Response of Biochemical Changes to Thiourea Application in Wheat (*Triticum aestivum* L.) Genotypes Under Water Stress at Vegetative Stage

गेहूँ (*ट्रिटिकम एस्टीवम* लि.) के जीनप्ररूप की कार्यिकीय अवस्था में जल तनाव स्थिति में थायोयूरिया उपयोग का जैव रसायन परिवर्तन को प्रेरित करने में अनुक्रिया

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Master of Science in Agriculture
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In the

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(Biochemistry)

By

Kamal Prasad Yadav

2016

Sri Karan Narendra Agriculture University, Jobner

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This is to certify that the thesis entitled **"Response of Biochemical Changes to Thiourea Application in Wheat (*Triticum aestivum* L.) Genotypes Under Water Stress at Vegetative Stage"** submitted for the degree of **Master of Science** in the subject of **Biochemistry** embodies bonafide research work carried out by **Mr. Kamal Prasad Yadav** 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 advisory committee on ----------.

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Place : Jobner ***(Kamal Prasad Yadav)***
Dated: / /2016

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ACRONYMS

@	:	At the rate of
%	:	Per cent
µl	:	Microlitre
^0C	:	Degree Celsius
$*O_2^-$	:	Superoxide anion
AICRP	:	All India Co-ordinated Research Project
CD	:	Critical difference
cm	:	Centimetre
DAS	:	Days after sowing
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
GOI	:	Government of India
LEAS	:	Late embryogenesis abundant
ROS	:	Reactive oxygen species
SOD	:	Superoxide dismutase
GR	:	Glutathione reductase
CAT	:	Catalase

POX	:	Peroxidase
APOX	:	Ascorbate peroxidase
AsA	:	Ascorbic acid
NR	:	Nitrate reductase
SH	:	Sulphydryl
H_2O_2	:	Hydrogen peroxide
MDA	:	Malondialdehyde
GB	:	Glycine betaine
RUBISCO	:	Ribulose-1,5-bisphosphate carboxylase oxygenase
ADP	:	Adenosine di phosphate
PCD	:	Programmed cell death
MPa	:	Mega pascal
ETR	:	Electron transport rate
ABA	:	Abscisic acid
SA	:	Salicyclic acid
PEG	:	Polyethylene glycol
MSI	:	Membrane stability index
POD	:	Peroxidase
O_2^-	:	Superoxide radical
OH-	:	Hydroxyl radical
RARI	:	Rajasthan agriculture research institute
ppm	:	Parts per million
DAIS	:	Days after imposing stress
RWC	:	Relative water content
SSA	:	Sulphosalicyclic acid
mg/ml	:	Milligram per milliliter
nm	:	Nanometre
mg	:	Milligram
DMSO	:	Dimethysulphoxide
A_{480}	:	Absorbance – 480
GSH	:	Glutathione reduced
EDTA	:	Ethylene Diammonium Tetra Acetic Acid
M	:	Mol
DTNB	:	Dithiobis nitro benzoic acid
DCPIP	:	2,6-dichlorophenol indophenols
TCA	:	Trichloroacetic acid
TBA	:	Thiobarbituric acid
v/v	:	Volume per volume
w/v	:	Weight per volume
PVP	:	Polyvinyl pyrolidone
Fr.wt	:	Fresh weight
m Mol/g	:	Millimol per gram
µg/g	:	Microgram per gram

Chapter – 1
INTRODUCTION

Wheat (*Triticum aestivum* L.) 2n = 42 is one of the most important staple food crop of the world including India. It is believed to be native of South Western Asia. It belongs to the family Gramineae. It is cultivated under diverse conditions of soil and climate. In India, it is the second most important food crop after rice. It is an excellent source of nutrients, containing approximately 78% carbohydrates, 12% protein, 2% fat, minerals and considerable amount of vitamins (Kumar *et al.,* 2011). About 80 to 85% of wheat grains are used as flour (*atta*) and consumed in the form of *chapaties*. Soft wheat is used for making *chapaties*, bread, cake, biscuits, pastry and other bakery products. Hard wheat is used for manufacturing *rawa*, *suji* and *sewaya*. In areas where rice is a staple food grain, wheat is eaten in the form of *puri* and *uppama*. It is also used for making flakes and sweet like *kheer* and *shira*. Wheat straw is mainly used as fodder for livestock.

India has the largest area under wheat (30.0 million hectares), but ranks second in production (93.5 million tonnes) after China with the average productivity of 3117 kg/ha (Economic Survey, 2014-15). It is cultivated mainly in the states of Uttar Pradesh, Madhya Pradesh, Punjab, Rajasthan, Haryana, Bihar, Gujarat and Maharashtra. Among the different states of India, Uttar Pradesh ranks first in area and total production, while Punjab ranks first in productivity (GOI, 2013-14). In Rajasthan, the crop occupies an area of 2.82 million hectares and production of 8.95 million tonnes with an average productivity of 3174 kg/ha (GOI, 2013-14).

Biotic and abiotic factors cause losses in crop production. The physiochemical factors include water and nutrient availability, temperature,

day length, soil p^H, salt content in the soil are the dominant factors regulating the productivity (Kramer and Boyer, 1995). Drought 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.,* 2005). Plants in general cannot store moisture hence, a control and tolerance mechanism is required to regulate the plant water status. Levitt (1982) has defined mechanism of escape, avoidance and drought tolerance. Different morpho-physiological and biochemical parameters have been reviewed earlier (Richard and Coleman, 1982; Boyer, 1996). Due to water stress (Morgan, 1984), low molecular weight protein known as 'stress protein' such as late embryogenesis abundants (LEAS) and dehydrins (Close and Lammers, 1993), osmotically active sugars (manitol, sorbitol, fructans and raffinose) are produced. Amino acids like proline, glycine betain and polyamines are osmolytes which may contribute towards osmotic adjustment, besides providing protection to macromolecules. There are certain chemicals which have potential to induce drought tolerance in crop plants. They are associated with several cellular and physiological processes and may be useful for protecting crop against abiotic stress.

Drought stress is one of the major limitations to crop productivity. In India, water deficit stress limits crop production in about 67% of net sown area. Wheat yield is reduced by 50-90% of its irrigated potential by drought in at least 60 million hectare in the developing world (Skovmand *et al*., 2001). Improving drought tolerance and productivity is one of the most difficult task for cereal breeders. The difficulty arises when the diverse strategies adopted by the plants themselves to combat drought stress, depending on the timing, severity and stage of crop growth fail (Nguyen *et al*., 2004 and Sairam *et al*., 1998). Drought impacts on biochemical and

molecular processes leads to stomatal closure with consecutive decrease in rates of transpiration, pigment content, photosynthesis that cause protein alteration leading to growth inhibition (Lawlor and Cornic, 2002; Zhu, 2002). Many techniques such as seed priming and exogenous application before and during cultivation have been efficiently used as methods of mitigations of drought stress.

Under drought stress, a variety of reactive oxygen species (ROS) are produced, which result in oxidative damage of cell membranes (Lin and Wang, 2002; Hameed *et al*, 2011). In order to scavenge these activated oxygen species, plant produce a number of enzymatic antioxidants such as super oxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), peroxidase (POX) as well as non-enzymatic antioxidants such as ascorbic acid (AsA), glutathione, α-tocopherol, flavonoides and caroteinoides (Blokhina *et al*., 2003; Hameed *et al*, 2009).

Most environmental stresses, including drought induce enhanced production of reactive oxygen species (ROS) (Pastory and Foyer, 2002; Karpinski *et al.*, 2003; Loloi *et al.*, 2004 and Ashraf *et al*., 2009). During optimal conditions, the balance between reactive oxygen species formation and consumption is tightly controlled by antioxidant enzymes and redox metabolites (Foyer *et al.*, 2005 and Kaur and Gupta, 2005). Drought tolerance is dependent on multiple genes and antioxidant enzymes. Antioxidant enzymes are important components in the mechanism of drought and desiccation tolerance (Ingram and Bartels, 1996; Noctor *et al.*, 2002; Miller *et al.*, 2010; Selote and Khanna-chopra, 2010; Chugh *et al*., 2011; Sharma and Dubey, 2005; Kaur *et al.*, 2009; Gill and Tuteja, 2010; Maia *et al*., 2010 and Rachana *et al*., 2012). Relationship between antioxidants levels and stress tolerance has been demonstrated in transgenic plants (Noctor *et al*., 1998).

Many stress alleviating molecules including thiols are crucial for enhancing the crop productivity as these improve the metabolic imbalance, developed in a cell during stress. Thiols are well-known to maintain the redox state (-SH/-S-S-ratio) in plant cells while maintains proper functioning under stress condition (Sahu *et al*., 2005; Nathawat *et al*., 2007; Srivastava *et al*., 2009; Anjum *et al.,* 2011 and Perveen *et al*., 2013). Thiourea is direct scavenger of superoxide radical as well as hydroxide radical and hydrogen peroxide under water stress (Kelner *et al*., 1990 and Lin and Kao, 1998). There is very little information available on the influence of exogenously applied thiourea on membrane stability and antioxidant defence system of wheat under water stress. Sulphydryl (SH) group is a dominant chemical group influencing metabolic reaction in the plant both under normal as well as stress conditions. Loss of reactive sulphydryl groups of membrane proteins is one of the major factors playing an important role in water stress injury. Seed soaking in sulphydryl compound solutions should therefore improve stress tolerance of plants. Thiourea; one of the fertilizers containing 36.8 and 42.1% of N and S, respectively has diverse biological activities in plants (Garg, 2007).

Recent studies have further shown that sucrose transport in plants requires special proteins known as “sucrose transport proteins” 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; Sahu *et al*, 2006), pearl millet (Parihar *et al*, 1998), mustard (Khafi *et al*, 1997) and clusterbean (Garg *et al*, 2006).

Several reactive oxygen species (ROS) are continuously produced in plants as byproducts of aerobic metabolism. Even under optimal conditions, many metabolic processes produce ROS. The production of toxic derivatives/ROS is increased as a result of different types of environmental stresses (Mahatma *et al,* 2009). Drought stress induces high production of ROS (Fu and Huang, 2001) and causes damage to mitochondria and chloroplast by increasing hydrogen peroxide (H_2O_2) concentration and lipid peroxidation (MDA) in plant tissues (Apel and Hirt, 2004). To counteract the toxicity of ROS a complex antioxidant system, composed of both non-enzymatic and enzymatic constituents is present in all plant cells. Antioxidant enzymes (e.g. superoxide dismutase, SOD; catalase, CAT; peroxidise, APX and ascorbic peroxidise, APOX) have been related to water deficiency and are considered the main components of antioxidative machinery for drought resistance in higher plants (Bergmann *et al*, 1999). Production of phenolics and flavonoids are considered as a cellular adaptive mechanism for ROS scavenging during stress, preventing subcellular damage (Rosemann *et al*, 1991).

Thiourea is a non-physiological thiol and has been employed by various researchers to impart stress tolerance and improve yield of crops like wheat (Sahu *et al*, 2006) and maize (Amin *et al*, 2013). Thiourea application improved the plant growth potential and photosynthetic efficiency. This was concomitant with the onset of early maturity and increased crop yield. All these effect could be related to ROS scavenging activity which has been first demonstrated in HL 60 cell lines (Kelner *et al*, 1990). Later this has also been proved in plants by demonstrating its ameliorative action towards

drought (Hassanein *et al*, 2012) and salinity (Srivastava *et al*, 2011) stress that are known to cause oxidative damage.

The present investigation was conducted to explore the effect of thiourea spray during vegetative stage before anthesis, on hydrogen peroxide, lipid peroxidation and some enzymatic and non-enzymatic antioxidants in water stressed and unstressed wheat plants. The present investigation was carried out during *rabi,* 2015-16 with the following objectives.

I. Analysis of different metabolites and antioxidants under water stress in wheat genotypes
II. Analysis of stress related enzymes during water stress in wheat genotypes.

Chapter – 2

REVIEW OF LITERATURE

The chapter contains the review of literature related to the work on effect of thiourea on biochemical attributes of water stress in wheat genotypes (*Triticum aestivum* L.).

The identification of biochemical responses that have adaptive value in conferring tolerance is of basic interest to plant biochemists whose observations over decades of intensive research 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, cross-talk between signaling systems may have important consequences for the plant's subsequent responses to other biological and environmental stress, and their survival (Cosgrove *et al.,* 2000).

One prominent areas of research is drought tolerance 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, where 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 and to accumulate an array of large and

small molecular weight compounds conferring drought tolerance (Zidenga, 2006).

Neha *et al.,* (2013) studied the effect of foliar applied glycine betaine (GB, 100 mM) on content of various osmolytes such as proline, choline, GB and sucrose under drought stress conditions in wheat and reported significant accumulation of Proline, GB, choline and sucrose under water stress conditions at tillering and anthesis stage.

Fahim *et al.,* (2013) reported that priming of seeds are an effective method for enhancing seed performance and improving tolerance of crops to abiotic stresses especially drought. One-hour priming increased total sugar content, total free amino acids and decreased soluble proteins in wheat cultivars. In another study, it was shown that application of salicylic acid increases moisture content, dry mass, carboxylase activity of Rubisco, Superoxide dismutase (SOD) activity and total chlorophyll, protected Nitrate reductase (NR) activity and maintained the protein and nitrogen content in wheat seedlings under water stress (Bhupindrer *et al.,* 2003).

Maghsoudlou *et al.,* (2014) reported the effects of drought stress on wheat leaf proteome pattern in susceptible (*Bahar*) and tolerant (*Kavir*) cultivars of spring wheat. Proteins involved in drought stress were identified as involved in photosynthesis, metabolic pathways, stress defense/response, photorespiration, protein synthesis and proteins with unknown functions. Winter wheat varieties have been shown differing in drought tolerance (strong, moderate and weak) and having variation in superoxide dismutase, catalase, proline, soluble protein and soluble sugars of the flag leaf after anthesis under water stress as compared to control (YuMei, 2006). Pereyra *et al.,* (2006) reported that *Azospirillum*-plant association is accompanied by biochemical changes in phospholipid

composition, fatty acid distribution profiles and degree of unsaturation of the major phospholipid classes in roots which promote plant-growth and tolerance to water stress. A comparative proteomic analysis of drought-responsive proteins during grain development of wheat varieties showed that newely synthesized proteins were mainly involved in carbohydrate metabolism like alpha-amylase inhibitor, ADP glucose pyrophos-phorylase and sucrose synthase, detoxification and defense like catalase and ascorbate peroxidase and storage proteins like late embryogenesis abundant (LEA)protein and WD 40 repeat protein (ShanShan *et a*l., 2012).

Ali *et al.,* (2013) reported that silicon maintained higher water status with increased leaf water potential, relative water content and higher chlorophyll content. Silicon is also beneficial to improve the growth of wheat and changes in the physiological and biochemical traits. In another study, it was shown that application of foliar selenium significantly lowered osmotic potential that markedly improved turgor pressure, enhanced transpiration rate, enhanced accumulation of total soluble sugars and free amino acids and activity of antioxidant system which ultimately increased the grain yield (Nawaj *et al.,* 2015). Iqbal *et al.,* (2012) reported that trehalose promoted a positive effect under the drought stress that has led to enhanced growth of wheat.

Hameed *et al.,* (2013) reported that two wheat (*Triticum aestivum* L.) genotypes with varying degree of drought tolerance showed programmed cell death (PCD) and related biochemical changes like higher peroxidase, superoxide dismutase, catalase, ascorbic acid, total phenolic content and unchanged lipid peroxidation under drought stress compared to sensitive genotype.

Amirjani *et al.,* (2013) reported the effect of drought stress on wheat (*Triticum aestivum*) seedlings under controlled condition. The seeds of wheat were subjected to five levels of water potential. 0 MPa (as control) and -2, -4, -6 and -8 MPa (as treatments) and germination percentage, mean germination time, proline, sugar, chlorophyll content, maximum photochemical efficiency of PS-II and electron transport rate (ETR) were examined. Total chlorophyll content was reduced in all studied treatments. Malondialdehyde, Ascorbic acid and Glutathione reduced contents increased in relation to the drought period. Activities of enzymatic antioxidant enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase, peroxidase and glutathione reductase increased to manage the oxidative stress. Mallick *et al.,* (2011) reported that the proline, reducing sugar, free amino acid, total polyphenol and antioxidant contents, and superoxide dismutase, catalase and peroxidase activities in leaves increased under water stress, whereas the relative water content decreased in wheat genotypes.

Marcin *et al.,* (2013) reported the role of salicylic acid (SA) and abscisic acid (ABA) in osmotic stress tolerance of wheat seedlings. In both drought susceptible and drought resistant wheat cultivars, significant physiological and biochemical changes were observed. While PEG treatment reduced gas exchange parameters, chlorophyll content in drought resistant cultivar, increased lipid peroxidation, soluble carbohydrates in drought susceptible, proline content in both cultivars and total antioxidants activity in drought susceptible. In another study, it was shown that the effect of pre-soaking of wheat genotypes in salicylic acid (SA) solution increased the total biomass, grain yield per plant, spikes per plant, 100 seed weight, proline, total soluble sugars, membrane stability index (MSI), superoxide dismutase (SOD) and ascorbate peroxidase (APOX) activity (Khan *et al.,* 2009).

Response of wheat (*Triticum aestivum L.) genotypes* to drought and exogenously applied abscisic acid was studied in pots (Sumera *et al.,* 2010). Marked decreases in leaf water potential as well as pigment content occurred under drought stress. The inhibitory effects of water stress on plant water status and biochemical contents were ameliorated by exogenous application of ABA. This ameliorating effect was found to be more significant at booting stage as compared to grain filling stage. Upon re-watering, recovery from drought stress was found to be greater in case of abscisic acid treated plants. Devi *et al.,* (2012) reported that biochemical markers such as changes in activities of antioxidant enzymes like catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POD) and superoxide dismutase (SOD) can be used for identifying genotypes before sowing for drought tolerance in wheat. Rajamani *et al.,* (2012) reported that an oxidative stress due to water deficiency triggers lipid peroxidation and accumulation of malondialdehyde and osmolytes (*viz.*, L-proline and soluble sugars). These low molecular weight metabolites could serve as useful biochemical markers for screening drought tolerance in wheat genotypes grown under arid and semi-arid conditions. Hameed *et al*., (2013) observed that the effect of hydrogen peroxide and thiourea application on wheat genotypes under water stress showed increased antioxidants and protease activity. Thiourea treatment prevented the protein loss, decreased the protease activity and enhanced the catalase and peroxidase activity, which results in protective effect.

Alhadi *et al.*, (1999) reported susceptible reaction to water stress during the vegetative growth stage in fenugreek. Drought cause substantial reduction in growth parameters such as height, weight and total leaf area. Photosynthetic pigment (chl.a, chl.b and carotenoid) in the leaves diminished by decreasing soil matric potential. Chaves *et al.,* (2003) reported that

negative responses of plants to water stress may be due to alteration in photosynthesis and carbohydrate metabolism mediated by stomatal closure and CO_2 exchange. Acharya *et al.,* (2006) reported considerable variability among sixteen fenugreek genotypes due to water stress. They differed in morphology, growth habit, bio mass and seed production capability. Chemical constituents of the seed, e.g. saponins, fibre, protein, amino acids and fatty acids contents also differed markedly. Malik and Tehlan (2009) observed significant differences in plant height, branches per plant, pods per plant, length of pod, seed per pod and seed yield in fenugreek genotypes under water stress.

Drought stress usually leads to oxidative stress due to stomatal closure which causes the over-reduction of photosynthetic electrons (Ben *et al.,* 2009). Karmakar *et al.,* (2014) reported that stress causes oxidative damage to plants either directly or indirectly by triggering an increased level of production of reactive oxygen species (ROS) which include superoxide radical. They cause damage to the biomolecules such as membrane lipid, proteins, enzymes, nucleic acid.

Pant *et al.,* (2014) reported that chlorophyll a and b, total chlorophyll, proline, total phenolic contents, total peroxidase and catalase activities increased under stress conditions, suggesting the tolerance of callus to drought stress. However, ascorbate peroxidase activities were found to decrease slightly. A slight disturbance was found in membrane stability index under drought condition.

Hassanein *et al* (2014-15) reported that combined application of Thiourea (2.5 or 5mM) and Salicyclic acid (1mM) through seed treatment and foliar spray was more effective in improving the wheat performance by enhancing antioxidant compound (Phenolic and flavonoides), membrane

stability, antioxidant enzyme (SOD and CAT) and reducing putrescine, MDA and hydrogen peroxide free radicals.

Abdelkader *et al* (2012) showed that pretreated wheat with salicyclic acid and/or thiourea, possessed a significant increase in carotenoids, antioxidant enzymes activities and some metabolites (growth promoters, photosynthetic pigments, carbohydrates, nitrogenous constituents and minerals) and decreased lipid peroxidation and hydrogen peroxide and also generally improved morphology and yield component in wheat crop.

Dawood (2016) showed that osmoregulator compounds mainly regulate osmotic pressure within the plant and protect cell wall, membranes of chloroplast and increase the photosynthesis efficiency, which leads to stability and the organization of permeability and play an important role in scavenging the free radicals that lead to mitigate the adverse impact of stress and improve growth, productivity and quality of wheat plants.

Asthir *et al* (2013) reported that Thiourea application ameliorated the heat induced damage by stimulating the total antioxidant activity through decreased in lipid peroxidation, membrane injury and increased total soluble proteins, amino acids and chlorophyll contentents in all tested genotypes and also increased plant height, peduncle length, peduncle weight and grain weight. Combined application of thiourea as seed treatment and foliar spray was more effective in improving the wheat performance by enhancing membrane stability, antioxidant potential and yield component.

Water stress lead to marked changes in endogenous levels of plant metabolites including proline, soluble sugars and proteins (Joyce *et al*., 1992). Some of these have been postulated to enhance drought resistance (Hanson and Hitz., 1982). Water stress affects almost every physiological and biochemical plant process (Hayek *et al*., 2000).

Davidson and Chevaliar (1992) reported that plant under water stress often store less amount of water soluble carbohydrates than non-stress plants, this could possibly be due to reduced photosynthesis. Battistelli *et al.,* (1992) observed that water stress decreased plant relative water content and photosynthetic rates of soybeans grown in the field. Four days after re-watering stressed plants, effects on relative water content were almost eliminated and photosynthesis was only 10% lower than those of non-stressed plants. Baisake *et al.,* (1994) reported that decline in chlorophyll content with water stress. Kraus *et al*., (1995) have also reported a decrease in chlorophyll content upon exposure to oxidation stress with comparatively higher chlorophyll content in tolerant genotypes under stress condition than in susceptible ones. Sairam (1994) reported that under moisture stress, chlorophyll content and chlorophyll stability index were higher in tolerant genotypes in comparision to susceptible genotypes.

Nyachiro *et al*., (2001) reported that water deficit decreased photosynthetic efficiency by 44.5 to 55.7% and caused a decrease in chlorophyll a, b and total chlorophyll content in wheat. The stability of chlorophyll may be considered a trait for tolerance to abiotic factors (Gupta *et al*., 2000). Under the stress condition, oxygen acts as an alternate acceptor of electron, resulting in the formation of the superoxide radical (O_2^-), H_2O_2 and the hydroxyl radical (OH^-) (Cadena, 1989). In plant cells chloroplast, mitochondria and peroxisomes are important intracellular generators of reactive oxygen spp (O_2^-, H_2O_2, OH^-) (Salim, 1991; Hernadez *et al*, 1994; Noctor and Foyer, 1998 and Dash and Panda, 2001).

Chapter – 3
MATERIALS AND METHODS

3.1 Plant materials

The present investigation was carried out on Wheat (*Triticum aestivum* L.). The seeds of three genotypes (RAJ-4037, RAJ-4079 and RAJ-1482)) were obtained from Incharge, AICRP on wheat, RARI, Rajasthan Agriculture Research Institute, Durgapura, Jaipur. A field experiment was carried out using three genotypes at Agronomy farm, S.K.N. Collage of Agriculture, Jobner during *Rabi* season 2015-2016 to investigate "Response of Biochemical Changes to Thiourea Application in Wheat Genotypes under Water Stress at Vegetative Stage". The proposed investigation was conducted in randomized block design (RBD). In order to achieve the objectives of present investigation the experiment was planned and executed as described below (Hameed *et al*., 2013).

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

Wheat genotypes were raised/sown in 2m x 3m plot, keeping row to row distance (22.5cm x 22.5cm) and plant to plant distance (10 cm x 10 cm). Sowing was done on 21 November 2015 using dibbler. Seeds were placed at a depth of 4-5 cm. The recommended dose of fertilizer (120 kg N, 60 kg P_2O_5, and 60 kg K_2O) were given to the crop. The sowing was done in randomised block design with three replication and three genotypes in four sets. Among these, two sets were used for stress by withholding irrigation and remaining sets were used as control by providing the normal irrigation.

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 after 40 days after sowing (DAS). Thiourea (1000 ppm) was applied through foliar spray at the same time *i.e.* 40 DAS after sowing (Hameed *et al*., 2013).

The observations were recorded after 47 DAS (7 days stress), 54 DAS (14 days stress) and 61 DAS (21 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 three stages viz. 47 DAS, 54 DAS and 61 DAS except yield which was recorded at maturity (120 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 Relative water content

3.5.2 Proline Content

3.5.3 Carotenoids content

3.5.4 Glutathione reduced

3.5.5 Ascorbic Acid

3.5.6 Total Phenol

3.5.7 Malondialdehyde

3.5.8 Total free amino acid

3.5.9 Membrane stability index

3.5.10 Peroxidase

3.5.11 Seed yield

3.6 Procedure and Techniques

3.6.1 Relative water content (%)

Fresh weight of the flag leaf was taken and then it was kept in distilled water for 4 hours (Barrs and Weatherly, 1962) to obtain turgid weight. The turgid weight was recorded after blotting the excess water on the surface of the leaf. Dry weight was obtained after drying the leaf in an oven at 60^{o}C. The RWC was taken then calculated by the formula (Slavik, 1974) as:-

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

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

Fresh leaf sample (0.2g) was extracted in 5.0 ml sulphosalicyclic acid (SSA 3%) [Appendix-IVA(a)] using mortar-pestle at room temperature. The homogenate was centrifuged at 8000 rpm for 10 minutes. The clear supernatants were collected in clear test tubes separately and the volume was made to 1ml with sulphosalicyclic acid. To 1.0 ml of supernatants was added 2 ml of glacial acetic acid and mixed thoroughly, followed by 2.0 ml acid ninhydrin [Appendix-IVA(b)] reagent 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. 4 ml of toluene solvent was added to each tube and mixed well using vortex mixture. The pink colour of L-proline as extracted in SSA and taken up by the solvent after incubation was separated. Toluene fractions were collected using a separating funnel and intensity of pink colour was read at 520 nm in a spectrophotometer. A standard curve was prepared using L-proline (0.1 mg/ml).

3.6.3 Carotenoid content (Wellburn, 1994)

Total chlorophyll in leaves was determined by DMSO (dimethylsulphoxide) method. Wheat leaves (50 mg) were taken in graduated test tubes. 10 ml of DMSO was added to each tube and incubated at 65^{o}C for 3 hours. After incubation the tubes were allowed to cool at room temperature and the volume was made up to 10 ml by adding DMSO. The optical density (OD) was recorded at 663 nm and 645 nm by taking DMSO as blank.

The amount of chlorophyll present in the sample was calculated using standard formula.

Total chlorophyll (mg/g) = 22.2 (OD at 663) + 8.02 (OD at 645):- The procedure was same for carotenoid as for total chlorophyll content except that absorbance was recorded at 480 nm. Carotenoid content was calculated using the following formula.

Carotenoid (µg/ml) = $(1000A_{480}-2.14\ C_a-70.16C_b/220)$

C_a (mg/lit) = $(12.7\ A_{663}-2.69\ A_{645})$

C_b (mg/lit) = $(22.9A_{645}\ -4.68\ A_{663})$

3.6.4 Glutathione Reduced (GSH) (Bailey, 1998)

Fresh leaf sample (0.25g) was extracted in mortar-pestle using 5.0 ml of 0.1 M phosphate buffer [Appendix-IVB(a)], pH 7.8 containing EDTA (1mM) [Appendix-IVB(c)], disodium salt was dissolved 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 then 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-IVB(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 in spectrophotometer against reagent black. A standard curve was prepared using GSH (0.1 mg/ml).

3.6.5 Ascorbic acid (Roe, 1964)

For the extraction of metabolite, 1 g of leaf sample each from control and stressed plants were ground in 5 ml of chilled 0.8 N $HClO_4$ and centrifuged at 10,000 rpm for 25 minutes. The clear supernatant was decanted carefully and used for the estimation of ascorbic acid. Ascorbic acid content was estimated by using the method of Roe (1964), which is based on the reduction of 2, 6-dichlorophenol indophenol (2, 6-DCPIP) dye by ascorbic acid. Sample was homogenized in 5 ml of 5% (w/v) meta phosphoric acid in glacial acetic acid and the homogenate was centrifuged at 10,000 x g for 25 minutes. The supernatant, thus, obtained was used for the estimation of ascorbic acid content. An aliquot (0.5 ml) was titrated with

2,6-DCPIP reagent until a pink end point is reached. The quantity of ascorbic acid was calculated by comparing the amount of 2,6-DCPIP reagent used for unknown sample with that used with known quantities of ascorbic acid (5-40 mg).

3.6.6 Total phenol (Malick and Singh, 1980)

500 mg leaves were grinded in a mortar-pestle with 5-6 ml of hot 80% alcohol [Appendix-IVE(b)]. The homogenate was centrifuged at 5000 rpm for 10 minutes. The clear supernatant was taken in 10 ml measuring cylinder and made up the volume to 10 ml with 80% alcohol. Pipetted out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard solution and 0.5 ml of the phenol extract in numbered test tube. Make up the volume to 1ml with distilled water. In control tube, take 1ml distilled water only. Add 0.5ml of Folin's reagent to each tube and keep at room temperature for 3 minutes. Add 1ml of 20% sodium carbonate solution [Appendix-IVE(a)], mix and make up the volume to 10ml with distilled water. Place the tubes in boiling water bath for 1minutes. After cooling the tubes, read absorbance at 650nm against the control.

3.6.7 Malondialdehyde (Heath and Packer, 1968)

Fresh leaf sample (0.2g) was extracted in 5.0 ml of 6% TCA solution [Appendix-IVD(a)] followed by centrifugation at 8,000 rpm for 10 minutes. Supernatant were collected in separate tubes. To 1ml of the supernatant taken in a clean, dry test tube, was added 2.0 ml of TBA reagent [Appendix-IVC(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 minutes and clear supernatant bearing yellow to light orange colour was read on spectrophotometer at two wavelength viz. 532 nm (major for MDA) and 600 nm (minor for interfering substance) Millimolar concentrance of MDA was calculated as follows.

MDA (mM) = (OD 532-OD 600) x 155 (extinction coefficient)

3.6.8 Total free amino acid (Mertz *et al*., 1974)

Amino acids were extracted with hot 80% ethanol from 100 mg leaves and prepared the extract in 2 ml 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. Made up the volume in each test tube to 1ml with distilled water. In other tube 1 ml distilled water only acted as control. Added 1ml of ninhydrin reagent to each tube mixed and kept the test tube in boiling water bath for 15 minutes. The tubes were cooled to room temperature and added 3 ml diluent [Appendix-IVF(a)] (n-propanol and water in 1:1 v/v) to each. OD was taken at 570 nm in spectrophotometer, adjusting zero OD with control. Using standard curve, amino acids was calculated and result expresses as mg amino acid per g of the sample.

3.6.9 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 distile water (50 ml). One set was kept at 40^0C 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 (100^0C) for 10 minutes and its conductivity (C_2) was recorded after cooling at room temperature. The MSI was calculated according to the formula:-

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

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

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

3.6.11 Seed yield per plant

For seed yield five plants were selected randomly of each genotypes and yield was calculated as seed yield per plant (g).

3.7. Statistical analysis

The experiment was conducted in Randomised Block Design (RBD). All the observation were taken in three replications for each genotype and treatment. The data were statistically analysis according to Panse and Sukhatme, 1985.

3.8. Facilities available:

Wheat crop was grown at Agronomy Farm, S.K.N. College of Agriculture, Jobner and biochemical analysis were carried out in the Department of Biochemistry.

Chapter-4
EXPERIMENTAL RESULTS

The results of the experiment entitled **"Response of Biochemical Changes to Thiourea Application in Wheat (*Triticum aestivum* L.) Genotypes Under Water Stress at Vegetative 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 conducted in three replications of each variety. The water stress was created by withholding irrigation at 40 days after sowing and the same time thiourea application was given to both the sets of the plants (control and water stress). The observations of different parameters were recorded at 7, 14 and 21 days after imposing stress (DAIS).

4.1 Relative water content (%)

The relative water content (RWC) of a leaf is a measurement of its hydration status (actual water content) relative to its maximal water holding capacity at full turgidity. RWC provides a measurement of the water deficit of the leaf and may indicate a degree of stress expressed under drought and heat stress. RWC integrates leaf water potential (ψ; another useful estimate of plant water status) with the effect of osmotic adjustment (a powerful mechanism of conserving cellular hydration) as a measurement of plant water status. A genotype with the ability to minimize stress by maintaining turgid leaves in stressed environments will have physiological advantages e.g., this allows turgor dependent processes such as growth and stomatal activity, and to protect and maintain the photosystem complex.

Table 4.1 shows the effect of water stress on relative water content (%) in three wheat varieties at three growth stages. Data presented in the

table showed that relative water content varied among varieties and treatments at vegetative stage. The relative water content decreased in all the three varieties with the growth stages. The relative water content decreased from 84.16 to 79.11 percent in variety RAJ-1482, from 83.21 to 76.23 percent in RAJ-4037 and from 76.51 to 71.49 percent in RAJ-4037 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 RAJ-1482 was 23.43, 24.32% and 26.39% respectively at 7, 14 and 21 days after imposing stress (DAIS) as compared to control. Similarly, the decrease in relative water content due to water stress as compared to control was 22.63%, 22.55% and 22.88% in variety RAJ-4079 at the three stages. The decrease in relative water content due to water stress in RAJ-4037 was 19.31%, 19.86% and 20.98% respectively at 7, 14 and 21 DAIS. The result thus shows that the decrease in relative water content due to water stress was maximum at 21 DAIS in all the three varieties. Among three varieties the maximum decrease in relative water content due to water stress was observed in RAJ-1482 followed by RAJ-4079 and RAJ-4037.

Application of thiourea (1000 ppm) through spray of the plants showed increase in relative water content under control (normal irrigation) as well as under water stress. The increase in relative water content due to application of thiourea under water stress was maximum at 21 DAIS in all the three varieties which was 7.04% in RAJ-1482, 4.78% in RAJ-4037 and 3.62% in RAJ-4079 as compared to water stress. Thus, the effect of thiourea in reversing the effect of water stress was maximum in RAJ-1482 followed by RAJ-4037 and RAJ-4079.

Table: 4.1 Effect of thiourea on Relative water content in wheat under water stress

Treatments / Varieties	Relative water content (%)		
	7 DAIS	**14 DAIS**	**21 DAIS**
RAJ-4037			
Control	83.21	80.13	76.23
Control + thiourea	86.53	82.22	79.14
Water stress	67.14 (-19.31)	64.21 (-19.86)	60.23 (-20.98)
Water stress+ thiourea	69.16 (3.00)	66.23 (3.14)	63.11 (4.78)
RAJ-4079			
Control	76.51	74.23	71.49
Control + thiourea	80.43	78.21	73.20
Water stress	59.19 (-22.63)	57.49 (-22.55)	55.13 (-22.88)
Waterstress+ thiourea	60.15 (1.62)	59.13 (2.85)	57.13 (3.62)
RAJ-1482			
Control	84.16	82.33	79.11
Control + thiourea	87.54	80.11	79.16
Water stress	64.44 (-23.43)	62.30 (-24.32)	58.23 (-26.39)
Water stress + thiourea	68.15 (5.75)	66.53 (6.78)	62.33 (7.04)
SEm±	**4.18**	**3.99**	**3.84**
CD (P=0.05)	**12.27**	**11.69**	**11.25**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

4.2 Proline (mg/g fr.wt.)

Proline, an amino acid, plays highly beneficial role in plants exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule. Review of the literature indicates that a stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance; stabilizing membranes thereby preventing electrolyte leakage; and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants.

The result showed that proline content varied among varieties and treatments at vegetative stage as shown in table 4.2. The proline content was almost same at all the growth periods. It ranged from 0.66 to 0.70 mg g^{-1} in variety RAJ-1482, from 0.57 to 0.52 mg g^{-1} in RAJ-4037 and 0.55 to 0.49 mg g^{-1} in RAJ-4079 from 7-21 DAIS under control condition (normal irrigation).

Proline content increased due to water stress in all the three varieties. The increase in proline due to water stress in variety RAJ-4037 was 8.77%, 16.66% and 26.92% respectively at 7, 14 and 21 days after imposing stress (DAIS) as compared to control. Similarly, the increase in proline due to water stress as compared to control was 7.27%, 13.20% and 26.53% in variety RAJ-4079 at the three stages. RAJ-1482 witnessed 3.03%, 4.41% and 5.71% increase in proline due to water stress respectively at 7, 14 and 21 DAIS. The result thus shows that the increase in proline due to water stress was maximum at 21 DAIS in all the three varieties. Among three varieties the maximum increase in proline due to water stress was observed in RAJ-4037 followed by RAJ-4079 and RAJ-1482.

Table: 4.2 Effect of thiourea on Proline in wheat under water stress

Treatments / Varieties	Proline (mg/g fr.wt.)		
	7 DAIS	14 DAIS	21 DAIS
RAJ-4037			
Control	0.57	0.54	0.52
Control + thiourea	0.50	0.49	0.48
Water stress	0.62 (8.77)	0.63 (16.66)	0.66(26.92)
Water stress+ thiourea	0.59 (-4.83)	0.58 (-7.93)	0.58 (-12.12)
RAJ-4079			
Control	0.55	0.53	0.49
Control + thiourea	0.46	0.45	0.44
Water stress	0.59 (7.27)	0.60 (13.20)	0.62(26.53)
Waterstress+ thiourea	0.58 (-1.69)	0.56 (-6.66)	0.54 (-12.90)
RAJ-1482			
Control	0.66	0.68	0.70
Control + thiourea	0.54	0.50	0.52
Water stress	0.68 (3.03)	0.71 (4.41)	0.74 (5.71)
Water stress + thiourea	0.66 (-2.94)	0.66 (-7.04)	0.64 (-13.51)
SEm±	**0.03**	**0.03**	**0.03**
CD (P=0.05)	**0.09**	**0.09**	**0.09**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

Application of thiourea (1000 ppm) through spray of the plants showed decrease in proline under control (normal irrigation) as well as under water stress. The decrease in proline due to application of thiourea under water stress was maximum at 21 DAIS in RAJ-4079, RAJ-4037 and RAJ-1482 varieties which was 12.90%, 12.12% and 11.11% respectively as compared to water stress. Thus, the effect of thiourea under water stress was maximum in RAJ-4079 followed by RAJ-4037 and RAJ-1482.

4.3 Total Carotenoids (mg/g)

Carotenoids are a class of phytonutrients ("plant chemicals") and are found in the cells of a wide variety of plants, algae and bacteria. They help plants absorb light energy for use in photosynthesis. They also have an important antioxidant function of deactivating free radicals — single oxygen atoms that can damage cells by reacting with other molecules.

Table 4.3 shows the effect of water stress on carotenoids in three wheat varieties at three growth stages. Result showed that carotenoids varied among varieties as well as treatments during vegetative stage. Content of carotenoids increased in all the three varieties with the growth stages from 2.98 to 3.16 mg g^{-1} in variety RAJ-4079, from 2.80 to 2.90 mg g^{-1} in RAJ-1482 and 2.69 to 2.71 mg g^{-1} in RAJ-4037 under control condition (normal irrigation).

Water stress led to decrease in carotenoids content in all the three varieties. The decrease in carotenoids due to water stress in variety RAJ-4079 was 9.06%, 23.91% and 29.11% respectively at 7, 14 and 21 days after imposing stress (DAIS) as compared to control. Similarly, the decrease in carotenoids due to water stress as compared to control was 10.71%, 23.48% and 28.62% in variety RAJ-1482 at the three stages. The decrease in carotenoids due to water stress in RAJ-4037 was 11.52%, 18.88% and

26.19% respectively at 7, 14 and 21 DAIS. The result thus shows that the decrease in carotenoids due to water stress was maximum at 14 DAIS in all the three varieties. Among three varieties, the maximum decrease in carotenoids due to water stress was observed in RAJ-4079 followed by RAJ-1482 and RAJ-4037.

Table: 4.3 Effect of thiourea on Carotenoids in wheat under water stress

Treatments / Varieties	**Carotenoids (mg/g)**		
	7 DAIS	**14 DAIS**	**21 DAIS**
RAJ-4037			
Control	2.69	2.70	2.71
Control + thiourea	2.73	2.72	2.72
Water stress	2.38(-11.52)	2.19 (-18.88)	2.00 (-26.19)
Water stress+ thiourea	2.59 (8.10)	2.39 (9.13)	2.18 (9.00)
RAJ-4079			
Control	2.98	3.22	3.16
Control + thiourea	3.22	3.21	3.20
Water stress	2.71 (-9.06)	2.45 (-23.91)	2.24 (-29.11)
Waterstress+ thiourea	2.93 (8.11)	2.68 (9.38)	2.46 (9.82)
RAJ-1482			
Control	2.80	2.98	2.90
Control + thiourea	3.22	3.18	3.16
Water stress	2.50(-10.71)	2.28 (-23.48)	2.07 (-28.62)
Water stress + thiourea	2.65 (6.00)	2.43 (6.57)	2.22 (7.24)
SEm±	**0.15**	**0.15**	**0.14**
CD (P=0.05)	**0.44**	**0.44**	**0.42**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

Application of thiourea (1000 ppm) through spray of the plants showed increase in carotenoids under control (normal irrigation) as well as under water stress. The increase in carotenoids due to application of thiourea under water stress was maximum at 21 DAIS in RAJ-4079, RAJ-4037 and RAJ-1482 varieties which was 9.82%, 9.00% and 7.24% respectively as compared to water stress. Thus, the effect of thiourea under water stress was maximum in RAJ-4079 followed by RAJ-4037 and RAJ-1482.

4.4 Glutathione Reduced (GSH) (mg/g)

Glutathione (GSH) is an important antioxidant in plants, animals, fungi, and some bacteria and archaea. GSH is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals.

Data presented in Table 4.4 showed that glutathione reduced varied among varieties and treatments during vegetative stage. Under normal irrigation as well as under stress the glutathione reduced increased in all the varieties with the growth stages except RAJ-1482. The glutathione reduced increased from 1.49 to 1.62 mg g^{-1} in variety RAJ-4079, from 1.38 to 1.47 mg g^{-1} in RAJ-1482 and decreased from 1.50 to 1.42 mg g^{-1} in RAJ-4037 under control condition (normal irrigation).

The glutathione was increased due to water stress in all the three varieties. The increase in glutathione due to water stress in variety RAJ-1482 was 5.47%, 5.55% and 7.48% respectively at 7, 14 and 21 days after imposing stress (DAIS) as compared to control. In variety RAJ-4079, the increase in glutathione due to water stress as compared to control was 4.02%, 5.06% and 5.55% at the three stages. The increase in glutathione due to water stress in RAJ-4037 was 2.66%, 2.73% and 3.52% respectively

at 7, 14 and 21 DAIS. The result thus showed that the increase in glutathione due to water stress was maximum at 21 DAIS in RAJ-1482 followed by RAJ-4079 and RAJ-4037. Among the three varieties the maximum increase in glutathione due to water stress was observed in RAJ-1482 followed by RAJ-4079 and RAJ-4037.

Table: 4.4 Effect of thiourea on GSH in wheat under water stress

Treatments / Varieties	GSH (mg/g)		
	7 DAIS	14 DAIS	21 DAIS
RAJ-4037			
Control	1.50	1.46	1.42
Control + thiourea	1.43	1.50	1.53
Water stress	1.54 (2.66)	1.50 (2.73)	1.47 (3.52)
Water stress+ thiourea	1.62 (5.10)	1.55 (3.33)	1.51 (2.72)
RAJ-4079			
Control	1.49	1.58	1.62
Control + thiourea	1.41	1.39	1.35
Water stress	1.55 (4.02)	1.66 (5.06)	1.71 (5.55)
Waterstress+ thiourea	1.62 (4.51)	1.70 (2.40)	1.74 (1.75)
RAJ-1482			
Control	1.38	1.44	1.47
Control + thiourea	1.36	1.33	1.30
Water stress	1.46 (5.47)	1.52 (5.55)	1.60 (7.48)
Water stress + thiourea	1.55 (6.16)	1.54 (1.31)	1.46 (0.63)
SEm±	**0.08**	**0.08**	**0.08**
CD (P=0.05)	**0.23**	**0.24**	**0.25**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

Application of thiourea (1000 ppm) through spray of the plants showed increase in glutathione under water stress. The increase in glutathione due to application of thiourea under water stress was maximum at 7 DAIS in all the three varieties which was 6.16% in RAJ-1482, 5.10% in RAJ-4037 and 4.51% in RAJ-4079 as compared to water stress. Thus, the effect of thiourea under water stress was maximum in RAJ-4037 followed by RAJ-1482 and RAJ-4079 at 7 DAIS.

4.5 Ascorbic acid (mmol/g)

Vitamin C (L-ascorbic acid) has important antioxidant and metabolic functions in plants. Plant-derived ascorbate is the major source of vitamin C in the human diet. L-ascorbate is abundant in plants and may have roles in photosynthesis and transmembrane electron transport. Being an antioxidant, ascorbic acid increases nutritional value of the plant products and is likely to enhance tolerance of the plants to abiotic stress.

Effect of water stress on ascorbic acid in three wheat varieties at three growth stages is shown on table 4.5. Results showed that ascorbic acid varied among varieties and treatments at vegetative stage. The ascorbic acid increased in all the three varieties with the growth stages. The ascorbic acid increased from 26.84 to 29.43 m mol/g in variety RAJ-4079, from 23.64 to 25.65 m mol/g in RAJ-1482 and from 22.24 to 25.23 m mol/g in RAJ-4037 during 7-21 DAIS growth period under control condition (normal irrigation).

Table: 4.5 Effect of thiourea on ascorbic acid in wheat under water stress

Treatments / Varieties	Ascorbic acid (mmol/g)		
	7 DAIS	**14 DAIS**	**21 DAIS**
RAJ-4037			
Control	22.24	24.53	25.23
Control + thiourea	18.54	20.32	22.22
Water stress	30.43**(36.82)**	35.65**(45.33)**	38.22**(51.48)**
Water stress+ thiourea	28.43 **(-6.57)**	33.22**(-6.81)**	35.12 **(-8.11)**
RAJ-4079			
Control	26.84	27.34	29.43
Control + thiourea	25.42	26.53	27.53
Water stress	38.54**(43.59)**	40.12**(46.74)**	43.23**(46.89)**
Waterstress+ thiourea	37.23 **(-3.39)**	36.7**(-8.44)**	35.43**(-19.03)**
RAJ-1482			
Control	23.64	24.54	25.65
Control + thiourea	20.34	22.43	23.42
Water stress	38.43**(62.56)**	40.54**(65.19)**	43.22**(68.49)**
Water stress + thiourea	37.25 **(-3.07)**	36.54**(-9.89)**	34.52**(-20.12)**
SEm±	**1.45**	**1.53**	**1.55**
CD (P=0.05)	**4.25**	**4.48**	**4.54**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

Effect of water stress showed increase in ascorbic acid due to the stress in all the three varieties. The increase in ascorbic acid due to water stress in variety RAJ-1482 was 62.56%, 65.19%, and 68.49% respectively at 7, 14 and 21 days after imposing stress (DAIS) as compared to control. Similarly, the increase in ascorbic acid due to water stress as compared to control was 36.82%, 45.33% and 51.48% in variety RAJ-4037 at the three stages. The increase in ascorbic acid due to water stress in RAJ-4079 was 43.59%, 46.74% and 46.89% respectively at 7, 14 and 21 DAIS. The result thus shows that the increase in ascorbic acid due to water stress was maximum at 21 DAIS in RAJ-1482 followed by RAJ-4079 and RAJ-4037. Among three varieties the maximum increase in ascorbic acid due to water stress was observed in RAJ-1482 followed by RAJ-4037 and RAJ-4079.

Application of thiourea (1000 ppm) through spray of the plants showed decrease in ascorbic acid under control (normal irrigation) as well as under water stress. The decrease in ascorbic acid due to application of thiourea under water stress was maximum at 21 DAIS in RAJ-1482 followed by RAJ-4079 and RAJ-4037 which was respectively 20.12%, 19.03% and 8.11% as compared to water stress.

4.6 Phenol content (µg/g)

Phenolic compounds are basically involved in plant metabolic system and widely spread throughout the plant kingdom. These compounds are very much essential for the growth of plant and involved in reproduction process of plants. These compounds are produced during the response process against pathogens and abiotic stress. Antioxidant action of phenolic compounds is because of their high tendency to chelate heavy metals like iron and copper.

Table: 4.6 Effect of thiourea on phenols in wheat under water stress

Treatments / Varieties	Phenol (µg/g fresh weight)		
	7 DAIS	14 DAIS	21 DAIS
RAJ-4037			
Control	4.12	4.15	4.14
Control + thiourea	4.11	4.18	4.17
Water stress	4.18 (1.21)	4.20 (1.20)	4.21 (1.60)
Water stress+ thiourea	4.22 (0.95)	4.23 (0.71)	4.25 (0.95)
RAJ-4079			
Control	4.19	4.18	4.17
Control + thiourea	4.21	4.20	4.19
Water stress	4.24 (1.19)	4.23 (1.19)	4.22 (1.19)
Waterstress+ thiourea	4.26 (0.47)	4.25 (0.47)	4.25 (0.71)
RAJ-1482			
Control	4.17	4.16	4.15
Control + thiourea	4.20	4.19	4.18
Water stress	4.22 (1.19)	4.21 (1.20)	4.20 (1.20)
Water stress + thiourea	4.24 (0.47)	4.23 (0.47)	4.23 (0.71)
SEm±	**0.22**	**0.22**	**6.07**
CD (P=0.05)	**0.65**	**0.65**	**0.65**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

In plant system, free radicals are formed due to stress which easily damage cells due to presence of free electrons. Green vegetables release some compounds which can neutralize free radicals and help in repairing the damage in cells caused due to free radicals. Generally antioxidants lose electron to a free radical and get paired. The pairing of electron with free radical makes it less harmful.

Results of present study showed that phenols varied among varieties and treatments at vegetative stage (Table 4.6). Content of phenols decreased in all the three varieties with the growth stages except RAJ-4037 under control condition (normal irrigation). Phenol decreased from 4.19 to 4.17 µg g^{-1} in variety RAJ-4079, from 4.17 to 4.15 µg g^{-1} in RAJ-1482 and increased from 4.12 to 4.14 µg g^{-1} in RAJ-4037 under control condition (normal irrigation).

The study showed very small increase in phenols due to water stress in all three varieties. The increase in phenols due to water stress in these three varieties ranged between 1.19% to 1.60%. The result thus shows that the increase in phenol due to water stress was maximum at 21 DAIS in RAJ-4037, 14 DAIS in RAJ-1482 and same in all growth stages in RAJ-4079 varieties. Among three varieties the maximum increase in phenol due to water stress was observed in RAJ-4037 followed by RAJ-1482 and RAJ-4079.

Application of thiourea (1000 ppm) through spray of the plants showed increase in phenols under control (normal irrigation) as well as under water stress. The increase in phenols due to application of thiourea under water stress was maximum at 21 DAIS in RAJ-4079 and RAJ-1482, and 14 DAIS in RAJ-4037. The effect of thiourea under water stress was same and negligeable in all three varities.

4.7 Malondialdehyde (m moles $g^{-1)}$

MDA (Malondialdehyde) is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. MDA readily combines with several functional groups on molecules including proteins, lipoproteins, and DNA. MDA-modified proteins may show altered physico-chemical behavior and antigenicity. Lipid peroxidation is a well-established mechanism of cellular injury in plants and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, among which malondialdehyde (MDA) is the most abundant. Therefore, measurement of malondialdehyde is widely used as an indicator of lipid peroxidation.

Table 4.7 shows the effect of water stress on malondialdehyde in three wheat varieties at three **growth** stages. Data presented in the table showed that malondialdehyde varied among varieties and treatments at vegetative stage. The malondialdehyde decreased in all the three varieties with the growth stages. The malondialdehyde decreased from 28.43 to 19.43 m moles g^{-1} in variety RAJ-4037, from 21.43 to 16.14 m moles g^{-1} in RAJ-1482 and from 20.60 to 17.25 m moles g^{-1} in RAJ-4079 under control condition (normal irrigation).

Table: 4.7 Effect of thiourea on MDA in wheat under water stress

Treatments / Varieties	MDA (m moles g^{-1})		
	7 DAIS	**14 DAIS**	**21 DAIS**
RAJ-4037			
Control	28.43	24.12	19.43
Control + thiourea	25.22	19.76	15.92
Water stress	32.12 (12.97)	29.33 (21.60)	25.96 (33.60)
Water stress+ thiourea	29.43(-8.37)	25.54(-12.92)	21.29(-17.98)
RAJ-4079			
Control	20.60	19.03	17.25
Control + thiourea	18.22	16.32	13.33
Water stress	25.50 (23.78)	24.22 (27.27)	22.93 (32.92)
Waterstress+ thiourea	24.12(-5.41)	22.52 (-7.01)	20.43(-10.90)
RAJ-1482			
Control	21.43	18.34	16.14
Control + thiourea	24.22	21.54	19.26
Water stress	31.58(47.36)	28.34(54.52)	25.43(57.55)
Water stress + thiourea	27.32(-13.48)	23.43 (-17.32)	19.34(-23.94)
SEm±	**1.26**	**1.13**	**1.07**
CD (P=0.05)	**3.70**	**3.32**	**3.14**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

The malondialdehyde increased due to water stress in all the three varieties. The increase in malondialdehyde due to water stress in variety RAJ-1482 was 47.36%, 54.52% and 57.55% respectively at 7, 14 and 21 days after imposing stress (DAIS) as compared to control. Similarly, the increase in malondialdehyde due to water stress as compared to control was 23.78%, 27.27% and 32.92% in variety RAJ-4079 at the three stages. The increase in malondialdehyde due to water stress in RAJ-4037 was 12.97%, 21.60%, and 33.60% respectively at 7, 14 and 21 DAIS. The result thus shows that the increase in malondialdehyde due to water stress was maximum at 21 DAIS in all the varieties. Among three varieties the maximum increase in malondialdehyde due to water stress was observed in RAJ-1482 followed by RAJ-4079 and RAJ-4037.

The application of thiourea (1000 ppm) through spray of the plants showed decrease in malondialdehyde under control (normal irrigation) as well as under water stress. The decrease in malondialdehyde due to application of thiourea under water stress was maximum at 21 DAIS in RAJ-1482, RAJ-4037 and RAJ-4079 which was 23.94%, 17.98% and 10.90% as compared to water stress. Thus, the effect of thiourea under water stress was maximum in RAJ-1482 followed by RAJ-4037 and RAJ-4079.

4.8 Total amino acid (mg g^{-1})

Total amino acids were estimated by ninhydrin method and the results showed that amino acids varied among varieties and treatments at vegetative stage (Table 4.8). The amino acids decreased in all the three varieties with the growth stages from 23.00 to 22.62 mg g^{-1} in variety RAJ-4079, from 21.97 to 21.60 mg g^{-1} in RAJ-4037 and 21.27 to 20.87 mg g^{-1} in RAJ-1482 under control condition (normal irrigation).

Table: 4.8 Effect of thiourea on total amino acid in wheat under water stress

Treatments / Varieties	Total amino acid (mg/g Fr.wt.)		
	7 DAIS	14 DAIS	21 DAIS
RAJ-4037			
Control	21.97	21.74	21.60
Control + thiourea	22.30	22.06	21.92
Water stress	23.18 (5.50)	23.27(7.03)	23.22(7.50)
Water stress+ thiourea	24.01 (3.58)	23.77(2.14)	23.60(1.63)
RAJ-4079			
Control	23.00	22.78	22.62
Control + thiourea	23.32	23.17	23.03
Water stress	23.68 (2.95)	23.56(3.42)	23.54(4.06)
Waterstress+ thiourea	24.01 (1.39)	23.82(1.10)	23.77(0.97)
RAJ-1482			
Control	21.27	21.03	20.87
Control + thiourea	21.57	21.34	21.34
Water stress	21.69 (1.97)	21.66(2.99)	21.59(3.44)
Water stress + thiourea	22.06 (1.70)	21.90(1.10)	21.72(0.60)
SEm±	**1.20**	**1.19**	**1.18**
CD (P=0.05)	**3.51**	**3.48**	**3.47**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

The amino acids increased due to water stress in all three varieties. The increase in amino acid due to water stress in variety RAJ-4037 was 5.50%, 7.03% and 7.50% respectively at 7, 14 and 21 days after imposing

stress (DAIS) as compared to control. Similarly, the increase in amino acid due to water stress as compared to control was 2.95%, 3.42% and 4.06% in variety RAJ-4079 at the three stages. The increase in amino acid due to water stress in RAJ-1482 was 1.97%, 2.99% and 3.44% respectively at 7, 14 and 21 DAIS. The result thus shows that the increase in amino acid due to water stress was maximum at 21 DAIS in all the three varieties. Among three varieties the maximum increase in amino acids due to water stress was observed in RAJ-4037 followed by RAJ-4079 and RAJ-1482.

The application of thiourea (1000 ppm) through spray of the plants showed increase in amino acid under water stress. The increase in amino acid due to application of thiourea under water stress was maximum at 7 DAIS in RAJ-4037 followed by RAJ-1482 and RAJ-4079 as compared to water stress. The effect of thiourea under water stress was maximum in RAJ-4037 followed by RAJ-1482 and RAJ-4079.

4.9 Membrane stability index (%)

Membrane stability index may be used as parameter to estimate the cellular injury caused 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. The increased levels of MDA in stress condition indicate the membrane sensitivity/ membrane damage while lower rate of increase of MDA indicates better membrane strength.

Table: 4.9 Effect of thiourea on MSI in wheat under water stress

Treatments / Varieties	MSI (% fw)		
	7 DAIS	**14 DAIS**	**21 DAIS**
RAJ-4037			
Control	72.69	69.15	66.07
Control + thiourea	84.31	80.76	79.20
Water stress	68.08 (-6.34)	64.01 (-7.43)	60.28 (-8.76)
Water stress+ thiourea	70.50 (3.55)	69.00 (7.79)	66.18(9.78)
RAJ-4079			
Control	73.15	70.51	66.11
Control + thiourea	76.16	71.16	69.14
Water stress	65.08(-11.03)	63.00(-10.65)	60.00(-9.24)
Waterstress+ thiourea	69.41 (6.65)	70.13(11.31)	72.15(20.25)
RAJ-1482			
Control	59.85	56.59	54.55
Control + thiourea	69.56	66.63	64.07
Water stress	51.63(-13.73)	46.84(-17.67)	43.63(-20.01)
Water stress + thiourea	61.55(19.21)	59.01(23.48)	60.30(38.20)
SEm±	**3.70**	**3.56**	**3.42**
CD (P=0.05)	**10.85**	**10.44**	**10.03**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

Table 4.9 shows the effect of water stress on membrane stability index (%) in three wheat varieties at three growth stages. Data presented in the table showed that membrane stability index varied among varieties and treatments at vegetative stage. The membrane stability index decreased in all the three varieties with the growth stages from 73.15 to 66.11 percent in variety RAJ-4079, from 72.69 to 66.07 percent in RAJ-4037 and 59.85 to 54.55 percent in RAJ-1482 under control condition (normal irrigation).

The membrane stability index decreased due to water stress in all three varieties. The decrease in membrane stability index due to water stress in variety RAJ-1482 was 13.73%, 17.67%, and 20.01% respectively at 7, 14 and 21 days after imposing stress (DAIS) as compared to control. Similarly, the decrease in MSI due to water stress as compared to control was 11.03%, 12.70% and 13.73% in variety RAJ-4079 at the three stages. The decrease in MSI due to water stress in RAJ-4037 was 6.34%, 7.43%, and 8.76% respectively at 7, 14 and 21 DAIS. The result thus shows that the decrease in MSI due to water stress was maximum at 21 DAIS in all the three varieties. Among three varieties the maximum decrease in MSI due to water stress was observed in RAJ-1482 followed by RAJ-4079 and RAJ-4037.

The application of thiourea (1000 ppm) through spray of the plants 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 21 DAIS in all the three varieties which was 38.20% in RAJ-1482, 20.25% in RAJ-4079 and 9.78% in RAJ-4037 as compared to water stress. Thus, the effect of thiourea in reversing the effect of water stress was maximum in RAJ-1482 followed by RAJ-4079 and RAJ-4037.

4.10 Peroxidase (EC. 1.11.1.7)

Peroxidases and their effects on plants have been analysed by many researcher. Peroxidases are involved in many physiological processes in plants, involving responses to biotic and abiotic stresses and the biosynthesis of lignin. Liglin is a polymer responsible for rendering the plants stronger, rigid and also making the cell walls hydrophobic. Peroxidases are involved in the polymerization of the precursors of lignin. They are also involved in the scavenging of Reactive Oxygen Species (ROS), which are partially reduced forms of atmospheric oxygen, highly reactive, and capable of causing oxidative damage to the cells. Peroxidases are haem-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyse a number of oxidative reactions. Plant peroxidases are monomeric glycoproteins containing 4 conserved disulphide bridges and 2 calcium ions. Plant peroxidases, which have multiple tissue-specific functions e.g., removal of hydrogen peroxide from chloroplasts and cytosol; oxidation of toxic compounds; biosynthesis of the cell wall; defence responses towards wounding; indole-3-acetic acid (IAA) catabolism; ethylene biosynthesis etc.

Table 4.10 shows the effect of water stress on peroxidase in three wheat varieties at three **growth** stages. Data presented in the table showed that peroxidase varied among varieties and treatments at vegetative stage. Peroxidase activity decreased in all the three varieties with the growth stages from 0.62 to 0.56 in variety RAJ-4037, from 0.54 to 0.49 in RAJ-1482 and 0.49 to 0.45 in RAJ-4079 under control condition (normal irrigation).

Table: 4.10 Effect of thiourea on Peroxidase in wheat under water stress

Treatments / Varieties	Peroxidase (enzyme units) x 10^3		
	7 DAIS	**14 DAIS**	**21 DAIS**
RAJ-4037			
Control	0.62	0.59	0.56
Control + thiourea	0.83	0.80	0.77
Water stress	0.92 (48.38)	0.90 (52.54)	0.89 (58.92)
Water stress+ thiourea	0.97 (5.43)	0.95 (5.55)	0.95 (6.74)
RAJ-4079			
Control	0.49	0.47	0.45
Control + thiourea	0.58	0.55	0.52
Water stress	0.67 (36.73)	0.66 (40.42)	0.65 (44.44)
Waterstress+ thiourea	0.70 (4.47)	0.68 (3.03)	0.67(4.68)
RAJ-1482			
Control	0.54	0.51	0.49
Control + thiourea	0.61	0.58	0.56
Water stress	0.69 (27.77)	0.67 (31.37)	0.66 (34.69)
Water stress + thiourea	0.72 (4.34)	0.70 (4.34)	0.69 (4.34)
SEm±	**0.04**	**0.04**	**0.04**
CD (P=0.05)	**0.11**	**0.11**	**s0.10**

DAIS = Days after imposing stress + = increase, - = decrease

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

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

Water stress led to increase in peroxidase activity in all the three varieties. The increase in peroxidase due to water stress in variety RAJ-4037 was 48.38%, 52.54% and 58.92% respectively at 7, 14 and 21 days after imposing stress (DAIS) as compared to control. Similarly, the increase in peroxidase due to water stress as compared to control was 36.73%, 40.42% and 44.44% in variety RAJ-4079 at the three stages. The increase in peroxidase due to water stress in RAJ-1482 was 27.77%, 31.37% and 34.69% respectively at 7, 14 and 21 DAIS. The result thus shows that the increase in peroxidase due to water stress was maximum at 21 DAIS in all the three varieties. Among three varieties the maximum increase in peroxidase due to water stress was observed in RAJ-4037 followed by RAJ-4079 and RAJ-1482.

The application of thiourea (1000 ppm) through spray of the plants showed increase in peroxidase under control (normal irrigation) as well as under water stress. The increase in peroxidase due to application of thiourea under water stress was maximum at 14 DAIS in RAJ-4079 and RAJ-4037 and same in all growth stages in RAJ-1482 varieties which was 6.74%, 4.68% and 4.34% respectively as compared to water stress. Thus, the effect of thiourea under water stress was maximum in RAJ-4079 followed by RAJ-4037 and RAJ-1482.

4.11 Seed Yield (q/ha)

Wheat grains were collected from mature plants grown in plot size of 2x3 m^2 for each variety in three replications. Average yield of each variety under each treatment was calculated as follows.

Yield/ha = Yield from a plot sized 6 m^2x10000/100x6

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

Treatments / Varieties	Seed yield (q/ha)
RAJ-4037	
Control	31.46
Control + thiourea	33.62 (6.86)
Water stress	26.32 (-16.33)
Water stress+ thiourea	30.22 (14.81)
RAJ-4079	
Control	28.08
Control + thiourea	31.20 (11.11)
Water stress	22.02 (-21.58)
Waterstress+ thiourea	26.98 (22.52)
RAJ-1482	
Control	26.00
Control + thiourea	30.03 (15.50)
Water stress	18.11 (-30.34)
Water stress + thiourea	20.12 (11.09)
SEm±	**1.55**
CD (P=0.05)	**4.54**

+ = increase, - = decrease

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

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

Table 4.11 shows the effect of water stress on seed yield in three wheat varieties. Data presented in the table showed that seed yield varied among varieties and treatments. The seed yield increased due to application of thiourea (1000 ppm) in all the three varieties under control condition as well as water stress. The maximum increase in yield due to thiourea application under normal irrigation was observed in RAJ-4037 (15.50%) followed by RAJ-1482 (11.11%) and RAJ-4079 (6.86%).

Result showed that seed yield decreased due to water stress in all three varieties. The decrease in seed yield due to water stress was 30.34% in RAJ-1482, 21.58% in RAJ-4079 and 9.98% in RAJ-4037 as compared to control. Among three varieties the maximum decrease in seed yield due to water stress was observed in RAJ-1482 followed by RAJ-4079 and RAJ-4037.

The application of thiourea (1000 ppm) through spray of the plants showed increase in seed yield under water stress. The increase in seed yield due to application of thiourea under water stress was maximum in RAJ-4079 (22.52%) followed by RAJ-4037 (14.81%) and RAJ-1482 (11.09%) as compared to water stress.

Chapter-5

DISCUSSION

Study on **"Response of Biochemical Changes to Thiourea Application in Wheat (*Triticum aestivum* L.) Genotypes Under Water Stress at Vegetative Stage"** was conducted in the Department of Biochemistry, S.K.N. College of Agriculture, Jobner during Nov to April 2015-16. Three genotypes of wheat RAJ-4037, RAJ-4079 and RAJ-1482 were used. These varieties were sown on November 15 in Agronomy farm, S.K.N. college of Agriculture, Jobner in a plot size of 2x3 m^2. The experiment was conducted in three replications for each variety and each treatment. Water stress was created by withholding water supply at 40 DAS to one set of plants. At the same time thiourea spray (1000 ppm) was given to all the three wheat genotypes grown under normal and water stress.

Water stress is one of the major limitations to crop productivity. In India, water deficit stress limits crop production in about 67% of net sown area. Wheat yield is reduced by 50-90% of its irrigated potential by drought in at least 60 million hectare in the developing world (Skovmand *et al*., 2001). Improving drought tolerance and productivity is one of the most difficult task for cereal breeders. The difficulty arises when the diverse strategies adopted by the plants themselves to combat drought stress, depending on the timing, severity and stage of crop growth fail (Nguyen *et al*., 2004 and Sairam *et al*., 1998). Drought impacts on biochemical and molecular processes leads to stomatal closure with consecutive decrease in rates of transpiration, pigment content, photosynthesis that cause protein alteration leading to growth inhibition (Lawlor and Cornic, 2002; Zhu, 2002). Many techniques such as seed priming and exogenous application before

and during cultivation have been efficiently used as methods of mitigations of drought stress.

Many stress alleviating molecules including thiols are crucial for enhancing the crop productivity as these improve the metabolic imbalance, developed in a cell during stress. Thiols are well-known to maintain the redox state (-SH/-S-S-ratio) in plant cells while maintains proper functioning under stress condition (Sahu *et al*., 2005; Nathawat *et al*., 2007; Srivastava *et al*., 2009; Anjum *et al.,* 2011 and Perveen *et al*., 2013). Thiourea is direct scavenger of superoxide radical as well as hydroxide radical and hydrogen peroxide under water stress (Kelner *et al*., 1990 and Lin and Kao, 1998). There is very little information available on the influence of exogenously applied thiourea on membrane stability and antioxidant defence system of wheat under water stress.

Recent studies have further shown that sucrose transport in plants requires special proteins known as “sucrose transport proteins” 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; Sahu *et al*, 2006), pearl millet (Parihar *et al*, 1998), mustard (Khafi *et al*, 1997) and clusterbean (Garg *et al*, 2006).

Thiourea is a non-physiological thiol and has been employed by various researchers to impart stress tolerance and improve yield of crops like

wheat (Sahu *et al*, 2006) and maize (Amin *et al*, 2013). Thiourea application improved the plant growth potential and photosynthetic efficiency. This was concomitant with the onset of early maturity and increased crop yield.

The present investigation was conducted to explore the effect of thiourea spray during vegetative stage before anthesis, on hydrogen peroxide, lipid peroxidation and some enzymatic and non-enzymatic antioxidants in drought stressed and unstressed wheat plants. In the present study, certain physio-biochemical parameters were analysed in three genotypes varying in field performance in response to water stress and thiourea application. Membrane stability index, Relative water content, MDA, GSH, Ascorbic acid, POX, Proline content, Total amino acids, Carotenoids and Phenols were analysed at three stages of development, i.e., 47 DAS, 54 DAS and 61 DAS in three wheat varieties, grown in the field and subjected to water stress and thiourea application. These Investigations were carried out in fully expanded leaves and yield was recorded at harvest. The experiment was undertaken to see if these physio-biochemical parameters could be related to tolerant in wheat plants.

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).

The relative water content (RWC) of a leaf is a measurement of its hydration status (actual water content) relative to its maximal water holding capacity at full turgidity .In the present study, it was observed that RWC decreased due to water stress in all the three varieties. Reductions in RWC due to water stress was found lowest in variety RAJ-4037 followed by RAJ-4079, thus indicating these to be tolerant to water stress at all growth stages. Maximum reduction in RWC was observed in varieties RAJ-1482 at all growth stages, indicating its susceptibility to water stress. Spray of thiourea reversed the decrease in RWC due to water stress to some extent in all the three varieties. With thiourea application maximum reversion was observed at 21 DAIS in RAJ-1482 followed by RAJ-4037 and then RAJ-4079. The results are supported by the earlier findings of Sumera *et al.,* 2010 who reported marked decrease in leaf water potential as well as pigment content under drought stress in wheat.

In the present study, the MSI reductions was lowest in variety RAJ-4037, thus indicating this to be tolerant to water stress at all growth stages. Maximum reduction in MSI values was observed in variety RAJ-1482 at all growth stages, indicating its susceptibility to water stress. The results are supported by the earlier findings of Hassanein *et al* (2014-15) who reported that combined application of Thiourea (2.5 or 5mM) and Salicylic acid (1mM) through seed treatment and foliar spray was more effective in improving the wheat performance by enhancing antioxidant compound (Phenolic and flavonoides), membrane stability, antioxidant enzyme (SOD and CAT) and reducing putrescine, MDH and hydrogen peroxide free radicals. Spray of thiourea reversed the decrease in MSI due to water stress to some extent in all the three varieties. With thiourea application maximum reversion was observed at 21 DAIS in RAJ-1482 followed by RAJ-4079 and then RAJ-4037. Foliar spray of thiourea (it has imino and thiol functional groups)

provides a ready sources of nitrogen and thiol which has great role in alleviating oxidative stress damage in physiologically important leaf tissue. Decrease in MSI due to water stress may be because of increase in fluidity of lipid bilayer of biological membrane (Savchenko et al.2002). Such alteration enhances permeability of membrane and results in loss of electrolytes.

Membrane stability index may be used as a parameter to estimate the cellular injury caused to membrane due to peroxidation of fatty acids of the membrane. The levels of membrane lipid peroxidation can be measured by estimating Malondialdehyde content. 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 the present study, it was observed that MDA content increased due to water stress and the increase was lowest in variety RAJ-4037 thus again indicating this to be tolerant variety to water stress. Maximum increase in MDA content due to water stress was observed in variety RAJ-1482, indicating its susceptibility to water stress. Spray of thiourea reversed the increased MDA content due to water stress to some extent in all the three varieties. With thiourea application maximum reversion was observed at 21 DAIS in RAJ-1482 followed by RAJ-4037 and then RAJ-4079. The result, thus, show that application of thiourea (1000 ppm) through spray is able to reverse the water stress to some extent. The results are supported by the earlier findings of Rajamani *et al.,* (2012) who reported that an oxidative stress due to water deficiency triggers lipid peroxidation and accumulation of malondialdehyde and osmolytes (*viz.*, L-proline and soluble sugars). These low molecular weight metabolites could serve as useful biochemical markers

for screening drought tolerance in wheat genotypes grown under arid and semi-arid conditions.

In order to maintain osmotic balance under abiotic stress, specific types of organic molecules accumulate in the cytoplasm which are termed as compatible solutes e.g. Proline glycine betain and total free amino acid. These solutes do not impair normal physiological functions even if accumulated at high concentrations. Amino acids like proline, glycine betain and polyamines are osmolytes which may contribute towards osmotic adjustment, besides providing protection to macromolecules. There are certain chemicals which have potential to induce drought tolerance in crop plants. They are associated with several cellular and physiological processes and may be useful for protecting crop against abiotic stress. Neha *et al.,* (2013) studied the effect of foliar applied glycine betaine (GB, 100 mM) on content of various osmolytes such as proline, choline, GB and sucrose under drought stress conditions in wheat and reported significant accumulation of Proline, GB, choline and sucrose under water stress conditions at tillering and anthesis stage.

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 high accumulation of L-proline and total free amino acid at 61 DAS compared to 47 DAS in the stressed tissues. RAJ-4037 and RAJ-4079 indicated 61 DAS stage to be a more responsive stage in terms of cellular osmotic adjustment. Comparing performance of the three varieties under water stress as compared to control showed that RAJ-4037 and RAJ-4079 are water stress tolerant. The variety RAJ-1482 exhibited susceptibility to water stress. Highest percent increase in RAJ-4037 and RAJ-4079 was noticed at 61 DAS under water stress as compared to control. Spray of

thiourea decreased proline content to some extent in all the three varieties under control and water stress. With thiourea application maximum reduction was observed at 61 DAS in RAJ-4079 followed by RAJ-4037 and then RAJ-1482.

While content of free amino acids increased due to water stress and the highest percent increase was noticed in RAJ-4037 at 61 DAS as compared to control. Spray of thiourea increased total amino acids to some extent in all the three varieties under water stress. With thiourea application maximum increase was observed at 61 DAS in RAJ-4037 at 47 DAS under followed by RAJ-4079 and then RAJ-1482. The results are supported by the earlier findings of Mallick *et al.,* (2011) who reported that proline, reducing sugar, free amino acid, total polyphenol and antioxidant contents, and superoxide dismutase, catalase and peroxidase activities in leaves increased under water stress, whereas the relative water content decreased in wheat genotypes.

Under drought stress, a variety of reactive oxygen species (ROS) are produced, which result in oxidative damage of cell membranes (Lin and Wang, 2002; Hameed *et al*, 2011). In order to scavenge these activated oxygen species, plant produce a number of enzymatic antioxidants such as super oxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), peroxidase (POX) as well as non-enzymatic antioxidants such as ascorbic acid (AsA), glutathione, α-tocopherol, flavonoides and caroteinoides (Blokhina *et al*., 2003; Hameed *et al*, 2009).

Several reactive oxygen species (ROS) are continuously produced in plants as byproducts of aerobic metabolism. Even under optimal conditions, many metabolic processes produce ROS. The production of toxic derivatives/ROS is increased as a result of different types of environmental

stresses (Mahatma *et al,* 2009). Drought stress induces high production of ROS (Fu and Huang, 2001) and causes damage to mitochondria and chloroplast by increasing hydrogen peroxide (H_2O_2) concentration and lipid peroxidation (MDA) in plant tissues (Apel and Hirt, 2004). To counteract the toxicity of ROS a complex antioxidant system, composed of both non-enzymatic and enzymatic constituents is present in all plant cells. Antioxidant enzymes (e.g. superoxide dismutase, SOD; catalase, CAT; peroxidise, APX and ascorbic peroxidise, APOX) have been related to water deficiency and are considered the main components of antioxidative machinery for drought resistance in higher plants (Bergmann *et al*, 1999). Production of phenolics and flavonoids are considered as a cellular adaptive mechanism for ROS scavenging during stress, preventing subcellular damage (Rosemann *et al*, 1991).

Effect of water stress in plants causes cellular dehydration leading to oxidative stress. A secondary effect of dehydration is the increase in cellular reactive oxygen species (Apel & Hirt, 2004). Plants cope with higher levels of ROS produced due to oxidative stress by synthesizing antioxidants and enhancing activities of antioxidative enzymes. Peroxidase (POX) enzyme is responsible for scavenging H_2O_2 during oxidative stress. In the present study, activity of POX increased drastically under water stress, showing that the enzymatic anti-oxidant system was operational in all wheat genotypes. Our results are supported by many other scientists. (Pant *et al*., 2014, Mittal *et al*., 2006 and Mittal 2010). They have reported that chlorophyll a and b, total chlorophyll, proline, total phenolic contents, total peroxidase and catalase activities increased under stress conditions while ascorbate peroxidase activity decreased slightly. Membrane stability index under drought condition also decreased.

Due to water stress, activity of POX increased and the highest percent increase was noticed at 61 DAS in RAJ-4037 and RAJ-1482 as compared to control. Spray of thiourea increased POX activity to some extent in all the three genotypes under water stress. With thiourea application maximum increase was observed at 61 DAS in RAJ-4079 followed by RAJ-4037 and then RAJ-1482. Increased activity of peroxidase due to thiourea suggests that there is need to scavange free radicals; however Yonova and Zozikova (2001) reported decrease in peroxidase activity and catalase activity due to thiourea treatment but increase in guaiacol peroxidase activity in barley.

Karmakar *et al*., (2014) worked on response of fenugreek (*Trigonella foenum-graecum* L.) seedlings under moisture and heavy metal stress with special reference to antioxidant system. They found increased POD activity, indicating that this enzyme serves as an intrinsic defence; to resist PEG induced oxidative damage. The similar results can be derived from the present investigation based on POX activity.

There was not much change in phenol content at different days after sowing in RAJ-4037, RAJ-4079 and RAJ-1482 under normal and water stress in all the three genotypes. Spray of thiourea also did not change the phenol content significantly in all the three genotypes under normal as well as water stress. It could be concluded from this observation that phenol content is not affected due to water stress as well as by application of thiourea so it can not be used as a parameters to find out tolerance/susceptibility of plants to water stress.

In the present study, highest percent increase of GSH contents during stress was observed, the magnitude of GSH percent being highest at 61 DAS reveals that the wheat plants had detoxified ROS intermediates ($^{\cdot}O_2^-$ →

H_2O_2) at this stage quickly. This could be due to operation of ASC-GSH cycle during water stress (Asada and Takahashi, 1987). GR has an important role in maintaining adequate GSH/GSSG ratio in favour of GSH. GR activity increased in stressed condition in all varieties. Increased GR activity might contribute for increased GSH/GSSG ratio, which could be essential for drought resistance of plants. The elevated level of antioxidants under abiotic stress could play an important role in preventing the stress-induced accumulation of ROS (foyer *et al*, 2005). Due to water stress, gutathione increased and the highest percent increase was noticed at 61 DAS in RAJ-1482 followed by RAJ-4079 and RAJ-4037 as compared to control. Spray of thiourea increase glutathione to some extent in all the three genotypes under water stress. With thiourea application maximum increase was observed at 7 DAS in RAJ-4037 followed by RAJ-1482 and then RAJ-4037

Amirjani *et al.,* (2013) reported the effect of drought stress on wheat (*Triticum aestivum*) seedlings under controlled condition. Malondialdehyde, Ascorbic acid and Glutathione reduced contents increased in relation to the drought period. In present study, the highest percent increase in ascorbic acid due to water stress was observed in RAJ-1482 followed by RAJ-4079 and RAJ-4037. Spray of thiourea reversed the increased in ascorbic acid due to water stress to some extent in all the three varieties. With thiourea application maximum reversion was observed at 21 DAIS in RAJ-1482 followed by RAJ-4079 and then RAJ-4037.

Reactive oxygen species have an adverse impact on photosynthesic apparatus of the cell by damaging chlorophyll and carotenoids (Mittova *et al*., 2000). In the present study, total carotenoid content decreased significantly in all the three genotypes at all the growth stages due to water stress. Low level of decrease in carotenoid in genotypes indicates that their

photosynthetic apparatus is able to resist adverse condition due to water stress. Similarly, accumulation of higher carotenoid content may be due to sugar synthesized via photosynthesis and its breaks down during respiration by plants that help in developing tolerance to water stress.

Carotenoid content decreased under water stress in all the three genotypes. Highest percent decrease was noticed in RAJ-4079 at 61 DAS under water stress as compared to control followed by RAJ-1482 and RAJ-4037. Spray of thiourea could increase carotenoid to some extent in all the three genotypes under control and water stress. Maximum increase was observed with 1000 ppm thiourea in all the genotypes at 61 DAS, however, the highest carotenoid content was noticed in RAJ-4079 due to thiourea application followed by RAJ-4037 and then RAJ-1482. Based on analysis of carotenoid, variety RAJ-4079 seems to be tolerant to water stress. The results are supported by the findings of Abdouli *et al*., 2012 and Aggrawal *et al*., 2013 who reported the similar observation based on carotenoid contents. Abdelkader *et al* (2012) showed that pretreated wheat with salicyclic acid and/or thiourea, possessed a significant increase in carotenoids, antioxidant enzymes activities and some metabolites (growth promoters, photosynthetic pigments, carbohydrates, nitrogenous constituents and minerals) and decreased lipid peroxidation and hydrogen peroxide and also improved morphology and yield component in wheat crop.

Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1988). Yield is the most important parameter for a crop. However, the yield contributing parameters are different in cereals, pulses, oilseed and seed spices crops. In case of wheat, we have measured yield under control, water stress and thiourea application under control and water stress. In the present study, there is reduction in yield due to water stress in all three genotypes. The decrease

was more in genotype RAJ-1482 under water stress as compared to control. In the present study, genotypes RAJ-4037 and RAJ-4079 were less affected by water stress. These results are supported by other parameters studied in this study.

Chapter-6
Summary and Conclusion

The study entitled **"Response of Biochemical Changes to Thiourea Application in Wheat (*Triticum aestivum* L.) Genotypes under Water Stress at Vegetative Stage"** was conducted during the *rabi* season 2015-2016 at Agronomy Farm and Department of Biochemistry, S.K.N. College of Agriculture, Jobner with following objectives.

(I) Analysis of different metabolites and antioxidants under water stress in wheat genotypes.

(II) Analysis of stress related enzymes during water stress in wheat genotypes.

Three wheat varieties RAJ-4037, RAJ-4079 and RAJ-1482 varying in field performance in response to water stress were grown under field condition. The water stress was created by withholding irrigation at 40 days after sowing and at the same time thiourea application was given to both the sets of the plants (control and water stress). The observations of different parameters were recorded at 7, 14 and 21 days after imposing stress (DAIS) at vegetative stage.

The parameters analyzed in the present study included physiological indices (membrane stability index and relative water content), biochemical parameters - Malondialdehyde, Glutathione reduced, Ascorbic acid, Peroxidase, Proline, Total amino acids, Carotenoids and Phenols. Fully expanded leaves were used for experimental purpose. Following observations/ conclusions were drawn from the present investigation:

1. Membrane stability index decreased in all the three varieties with the growth stages and also due to water stress. Among three varieties the maximum decrease in MSI due to water stress was observed in RAJ-1482 followed by RAJ-4079 and RAJ-4037. Spray of thiourea could restore the MSI to some extent under water stress. The effect of thiourea in reversing the effect of water stress was maximum in RAJ-1482 followed by RAJ-4037 and RAJ-4079.

2. Peroxidase activity decreased in all the three varieties with the growth stages, and increased due to water stress. Among three varieties the maximum increase in POX activity due to water stress was observed in RAJ-4037 followed by RAJ-4079 and RAJ-1482 as compared to control. Peroxidase activity further increased due to application of thiourea under water stress which was maximum in RAJ-4037 followed by RAJ-4079 and RAJ-1482.

3. Relative water content decreased in all the three varieties with the growth stages and also due to water stress. Among three varieties the maximum decrease in relative water content due to water stress was observed in RAJ-1482 followed by RAJ-4079 and RAJ-4037 as compared to control. Spray of thiourea could restore the RWC to some extent under water stress. The effect of thiourea in reversing the effect of water stress was maximum in RAJ-1482 followed by RAJ-4037 and RAJ-4079.

4. Proline increased in all the three varieties with the growth stages and also due to water stress. Among three varieties, the maximum increase in proline due to water stress was observed in RAJ-4037 followed by RAJ-4079 and RAJ-1482 as compared to control. Spray of thiourea reduced proline content under water stress. The effect of thiourea in

reversing the effect of water stress was maximum in RAJ-4079 followed by RAJ-4037 and RAJ-1482.

5. Carotenoids increased in all the three varieties with the growth stages. Water stress decreased content of carotenoids. Among three varieties, the highest percent decrease due to water stress as compared to control was noticed in RAJ-4079 at 61 DAS followed by RAJ-1482 and RAJ-4037. Spray of thiourea increased carotenoids content under water stress. The effect of thiourea in reversing the effect of water stress was maximum in RAJ-4079 followed by RAJ-4037 and RAJ-1482.

6. Glutathione increased in all varieties with the growth stages except RAJ-4037. Water stress also increased glutathione in these varieties. The maximum increase in glutathione due to water stress was observed in RAJ-4037 followed by RAJ-4079 and RAJ-1482. Spray of thiourea decreased GSH to under water stress. The effect of thiourea in reversing the effect of water stress was maximum in RAJ-4037 followed by RAJ-1482 and RAJ-4037 at 7 DAIS.

7. Ascorbic acid content increased in all the three varieties with the growth stages and also due to water stress. Among three varieties, the maximum increase in ascorbic acid due to water stress was observed in RAJ-1482 followed by RAJ-4037 and RAJ-4079. Spray of thiourea decreased the content of ascorbic acid under water stress. The effect of thiourea in reversing the effect of water stress on ascorbic acid was maximum in RAJ-1482 followed by RAJ-4037 and RAJ-1482.

8. There was not much change in phenol content at different days after sowing in all the three varieties under normal as well as water stress condition. Spray of thiourea also did not change the phenol content

significantly in all the three genotypes under both normal and water stress condition.

9. Malondialdehyde decreased in all the three varieties with the growth stage, while it increased due to water stress. Among three varieties, the maximum increase in malondialdehyde due to water stress was observed in RAJ-1482 followed by RAJ-4037 and RAJ-4079. Spray of thiourea could restore the MDA to some extent under water stress. The effect of thiourea in reversing the effect of water stress was maximum in RAJ-1482 followed by RAJ-4037 and RAJ-4079.

10. Amino acids decreased in all the three varieties with the growth stages but increased due to water stress. Among three varieties, the maximum increase in amino acid due to water stress was observed in RAJ-4037 followed by RAJ-4079 and RAJ-1482. Spray of thiourea decreased amino acids content under water stress. The effect of thiourea in reversing the effect of water stress on amino acid was maximum in RAJ-4037 followed by RAJ-1482 and RAJ-4079.

11. There is reduction in yield due to water stress in all the three genotypes. The decrease was maximum in genotype RAJ-1482 under water stress as compared to control. Genotypes RAJ-4037 and RAJ-4079 were less affected by water stress. Spray of thiourea could restore the yield to some extent under water stress. The effect of thiourea in reversing the effect of water stress on yield was maximum in RAJ-4079 followed by RAJ-4037 and RAJ-1482.

From the present study, it may be concluded that the physio-biochemical traits like relative water content (RWC), carotenoids and membrane stability index and biochemical traits like MDA, peroxidase, phenol, total amino acid, proline, GSH and ascorbic acid are important

characters for studying effect of water stress in plants. Based on yield data wheat variety RAJ-4037 behaved as water stress tolerant and was associated with increased peroxidase activity, total amino acids, ascorbic acid, GSH, proline, MDA content and decreased content of carotenoid, RWC and MSI under water stress. The results also showed that application of thiourea was able to reverse the effect of water stress to some extent. Better performing variety RAJ-4037 over others seems to be due to its higher relative water content, proline content, carotenoid content under water stress condition. It seems these traits help the plants in maintaining higher membrane stability index and osmotic adjustment under water stress condition.

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Response of Biochemical Changes to Thiourea Application in Wheat (*Triticum aestivum* L.) Genotypes Under Water Stress at Vegetative Stage

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Abstract

Drought is one of the most important abiotic stresses throughout the world. Wheat as a major crop is mostly cultivated in area that encounter with drought stress. The response of three wheat (*Triticum aestivum L*.) genotypes viz. RAJ-4037, RAJ-4079 (drought tolerant) and RAJ-1482 (drought susceptible) to water stress and exogenously applied thiourea (1000 ppm) was determined in Department of Biochemistry in field grown plants at S.K.N. college of Agriculture, Jobner, Jaipur during wheat season 2015-16. For this purpose, physiological indices (membrane stability index and relative water content), biochemical parameters Malondialdehyde, Glutathione reduced, Ascorbic acid, Peroxidase, Proline, Total amino acids, Carotenoids and Phenols were measured in fully expanded leaves. Sampling was done at 7, 14 and 21 days after water stress induction. Marked decreases in leaf relative water content (RWC), MSI as well as carotenoid content occured under water stress. The study revealed increase in total phenol, total amino acid, proline, MDA, peroxidase activity (POX), GSH and Ascorbic acid content in all the three wheat genotypes at all stages due to water stress. Genotypes RAJ-1482 was found to be the most water stress sensitive among these genotypes. The inhibitory effect of water stress on plant water stress and biochemical content were ameliorated by exogenous application of thiourea (1000 ppm). This ameliorating effect was found to be more significant at all growth stages particularly in tolerant genotypes RAJ-4037. The yield increase due to Thiourea application in water stress tolerant variety RAJ-4037 was more as compared to other genotypes. Results signify the role of thiourea in regulating the water stress response of wheat. Thiourea could be used as a potential growth regulator under water stress condition.

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गेहूँ [*ट्रिटिकम एस्टीवम* (एल.)] के जीनप्ररूप की कार्यिकीय अवस्था में जल तनाव स्थिति में थायोयूरिया उपयोग का जैव रसायन परिवर्तन को प्रेरित करने में अनुक्रिया

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सारांश

सुखा पुरे विश्व में एक महत्वपूर्ण अजैविक तनाव है। एक महतवपूर्ण फसल के रूप में गेहूँ ऐसे क्षेत्र में उगाया जाता है जो कम से कम सुखे अवधि में सुखाग्रस्त तनाव का सामना करना पड़ता है। तीन गेहूँ प्रारूपों में राज.–4037, राज.–4079 (सुखा प्रतिरोधकता) और राज–1482 (सुखा सहनशीलता) पर पादप जैव रासायनिक विभाग श्री कर्ण नरेन्द्र कृषि महाविद्यालय, जोबनेर, जयपुर में गेहूँ ऋतु 2014–15 तक सुखा और थायोयूरिया उपचार की अनुक्रिया।

इस उद्देश्य के लिए कार्यिकीय सूचक जैसे आपेक्षिक जल सामग्री, झिल्ली स्थारांक सूचक और जैव रासायनिक सूचक जैसे मेलानडाई एल्डिहाईड (एम.डी.ए.), ग्लूटाथियोन रिडयूसड (जी.एस.एच.), एस्कोर्बिक अम्ल, परऑक्सीडेज (पी.ओ.एक्स.), प्रोलिन, कुल अमीनों अम्ल, केरोटिनायॅड्स और फीनोल का पूरी फूली पत्ती में मापन किया। नमुना जल तनाव प्रेरित करने के बाद, 7, 14 और 21 दिन बाद लिया। जल तनाव स्थिति ने पत्ति में आपेक्षिक जल सामग्री, झिल्ली स्थरांक सूचक और केरोटिनायॅड्स सामग्री कम पाया गया। जल तनाव के कारण पूरी वृद्वि अवस्था में पूरे तीनों गेहूँ जीन प्ररूपों में कुल फीनोल, कुल अमीनों अम्ल, प्रोलिन, मेलोनडाई एल्डिहाईड (एम.डी.ए.) ग्लुटाथियोन रिडयूसड (जी.एस.एच.), एस्कोर्बिक अम्ल, परऑक्सीडेज (पी.ओ.एक्स) में वृद्धि का अध्ययन दिखाया गया है। इस जीन प्ररूपों के बीच में जीन प्ररूप राज–1482 जल तनाव संवेदनिशील पाया गया। पादप और जैव रासायनिक सामग्री पर जल तनाव का रोकने योग्य प्रभाव का बाहरी थायोयूरिया का उपयोग के द्वारा सुधारा गया।

यह सुधार प्रभाव सभी वृद्धि अवस्था में विशेषतौर पर सहनशील जीनप्ररूप राज.–4037 में अधिक महत्वपूर्ण पाया गया। दूसरे जीन प्ररूप की तुलना में सहनशील जीन प्ररूप राज. –4037 में जल तनाव में थायोयूरिया के उपयोग के कारण उपज बढ़ती हुई पाई गई है। जल तनाव के नियंत्रण में थायूरिसा की भूमिका का परिणाम महतवपूर्ण पाया गया। थायोयूरिया का उपयोग जल तनाव स्थिति के अन्दर सामथ्य वृद्धि नियंत्रक के रूप में किया जाता है।

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Appendix – I

Analysis of variance for thiourea on wheat under water stress at 7 days after sowing

Source of variance	d.f.	Mean sum of square									
		RWC	Proline	Carotenoid	GSH	Ascrobic Acid	Phenols	MDA	Total amino acid	MSI	Peroxidase
Stressed	2	23.340**	0.002	0.044	0.016	7.923	0.110	5.149	3.541*	32.192	0.005
Genotypes	11	322.671**	0.013*	0.206*	0.023*	162.104**	0.006*	55.889**	2.872*	210.915**	0.065*
Error	22	49.149	0.003	0.067	0.019	6.589	0.147	5.028	4.382	45.660	0.004
SEm±		**4.05**	**0.03**	**0.15**	**0.08**	**1.48**	**0.22**	**1.29**	**1.21**	**3.90**	**0.04**
CD (p = 0.05)		**11.87**	**0.09**	**0.44**	**0.23**	**4.35**	**0.65**	**3.80**	**3.54**	**11.44**	**0.11**
CV		**9.49**	**8.90**	**9.30**	**9.22**	**8.87**	**9.14**	**8.73**	**9.23**	**9.86**	**9.27**

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

Appendix – II

Analysis of variance for thiourea on wheat under water stress at 14 days after sowing

Source of variance	d.f.	Mean sum of square									
		RWC	**Proline**	**Carotenoid**	**GSH**	**Ascrobic Acid**	**Phenols**	**MDA**	**Total amino acid**	**MSI**	**Peroxidase**
Stressed	2	21.863*	0.002	0.035	0.017	8.658*	0.111	4.007*	3.503*	30.952**	0.005
Genotypes	11	264.781**	0.020	0.411	0.033	154.732**	0.003	46.881**	2.984*	222.658**	0.066
Error	22	45.665	0.003	0.066	0.020	7.014	0.148	3.806	4.305	42.843	0.004
SEm±		**3.90**	**0.03**	**0.15**	**0.08**	**1.53**	**0.22**	**1.13**	**1.20**	**3.78**	**0.04**
CD (p = 0.05)		**11.44**	**0.09**	**0.44**	**0.24**	**4.48**	**0.65**	**3.30**	**3.51**	**11.08**	**0.11**
CV		**9.51**	**8.84**	**9.54**	**9.45**	**8.63**	**9.17**	**8.59**	**9.22**	**9.98**	**9.26**

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

Appendix – III

Analysis of variance for thiourea on wheat under water stress at 21 days after sowing

Source of variance	d.f.	Mean sum of square										
		RWC	Proline	Carotenoid	GSH	Ascrobic Acid	Phenols	MDA	Total amino acid	MSI	Peroxidase	Yield
Stressed	2	19.526*	0.002	0.029	0.017	8.763*	0.111	2.956*	3.478*	31.113**	0.005	4.498*
Genotypes	11	265.778**	0.027	0.603	0.054	163.771**	0.004	43.485**	3.051*	240.495**	0.072	69.463**
Error	22	42.147	0.003	0.062	0.021	7.414	0.148	2.871	4.259	41.126	0.004	7.201
SEm±		**3.75**	**0.03**	**0.14**	**0.08**	**1.57**	**0.22**	**0.98**	**1.19**	**3.70**	**0.03**	**1.55**
CD (p = 0.05)		**10.99**	**0.08**	**0.42**	**0.25**	**4.61**	**0.65**	**2.87**	**3.49**	**10.86**	**0.10**	**4.54**
CV		**9.56**	**8.66**	**9.65**	**9.60**	**8.53**	**9.17**	**8.59**	**9.21**	**10.10**	**9.23**	**9.93**

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

APPENDIX-IV
(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.