.8615	0.0001	0.0563	0.0406	0.0001	0.0796	2.5640	0.0454	46.3879
SEm±		4.3848	3.8237	0.0027	0.1370	0.1163	0.0030	0.1629	0.9245	0.1230	3.9323
CD (p = 0.05)		12.8582	11.2128	0.0080	0.4018	0.3411	0.0088	0.4777	2.7110	0.3606	11.5312
CV		9.4145	9.3279	8.9455	8.7889	9.5182	8.8033	9.7967	8.3153	9.0628	9.1482

** and * significantly at 1% and 5%, respectively

Appendix – IV

Analysis of variance for thioura on membrane stability index in chickpea under water stress at 98 days after sowing

Source of variance	d.f.	Mean sum of square										
		MSI	RWC	Total amino acid	Proline content	GSH	POX	Carotenoids	MDA	Phenol content	Protein content	Yield
Stressed	2	44.4953	29.6138	0.0002	0.0523	0.0448	0.0010	0.0734	8.0090	0.0391	32.4773	0.0034
Genotypes	11	22.0996	54.6920	0.0005	0.0868	0.0687	0.0002	0.4959	46.2059	0.0579	3.3928	0.2679
Error	22	61.2011	40.4391	0.0002	0.0694	0.0620	0.0001	0.1041	11.0461	0.0529	44.8454	0.0080
SEm±		4.5167	3.6715	0.0076	0.1521	0.1437	0.0043	0.1863	1.9189	0.1328	3.8663	0.0517
CD (p = 0.05)		13.2450	10.7664	0.0222	0.4459	0.4215	0.0126	0.5462	5.6270	0.3894	11.3378	0.1515
CV		9.3690	9.2162	8.9644	8.8089	9.5022	8.8490	9.4889	9.3490	9.1279	9.2101	12.2580

** and * significantly at 1% and 5%, respectively

APPENDIX-V
(Reagents and solutions)

(A) L-proline

(a) Sulphosalicylic acid (SSA) 3%:-

3.0 g of sulphosalicylic acid was dissolved in 100 ml distilled water.

(b) Ninhydrin reagent:-

1.25 g of ninhydrin dissolved in 50 ml the acid mixture which was prepared by mixing 100 ml of 85% ortho-phosphoric acid to 150 ml of glacial acetic acid.

(B) Glutathione Reduced (GSH)

(a) Phosphate buffer (pH 8.00, 0.1M)

1.19 g sodium dihydrogen orthophosphate was dissolved in 100 ml distilled water. 400 mg NaOH was dissolved in 100 ml distilled water. The later was added to the monobasic sodium salt solution so as to get pH of 8.00.

(b) DTNB solution:-

20 mg 5,5'-dithiobis nitrobenzoic acid was dissolved in 5.0 ml of 0.1M phosphate buffer(pH 8.00).

(c) Ethylenediamine tetraacetic acid (EDTA), sodium salt (1mM):

50 mg EDTA was dissolved in 10 ml 0.1M phosphate buffer pH 8.0.

(C) Peroxidase

(a) Phosphate buffer (0.1 M, pH 7.2)-

3.12 gm of monobasic sodium salt dissolve in 100 ml distilled water (a) and 2.84 gm dibasic sodium salt dissolve in 100 ml distilled water (b). 2.8 ml of (a) and 7.2 ml of (b) were mixed and final volume was made to 100 ml with distilled water to make the buffer of pH 7.2.

(D) Malondialdehyde

(a) Trichloroacetic acid (TCA) 5%-

5 g TCA was dissolved in 100 ml distilled water.

(b) Thiobarbituric acid reagent (TBA) 0.67%-

670 mg TBA was dissolved in 100 ml of 5% TCA solution.

(E) Total Phenol

(a) Sodium carbonate solution (20%) :- 20 g Na_2CO_3 was dissolved in 100 ml distilled water.

(b) Alcohal solution (80%) :- 80 ml ethanol was dissolved in 20 ml distilled water

(F) Total free amino acid

(a) Diluent :- 100 ml n-propanol was dissolved in 100 ml distilled water.