THIOUREA-INDUCED DROUGHT STRESS TOLERANCE IN TWO CHICKPEA VARIETIES

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CERTIFICATE

*This is to certify that the thesis entitled **"THIOUREA-INDUCED DROUGHT STRESS TOLERANCE IN TWO CHICKPEA VARIETIES"** submitted to the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in AGRONOMY**, embodies the result of a piece of bonafide research work carried out by **NAZNIN AHMED**, Registration No. **13-05291** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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The Author

THIOUREA-INDUCED DROUGHT STRESS TOLERANCE IN TWO CHICKPEA VARIETIES

ABSTRACT

Drought stress is one of the major constraints for crop production around the world; hence, a number of mechanistic approaches are required to mitigate the negative impact of drought stress. Two chickpea varieties BARI Chola-7 and BARI Chola-9 were studied to understand the effect of drought stress and the protective role of thiourea (TU) in improving drought stress tolerance. The experiment was conducted at the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, from December 2019 to March 2020. The experiment consisted of sixteen treatments with four levels of drought stress: D_0 = no drought stress i.e. soil moisture at 100% field capacity (FC), D_1 = mild drought stress (25% depletiom from FC), D_2 = moderate drought stress (50% depletion from FC), D_3 = severe drought stress (75% depletion from FC) with and without 5 mM TU application. This study was carried out in a completely randomized design (CRD) with three replications. All the obtained data were subjected to one-way analysis of variance (ANOVA). In the current study, the highest reduction of plant height, root length, fresh weight and dry weight of shoot and root, number of branches plant^{-1}, RWC and chlorophyll (chl) content was found under severe drought stress in the two chickpea varieties compared to control. A sharp increase of malondialdehyde (MDA), H_2O_2 and proline (Pro) content was observed under mild, moderate and severe drought stress. However, foliar spray of TU mitigated the oxidative damages under drought stress as reflected in improved growth and physiological parameters under mild and moderately stressed plants of both the varieties. Ascorbate (AsA) content was decreased and glutathione (GSH) and glutathione disulfide (GSSG) content were increased under D_1, D_2 and D_3. Between the two varieties, BARI Chola-9 was more tolerant compared to BARI Chola-7. Besides, TU has proved its beneficial effect against drought stress by increasing MDA, H_2O_2 and Pro in mild and moderately stressed plants through modulating non-enzymatic antioxidants. Drought stress lowered the weight of 100-seed, seed yield plant^{-1}, stover yield plant^{-1} and biological yield plant^{-1}, which were further improved by foliar spray of TU under mild and moderate drought stress of both the chickpea varieties. Noteworthy that BARI Chola-7 could not survive till maturity and plant death in two treatments ($V_1D_3TU_0$ and V_1D_3TU) occured finally. Those two treatments were not considered for measuring yield parameters under drought stress. Nonetheless, TU did not show any significant effect in improving stress-inducing damage under severe drought stress. However, the effect of TU was more promising in ameliorating oxidative stress under mild and moderately stressed plants of BARI Chola-9 compared to BARI Chola-7. Thus it was concluded that TU foliar spray improved morphological, physiological, biochemical and yield parameters under mild and moderate drought stress of chickpea.

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ABBREVIATIONS AND ACCRONYMS

ANOVA	Analysis of variance
AsA	Ascorbic acid (ascorbate)
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistcs
CAT	Catalase
Chl	Chlorophyll
CRD	Completely randomized design
CV	Coefficient of variance
cv.	Cultivar
DAS	Days after sowing
DHA	Dehydroascorbate
et al.	*et alibi* (and others)
FAO	Food and Agriculture Organization
FC	Field capacity
GSH	Reduced glutathione
GSSG	Glutathione disulfide
i.e.	id est (That is)
LSD	Least significance difference
MDA	Malondialdehyde
Pro	Proline
ROS	Reactive oxygen species
RuBisCo	Ribulose -1, 5- bisphosphate caroxylase or oxygenase
RWC	Relative water content
TU	Thiourea
viz.	Namely

ABBREVIATIONS AND ACCRONYMS (Cont'd)

°C	Degree Celsius
µg	Microgram
cm	Centimeter
g	Gram
ha	Hectare
kg	Kilogram
m	Meter
mg	Milligram
mM	Millimole

Chapter I

INTRODUCTION

The climate change is increasing to such an extent that it has already exerted a negative impact on the required quantity of water for its inhabitants, though it covers 71% of the earth's surface (Alam *et al.*, 2014a). Climate change increases the risks of rising temperature, which is projected to be higher at 1.5 °C (Hoegh-Guldberg *et al.*, 2018), whereas Assad *et al.* (2019) stated that the average temperature of the world would raise by 1.4 to 5.8 °C until the end of the century. Drought stress increased with the infrequent patterns of precipitation, increased temperature, and uncontrolled use of water by urbanization and industrialization in every country of the world (Johal and Hagroo, 2019).

United Nations (2019) reported that the world population is supposed to reach 8.6, 9.8 and 11.2 billion by 2030, 2050 and 2100, respectively, but agricultural lands will not be able to produce at the same speed. Furthermore, every year, 83 million peoples are added to the world's population (UN, 2019). Already one in nine people around the world suffer from hunger, and the only way to feed them is by doubling food production in a sustainable way (OECD/FAO, 2019). To meet the challenge of growing populace the requirement of food must be increased by 70% by the year 2050 (Hasanuzzaman *et al.*, 2018a). As Bangladesh is an agriculture-based country, it is also battleing to adapt to the climate change and to feed the increasing population to achieve food security (Islam *et al.*, 2017).

Since the first evolution of a plant, the earth has been experiencing a repeatedly changing climate. Being a sessile organism, having no locomotive structures, they frequently face a number of adverse environmental conditions known as abiotic stress, including drought, salinity, temperature extremes, toxic metals, UV radiation, etc., which has a detrimental effect on plant survival, biomass production and yield of plants (Hasanuzzaman *et al.*, 2012a). Almost 90% of cultivable lands are subjected to both biotic and abiotic stresses, which cause maximum 70% yield losses in major food crops (Waqas *et al.,* 2019). Worldwide, 64% of land areas are affected by water stress (Yadav *et al.,* 2020a). Drought can alone decrease crop productivity and yield as much as 50% in different field crops of drought-prone areas of different countries (Lamaoui *et al., 2*018).

From different abiotic stresses in the envoronment, drought stress has been distinguished to cause significant growth reduction and yield loss of crops. Throughout the life cycle, plants require a great quantity of water and nutrients for triggering germination, cell division, cell elongation, which leads to further promotion of plant growth and other metabolic actions, for instance, production of organic components, photosynthesis, respiration and other physiological and biochemical processes (Farooq *et al.,* 2012; Alam *et al.,* 2014b). Drought stress created imbalanced osmotic pressure and notable reduction of relative and absolute water content and loss of turgidity (Nahar *et al.,* 2017). Mild to moderate drought stress commonly resulted in impairment of these physiological and biochemical processes through poor water, ion and nutrients uptake from soil matrix by root, modified carbon and nitrogen cycle, stomatal closure, photosynthesis inhibition, reduced carbohydrate synthesis, increased respiration, reduced cell division, and elongation (Hasanuzzaman *et al.,* 2018b; Bhuiyan *et al.,* 2019). Drought stress mostly occurred in reproductive stage of some crops grown in dry season such as wheat, chickpea, maize, and sorghum (Lamaoui *et al.,* 2018). Thus, the limitation of the crop production by drought stress has been recognized as more severe than any other abiotic stress in the world.

Chickpea (*Cicer arietinum* L.) belongs to the family Fabaceae and the most important protein-rich-food legume. However, its production has greatly been hampered because of environmental threat such as abiotic stress in different tropical and subtropical areas. Chickpea experiences drought stress at the pre and post-anthesis stage causes serious yield decline by 45 to 50% (Rani *et al.*, 2019).

North-western and south-western region of Bangladesh have been experienced the extreme level of contingent drought risk due to the shortage of frequent rainfall and unavailable groundwater from the year 1984 till 2013, and caused 25 to 30% yield reduction of different crops (Habiba *et al*., 2013). Chickpea in the northern region faces great challenges due to drought stress at rabi and pre-kharif dry period due to the cumulative effect of dry days (Ramamasy and Baas, 2007) and consequences of early maturity through early flowering and pod abortion, which significantly reduces chickpea yields every year (Kadiyala *et al.*, 2016). In chickpea, drought stress affects different morphological, physiological and biochemical traits (Sabaghpour *et al.,* 2006; Rahbarian *et al.,* 2011; Ramamoorthy *et al.*, 2016).

Recently, due to the climate change, irregular rainfall causes an increment of the intensity of drought stress and has become a serious threat to the country's food security as it damages the major economic crop production in Bangladesh. Drought stress has a major influence on agricultural production that has drawn a considerable attention of the farmers, researchers and policy makers and crop scientists have increased their research area to the crop adaptation to drought stress as well as to develop drought-tolerant crop varieties (Islam *et al.*, 2017). Several experiments on drought-stressed chickpea have shown growth reduction resulted from significantly decreased photosynthesis, relative water content (RWC), carbohydrate accumulation etc. (Randhawa *et al.,* 2014; Hussain *et al.,* 2015). Therefore, approaches for screening and breeding of suitable crop varieties combining the molecular, physiological, biochemical, and metabolic aspects of drought tolerance are essential and one of the most important tasks for the plant biologists. But this approach is time-consuming, and searching for other options could be effective for sustainable crop production. In recent years, application of osmoregulators, plant hormones, stress signaling molecules, polyamines etc. were found effective for alleviating drought stress (Alam *et al.*, 2013; Hasanuzzaman *et al.,* 2017b; Nahar *et al.,* 2017; Bhuiyan *et al.,* 2019).

Thiourea (TU) is a synthetic stress-alleviating chemical containing nitrogen (as $-NH_2$) and sulfur (as -SH) by 36 and 42%, respectively (Waqas *et al.,* 2019). It is an important plant growth regulator which improves plant growth and developmental cascade (Garg *et al.,* 2006) and stimulates defense system in plants against different abiotic stresses. Exogenous application of TU can modulate important physiological responses, including photosynthesis, proline metabolism, and plant water relationships under different abiotic stresses (Kaya *et al.,* 2015; Vineeth *et al.*, 2016; Wakchaure *et al.,* 2018; Kaya *et al.,* 2019). Under drought stress, TU foliar spray improves phloem translocation of photosynthates, increases leaf RWC and enables plants to make better utilization of water under moderate and severe drought stress (Bhunia *et al.,* 2015; Singh and Singh, 2017; Pasala, 2017). Foliar application of TU is also capable of scavenging ROS and declines the overproduced MDA and H_2O_2 content under drought stress (Hassanein *et al.,* 2015). Thus, the exogenous spray of TU enhances WUE, economic yield, and quality of different crops as well (Wakchaure *et al.,* 2018; Waqas *et al.,* 2019).

Many studies have been published on the plant responses and tolerance capacity to drought stress, but comparative studies on the effect of different levels of drought stress on different chickpea varieties under different levels of drought stress are not remarkable. Furthermore, studies on exogenously applied TU to increase drought stress tolerance in different chickpea varieties are also scarce in number.

In this experiment, the effect of exogenous TU application to induce drought tolerance on two chickpea varieties (BARI Chola-7 and BARI Chola-9) were studied under different levels of drought stress, and responses were observed on some morphological, physiological, biochemical, and yield parameters of plant to compare the performance of the two varieties.

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Considering these facts, the present study was undertaken with the following objectives:

i. To compare the morphological, physiological, and biochemical responses between two contrasting chickpea varieties under drought stress.

ii. To investigate the possible role of TU in conferring drought stress tolerance in both the chickpea varieties.

Chapter II

REVIEW OF LITERATURE

2.1 Chickpea

Chickpea (*Cicer arietinum* L.) is one of the most significant leguminous crops grown for human utilization and produced in around 57 countries of the world under different natural conditions (Kumar *et al.*, 2018). Mean yearly production share of chickpea by locale from 2008 to 2017 revealed that Asia contributes 83% globally (Merga and Haji, 2019) and in 2018, the most noteworthy chickpea delivering nation in Asia was India yet the least one was Bangladesh (Statista, 2020).

2.1.1 Botany

Chickpea is a cool-season food legume with bushy 60 cm plants bearing fluffy pinnately compound leaves. The rooting pattern of chickpea is characterized by a taproot system containing first-order lateral roots and second-order branches. Root growth is a lot quicker than shoot growth (Sajja *et al.*, 2017). The small white or reddish flowers are usually self-pollinated. The number of flowers and pods per plant relies upon the genotypes and various ecological conditions. Each pod contains one or two seeds. Chickpea is principally categorized into desi and kabuli type with various qualities (Rawal and Navarro, 2019).

Desi chickpea contains little brown colored seeds with a rough pigmented seed coat. It mostly cultivated in relatively warm climates in Asia and Africa. Whereas, Kabuly chickpea grown in temperate-region countries which has bigger round seeds with a smooth light beige-shaded seed coat. Overall, desi type pre-dominates world chickpea production contributing 80% of production where staying 20% dedicated to kabuli type (Merga and Haji, 2019). However, with the development of new varieties, two kinds of chickpea can be grown in countries like Canada, Australia, India, Pakistan and Myanmar (Sofi *et al.*, 2020).

2.1.2 Importance

Like all other legumes, chickpea can contribute in accomplishing the goals of sustainable food and natural security by biological nitrogen fixation, compression of weeds and controlling erosion as a cover crop which improve the soil status and most significantly reduce the malnutrition in the third countries of the third world (Meena *et al.,* 2018).

Sharasia *et al.* (2017) stated that chickpeas are rich in protein and energy, which makes them great for animal feed. Particularly, cereal straw contains lowest amount of nutrition than the chickpea where it contains nearly about 44 to 46% digestible nutrition on the basis of dry matter and more palatable than wheat straw, having no adverse effect on livestock it allows animals to grow and produce milk equally as soy or cereal.

Rawal and Navarro (2019) showed that desi chickpea contains 21.2 g protein, 5.0 g fat, 21.2 g dietary fiber and 40.0 g available carbohydrate per 100 g edible portion on a fresh weight basis where, kabuli chickpea contains 20.8 g protein, 6.1 g fat, 13.1 g dietary fibre and 48.9 g available carbohydrate. In addition, chickpea has a low glycemic index (28±9) than rice (87±2), which is a proportion of how rapidly an item of food can expand blood sugar level thus control diabetes reducing cardiovascular and cancer risks.

Houshmandfar *et al*. (2019) showed that chickpea can be grown in rotation with wheat, barley, and other winter crops in the semi-arid tropics as chickpea had the lowest non-productive water use and arrived at its maximum yield at a lower water supply than the other species.

In Bangladesh, chickpea stands 5th among the pulse crops in regard of area (5914.5 ha) and yield (6875 tons), with concomitantly observed the average crop production close to 1.2 t ha^{-1} (BBS, 2017). However, the most potential pulse producing countries of the whole world, but it has not yet been able to attain self-sufficiency in pulse production and almost entirely reliant on imports for its chickpea consumption due to its high demand and low productivity (Sharasia *et al*., 2017). Since the last two decades, imports of chickpea have been raised gradually, which is mainly due to chickpea production in the country declining from 67,687 tons in 1991-93 to 11,000 tons in 2001-03 and further to 6,895 tons in 2011-17 (Rawal and Navarro, 2019).

Different sorts of biotic and abiotic stresses are liable for an enormous gap between potential and actual yields of chickpea in many chickpea producing countries. Farooq *et al*. (2017a) concluded that water stress declined the chickpea production when plant faced this stress at late ripening (49-54%), anthesis (27-40%) and reproductive (45-69%) periods. Merga and Haji (2019) recognized a yearly reduction of 6.4 million tons in potential worldwide chickpea production to abiotic stresses, viz. heat, drought, alkalinity, cold, salinity, waterlogging, and nutrient deficiencies are the significant abiotic factors that influence the yield of global chickpea.

Yet, in Bangladesh, among many reasons, less water availability induced drought stress during its growing period is one of the most yield-limiting factors for chickpea as 90% of its area is under the rainfed condition without irrigation. Moreover, chickpea is to a great extent, produced as an important rational pulse crop in the cropping system on leftover soil moisture. This regularly results in continuous exposure of chickpea to increasing drought during flowering and maturity stages (terminal drought) which significantly affect chickpea yield every year (Salma *et al*., 2016).

There are many studies reporting the impact of different moisture level and water regimes on chickpea growth and yield. In any case, research works related to the oxidative stress caused by drought and the related enzymatic and non-enzymatic activities in chickpea are very limited in number. However, a portion of the research findings which are extremely applicable to our investigation and given useful data are reviewed in this chapter.

2.2 Plant responses and tolerance to abiotic stress

Larger portion of the world's arable lands are exposed to most of the abiotic stresses like water scarcity, waterlogging, high salinity, extreme temperature, metal/metalloid stress etc., which cause physical harm to plant. If the stress continues for an extended period of time or becomes high, it may lead to an irreparable programmed cell death, reduced growth, and in extreme cases, brings about plant death (Hasanuzzaman *et al.*, 2012a), which results in about 70% reduction of global crop production (Nahar *et al*., 2017; Hasanuzzaman *et al.*, 2018b) by affecting critical plant growth patterns and physiological responses. Plant responses to different abiotic stresses depend heavily on crop genotypes, crop developmental stages, stress type, stress severity, and duration etc.

Plant growth inhibition increased with the increase of stress severity. To show the effect of salt stress mustard (*Brassica juncea* L.) was exposed to 100 and 150 mM NaCl by Ahmad (2010) for 45 days and resulted that higher salinity stress (150 mM, NaCl) causes reduction of shoot fresh weight (32%) and root fresh weight (44%), which were higher than the reduction of shoot fresh weight (16.9%) and root fresh weight (27%) under lower concentration of NaCl (100 mM) the growth and biomass yield of mustard decreased with the increase of salt stress. Yet, in rice, leaf mortality increased with the increase of salt (100 mM and 150 mM, NaCl) stress at an early stage and it is about 0 to 300% after one week. However, a critical decrease in plant biomass production like, length of shoots, number of roots including length of roots happened under increased salt stress (Jamil *et al*., 2006; Jiang *et al*., 2010; Hussain *et al*., 2017).

To explore the impact of waterlogging on the development and yield of summer maize Ren *et al.* (2014) stated that plant morphology such as shoot length, ear length, and leaf area index (LAI) decreased with the increase of waterlogging duration. Furthermore, dry matter accumulation and its distribution proportion of grain also declined simultaneously under waterlogging.

The effect of combined stress in mung bean (*Vigna radiata* L. cv. BARI Mung-2) was demonstrated by Nahar *et al.* (2017) and stated that combined exposure of plants to drought and heat stress (40 ºC) resulted in highest reduction of plant height, root length, leaf area and dry weight of seedlings by 20, 24 and 41%, respectively, compared to control plants.

Sesame (*Sesamum indicum* cv. BARI til-4) exposed to 2, 4, 6, and 8 days of waterlogged soil by Anee *et al*. (2019) and showed that maximum reduction of RWC (70%) and proline (Pro) content (20%) of leaves were observed under a prolonged period of waterlogging (8 days), over short durated waterlogging.

Molecular studies showed that plants produce deleterious abiotic stress signaling chemical entities called reactive oxygen species (ROS) like singlet such as, singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) etc. that may be generated in plant at a lower level under controlled conditions as a usual cellular metabolism utilizing only 1-2% of the O_2 which only favours the usual growth and development without causing any major physiological damage (Noctor *et al.*, 2018). But plants exposed to abiotic stress generated uncontrolled ROS, which causes abrupt ROS burst and the systems adequately surrenders to death due to the absence of ROS scavengers in the sensitive crop genotypes. However, the crop varieties tolerant for the particular stressor have a high constitutive expression of the ROS scavenging genes even under controlled conditions and endure the oxidative stress through the synthesis of different non-enzymatic and enzymatic antioxidant (Mhamdi and Van Breusegem, 2018).

According to Ahmed (2010), salt stress markedly increased the lipid peroxidation content in mustard (*B. juncea* L.) and showed that under NaCl-induced salt stress, lipid peroxidation increased by 21% (150 mM, NaCl) and 17% (100 mM, NaCl).

Alzahrani *et al*. (2019) demonstrated that salinity-induced (150 mM, NaCl) oxidative stress in faba bean is depicted by the enhanced generation of ROS, including H_2O_2 and increased the activities of malondialdehyde (MDA) and electrolyte leakage (EL) compared to unstressed plants. In mung bean salt stress (100 mM, NaCl) increased 2 fold of H_2O_2, MDA, EL, and $O_2^{\bullet-}$ (Ahmad *et al.*, 2019). However, plants redox homeostasis gets disturbed as a result of the excessive ions accumulation and over-production of ROS under salinity stress (Tariq and Shahbaz, 2020).

Nahar *et al*. (2016) stated that MDA increased significantly by 125% along with the increase of H_2O_2 under cadmium (Cd) stress (1.5 mM, $CdCl_2$) in mung bean. In another experiment conducted with *Brassica napus,* Hasanuzzaman *et al*. (2017a) showed that Cd stress (1mM, 2d) increased H_2O_2 content (60%) and activity of LOX (145%) as compared to control plants.

2.3 Drought stress

Drought and water stress has a complex impact on plant water relation traits such as leaf water potential, RWC, stomatal conductance, temperature of canopy and transpirational rate of different plant species (Siddique *et al.,* 2001).

An experiment on wheat (*Triticum aestivum* L.) was carried out by Zhang *et al.* (2004) demonstrated that the proportion between dry matter produced and water consumed increased, which resulted in the increment of water use efficiency (WUE) of wheat under drought stress over control.

Measuring morphological traits, leaf area, and transpiration rate at various growth stages, Lazaridou and Kotroubus (2004) showed that WUE increased with lowered water loss through declining leaf area and transpiration rate in clover (*Trifolium alexandrinum*) under drought-stressed condition compared to well-watered treatments.

2.4 Crop responses to drought

The severity of drought-induced damage on crop and their responses varies depending on plant genotypes and growth stages such as germination, vegetative growth stage, reproductive growth stages and maturity stage, which resulted in yield reduction**.**

2.4.1 Effect on seed germination and seedling establishment

Seed germination and seedling establishment negatively influenced by drought stress. According to Kaya *et al*. (2006), mean germination time delayed by 2 days under severe drought stress (−0.9 MPa) compared to no drought stress in sunflower

(*Helianthus annuus* L.). In addition, germination percentage also decreased by 72% over control when seeds exposed to severe drought stress. Water deficit condition delays the imbibition process and brings about diminished germination rates and reduced seedling vigor. For example, Liu *et al*. (2019) showed that drought stress (20% PEG-6000) suppressed rice seed germination and germination remained below 60% (by day five), compared to control.

Bhuiyan *et al*. (2019) stated that under drought stress (20% PEG) the height of seedlings reduced by 20% in comparison to control in rapeseed with a significant reduction of the fresh weight and dry weight of seedlings.

2.4.2 Effect on growth

According to Lum *et al*. (2014), PEG-induced drought stress (−8 bar) reduced shoot length, root length and dry matter significantly in drought-sensitive upland rice variety (Kusam) by 76, 56, and 50%, respectively, in comparison to control (0 bar) due to low water potential. Conducting an experiment with mung bean, Nahar *et al.* (2017) stated that notable decline of plant height (15%), root length (18%), leaf area (27%), and dry weight (26%) was observed under drought-induced oxidative stress (5% PEG). Anjum *et al*. (2017) showed that the leaf area, shoot fresh weights, shoot dry weights, number of leaves plant^{-1} significantly reduced in three maize cultivars under severe drought stress (40% FC) by 3, 8, 13 and 6% in Dong Dan 80, 5, 9, 16, and 10% in Wan Dan 13 and 9, 17, 30, and 31% in Run Nong 35, compared to well-watered control, respectively.

Likewise, Hussain *et al*. (2019) reported that in two different maize cultivars, drought stress (50% FC) for 15 days reduced the plant height, shoot fresh weight plant^{-1}, shoot dry weight plant^{-1}, stem diameter plant^{-1}, and leaf area by 10%, 11%, 16%, 10%, and 4% in Xida 319, and 8, 18.17,10, 10, and 8% in Xida 889, respectively.

Negative result of water stress on plant growth of five rice genotypes was investigated by Saha *et al.* (2019) and reported that root length decreased in all varieties by 24 to 45%, but in BRRI Dhan-56 showed the least decline in root length at 10 days after treatment. In addition, shoot and root ratio, shoot length, shoot and root fresh and dry weight decreased in all the five rice varieties. Similarly, conducting an experiment with rice (BRRI Dhan-24) Nasrin *et al.* (2020) described that plants exposed to drought stress for 12 to 21 days reduced root length (49-68%), shoot length (28-47%), root fresh weight (95-98%), shoot fresh weight (84-93%), root dry matter (90-94%), and shoot dry matter (47-82%), over control. Drought stress also declined leaf area by reducing leaf length and leaf breadth by 31-36% and 22-56% at a different duration of water stress, over control.

2.4.3 Effect on physiology and metabolism

The effect of drought on plant physiology is a complex event and has been considered as a disturbance of the water balance. Stomatal conductance declines transpiration and plays an essential function in regulating plant water balance and stomatal closure reduces plant biomass and yield (Pirastech-Anosheh *et al.*, 2016).

An experiment was conducted with two wheat cultivars (Xinong 9871 and Changhan 58) by Li *et al*. (2017) to determine the effect of water stress on photosynthetic rate, stomatal characteristics and WUE and resulted that water stress (40% MC) reduced the photosynthetic rate of the two cultivars along with stomatal density and stomatal width decreased which inhibited the transpiration rate and increased the WUE.

The exposure to drought stress significantly reduced chl *a* and chl *b* including total amount of chl contentwhich also associated with carotenoid (Car) by 71, 60, 75, and 83%, respectively, comparision to control (Nawaz *et al*., 2016). Hasanuzzaman *et al*. (2018c) stated that the crop plants revealed acute water stress (20% PEG) which caused the chl *a* and total chl content reduced upto 52% and 43%, respectively in leaves of rapeseed plants over control. However, heat and drought stress (50% FC) combinedly declined the chl *a*, chl *b*, with total chl content (Hussain *et al*., 2019).

Drought stress at mild to severe level reduced the RWC of leaves in different crops. Nawaz *et al*. (2016) reported that the water deficit stress (60% FC) declined the plants leaf RWC and excised leaf water retention 34 and 22% compared to control (100% FC) of maize plants and excised leaf water loss increased from 15% (control) to 70% (drought-stressed). According to Hasanuzzaman *et al*. (2018c), drought stress reduced the RWC of rapeseed leaves at moderate (10 % PEG) and severe stressed (20% PEG) seedlings by 14 and 36%, respectively in comparison to the control. Supporting this result, Hussain *et al*. (2019) stated that the water stress signifcantly declined the RWC in the hybrid maize Xida 319 (31.5%), which was greater than that in Xida 889 (13.6%). However, upon exposure to drought stress leaf RWC reduced by 23% in comparison to control in rapeseed seedlings (Bhuiyan *et al*., 2019).

Drought-tolerant rice variety (Pulot Wangi) resulted in highest Pro content (581.78 mg g^{-1} FW) upon exposure to severe drought stress (−8 bar) by balancing the osmotic potentiality of cell and external environment (Lum *et al*., 2014). With free Pro content soluble protein content also increased under drought stress (Hussain *et al*., 2019). However, the Pro accumulation was observed by 71% and 125%, respectively in comparision to the untreated control in rapeseed seedlings (10 and 20% PEG) (Hasanuzzaman *et al*., 2018c). Bhuiyan *et al*. (2019) experimented that Pro content was enhanced by 10-fold in comparision to untreated control. The same observation stated by Rezayian *et al*. (2020) that Pro content was remarkably enhanced by 61, 81 and 71% in the plants under drought (15% PEG, 3 weeks) as compared with control in soybean.

2.4.4 Effect on yield and yield attributes

Under drought stress, nutrient uptake and translocation decreased with the reduction of water potentiality, which results in yield decline. Thus, in wheat grain yield, 1000-grain weight, spike length, harvest index decreased when plants were exposed to drought stress at 21 days after emergence and 42 days after emergence. Decrease of grain yield $plant^{-1}$ was measured by 24 and 60% in early drought-stressed plants and late drought-stressed plants, respectively, over control (Newaz *et al*., 2012). Allahverdiyev *et al*. (2015) showed that the reduction of physiological parameters such as photosynthesis rate, total dry biomass and RWC of two wheat genotypes (Durum and Bread wheat) under rainfed condition which resulted in the reduction reduced spike weight, grain no. $spike^{-1}$, 1000-kernel weight compared to irrigated wheat. In contrast, plant height, spike length and width of wheat did not influence by drought stress under rainfed condition (Allahverdiyev *et al*., 2015). Anjum *et al*. (2017) reported that kernels ear^{-1}, weight of 100-grain, grain yield $plant^{-1}$, and biological yield $plant^{-1}$ of three maize hybrids reduced under severe drought stress (40% FC) by 2, 10, 13, and 6% in Dong Dan 80; 5, 14, 22, and 7% in Wan Dan 13 and 19, 24, 43, and, 16% in Run Nong 35, respectively, compared to well-watered control. Thus, the overall performance of all maize hybrids under drought stress was recorded significant yield reduction in Dong Dan 80 (6%), Wan Dan 13 (7%) and Run Nong 35 (16%). Similarly, Hussain *et al*. (2019) concluded that compared to control kernel ear^{-1}, weight of 100-kernel, and grain yield $plant^{-1}$ decreased in both maize cultivars by 15, 4, and 20% in Xida 319 and 15, 10, and 17% in Xida 889, respectively.

2.5 Drought-induced oxidative stress and antioxidant defense system

2.5.1 Oxidative stress under drought-stressed condition

Drought or water deficit stress significantly increased the total amount of TBARS, H_2O_2, MDA and EL in the three studied varieties of maize viz. Wan Dan 13 and Run Nong 35 including Dong Dan 80 by 10-46, 9-34, and 5-24%, respectively, compared to control (Anjum *et al*., 2017).

The MDA and H_2O_2 content were identified as stress marker and showed sharp increase in rapeseed seedlings under moderate drought stress (10% PEG) by 65 and 53%, respectively, whereas under acute water deficit stress (20% PEG) the increment was 123 and 93%, respectively, over control.

In wheat, the maximum increase of EL (64%), H_2O_2 content (25%) and MDA (54%) was observed by Amoah *et al*. (2019). Liu *et al*. (2019) compared the control with drought-stressed plants (20% PEG) for 5d and showed that the MDA contents increased significantly by 16% and caused ROS over-generation and cell lipid peroxidation in the imbibing seeds. Bhuiyan *et al*. (2019) reported that markedly improved MDA and H_2O_2 content were perceptible under water stress revealed rapeseed seedlings by 82 and 131%, comparing with the control. Drought stress treatments improved the MDA and H_2O_2 levels in maize hybrids Xida 319 and Xida 889 over control (Hussain *et al*., 2019).

2.5.2 Antioxidant defense system and its role in drought tolerance

Newaz *et al*. (2016) reported that the activities of SOD, CAT, POX, and APX increased by 2.6, 1, 3, and 8.5 fold, respectively in drought-stressed plants (60% FC), over control (100% FC) as a plant defense system to minimize the excessive accumulation of ROS. Significantly higher SOD, ASA and DHA contents were found under different water stress levels stress in maize (Anjum *et al*., 2017).

In the case of rapeseed seedlings, drought stress resulted in a notable increase of APX (23%), GR (81%), GPX (26%) and CAT (29%) activities while DHAR activity decreased by 22% in comparison with control (Bhuiyan *et al*., 2019). A similar result was established by Liu *et al*. (2019) that, to alleviate the oxidative stress-induced by drought (20% PEG) in rice seeds SOD (36.5%), APX (51.1%), POD (58.9%), and CAT (28.2%) activities increased remarkably compared to control.

According to Hussain *et al.* (2019), performance of APX and CAT in two maize varieties, namely, Xida 889 and Xida 319 reduced under drought stress condition.

Rezayian *et al*. (2020) reported that maximum performance of APX, CAT and POX was detected at low level of drought (5% PEG), but SOD and POX activities were higher at high level of drought (15% PEG).

2.6 Effect of drought on chickpea

2.6.1 Effect on seed germination and seedling establishment

The capacity of germination with seedling emergence in all genotypes chosen by Yücel *et al*. (2010) stopped completely in severe water stress (−0.8 Mpa) induced by PEG-6000. In another experiment, chickpea cultivars (Arman and Azar) were exposed to different drought stress levels by Ajirloo *et al*. (2011) and resulted in the reduction of germination, length of radical, and plumule where the dry weight of seedlings were increased with the increase of PEG-induced drought stress from o to −1.2 Mpa.

At 5% water holding capacity (WHC), small seeds of chickpea cultivar effectively germinated, but the emergence of large crop seeds was suppressed and failed (Vessal *et al*., 2012).

Highly decreased seed germination percentage with increased PEG concentration was observed in chickpea cultivar Vishal (62% at 4% PEG) and seed vigor index was reduced by 18.80% under the control, which was lower than another cultivar Virat which showed maximum seed vigor index 9% than other varieties (Tidke *et al*., 2019).

When chickpea seeds were exposed to 50% PEG-induced drought stress, the germination percentage declined from 94% to 2% in control and stressed plants, respectively (Koskosidis *et al*., 2020).

2.6.2 Effect on growth

According to Gunes *et al.* (2006), drought-induced oxidative stress at 40% FC resulted 15-45% (at pre-anthesis stage) and 35-55% (at post-anthesis stage) growth reduction in 11 chickpea cultivars compared to control (60% FC). Upon exposure to 40% FC, plant dry weight of 10 chickpea cultivars was decreased compared to plants grown at 60% FC (Gunes *et al.,* 2007). Dry weight of plants used to measure drought susceptibility index and Gunes *et al*. (2008) showed notable reduction of plant dry weight at both early and terminal drought stress compared to control, but the reduction was more prominent in the case of terminal drought stress.

According to Sohrabi *et al*. (2012), under severe drought stress, growth parameters like plant height, total dry weight and shoot dry weight decreased by 13, 23, and 28%, respectively, compared to well-watered plants.

The drought stress influences the plant growth stages. Twenty chickpea genotypes were chosen by Randhawa *et al.* (2014) and reported that stem dry weight plant $^{-1}$, root dry weight plant^{-1},and leaves dry weight plant^{-1} reduced by 16-29, 15-25, and 17-23%, respectively when the crop plants revealed water stress at the stage of pod emergence and flowering, over control (normal irrigation).

According to Hussain *et al.* (2015), under rainfed condition plant height, primary branches and secondary branches decreased by 16, 6, and 13% over normal irrigation (irrigation at flowering and pod formation). Drought stress reduced leaf area and root length compared to control. However, drought-tolerant chickpea varieties showed higher leaf area and root length under stressed conditions compared to drought susceptible varieties (Khan *et al*., 2018).

2.6.3 Effect on physiology and metabolism

Water stress reduces nutrient uptake, nutrient translocation and nutrient concentration in plants. In chickpea, drought stress (40% FC) at pre-anthesis stage resulted in higher reduction of N, P, K, Ca, Mg, Fe, Zn, Mn and B uptake compared to control, and drought stress at post-anthesis period. Total nutrient uptake efficiency was decreased by 70-98% in 11 chickpea cultivars at early drought stress compared to control, while the reduction was 50-75% at late drought stress (Gunes *et al*., 2006). In another experiment, Gunes *et al*. (2008) demonstrated that terminal drought stress caused maximum excised-leaf relative water loss and minimum RWC in drought-susceptible chickpea cultivars.

Plants exposed to drought stress at vegetative stage reduced the chl *a* (18%), chl *b* (27%) and total chl content (18%) and increased Pro content (485%) in chickpea compared to normal irrigated plants (Mafakheri *et al*., 2010).

Rahbarian *et al*. (2011) stated that the shoot dry weight, leaf internal CO_2 concentration decreased in the podding stage but CO_2 assimilation rate and transpiration rate decreased in all stages in all genotypes under severe drought stress (25% FC) compared to control (100% FC). However, WUE increased in the seedling stage and early flowering stage but decreased in pod filling stage.

Talebi *et al.* (2013) established that under water stress (50 mm water 2 times after flowering) RWC decreased by 17% and Car content decreased by 30%, over control (50 mm water 6 times after flowering) where drought susceptibility index (DSI) varied from 0.46 to 1.77 with an average of 0.96. All the genotypes in that experiment had lower values of DSI with relatively higher RWC values.

Under moderate drought stress, stomatal conductance is reduced. Supporting the result, Pouresmael *et al.* (2013) demonstrated that stomatal conductance was reduced in the range from 31 to 91% under moderate stress (55-60% FC), but under severe stress (25-30% FC) it reduced by 61 to 93%, and showed highest reduction upto 95% in drought-susceptible genotype (ILC3279).

Randhawa *et al*. (2014) reported that leaf area and leaf area index (LAI) reduced by 14-32% and 29-45%, respectively under different irrigation treatments (one pre-sowing irrigation, no irrigation at flowering stage and no irrigation at pod formation stage), over control (normal irrigation).

Dalvi *et al*. (2018) demonstrated that compared to control the mean value of leaf Pro content of chickpea genotypes under drought stress was increased by 2.39 fold and 2.1 fold at pre and post flowering stage, respectively. A similar result was founded by Khan *et al.* (2019) and concluded that the the tolerant variety showed a higher increase in Pro (14%) and leaf sugar content (45%) than the sensitive variety.

Hashem *et al*. (2019) demonstrated that with the reduction of chl *a*, chl *b*, total chl content (54, 46, and 39%); stomatal pore aperture, stomatal density, the photosynthetic rate also declined (50, 33, and 50%), respectively, compared to control. In line with this, Khan *et al*. (2019) stated that in drought-sensitive variety of chickpea chl content and photochemical efficiency reduced by 61 and 64% and in tolerant variety the decrease was 42 and 26%, respectively, over well-watered plant.

2.6.4 Effect on nodule formation

Plants require water to form nodule and fix of nitrogen which is restricted under early drought stress. Labidi *et al*. (2009) conducted an experiment on 5 chickpea lines and reported that individual nodule biomass, specific N fixation reduced by 18 and 24% and empty nodule numbers increased by 35% under drought stress (33% FC) over control (100% FC).

Istanbuli *et al*. (2019) established a positive relation between yield and nodule traits of chickpea genotype under drought stress (rainfed condition). With the reduction of nodule fresh weight (18%), nodule dry weight (21%) and nodule biomass (21%) under drought stress, the grain yield, biological yield, and 100-seed weight also reduced by 13, 15, and 1.7%, respectively, comparing with control.

2.6.5 Effect on yield

Drought-induced oxidative damage causes significant yield reduction in chickpea cultivars. According to Leport *et al*. (2006), above-ground dry weight plant^{-1} and seed yield plant^{-1} decreased significantly when four chickpea cultivars exposed to drought stress at pre and post flowering stages. The decline of seed yield was higher compared to total biomass which resulted in the reduction of harvest index as well. However, drought stress at pod setting stage showed 50-75% increase of pod abortion while pod abortion was higher on secondary branches compared to primary branches.

Flower abortion and pod abortion increased under drought stress, which negatively affects the seed yield of chickpea. Supporting this Fang *et al*. (2010) reported that in chickpea (cv. Rupali) flower and pod abortion was increased by 36 and 54%, and resulted lower seed yield (4 g plant^{-1}) compared to control plant (12.3 g plant^{-1}).

Conducting an experiment with three chickpea varieties, Mafakheri *et al.* (2010) reported that drought stress at vegetative and anthesis period resulted in a notable decline of yield by 44, 61, and 65% in Bivaniej, ILC482 (drought-tolerant) and Pirouz (drought susceptible), respectively with reduced chl content, compared to well-watered control. Total number of pods reduced by 24% (one pre-sowing irrigation), 19% (no irrigation at flower initiation stage), and 22% (no irrigation at pod initiation stage) compared to normal irrigation (Randhawa *et al*., 2014). In another experiment, Hussain *et al*. (2015) showed the reduction of 100-grain weight (8%), yield plant^{-1} (19%), and yield kg ha^{-1} (13%) under rainfed condition over control (irrigation at flowering and pod formation stages).

2.6.6 Oxidative stress under drought

According to Gunes *et al*. (2007), drought stress (40% FC) increased H_2O_2 concentration (55-133%) and MDA content (25-68.7%) over control plants (60% FC).

With the aim of investigating the most sensitive growth stage of chickpea genotypes to drought stress Patel *et al*. (2012) designed an experiment by exposing four chickpea cultivars under early drought stress (EDS) and late drought stress (LDS) and showed that H_2O_2 and MDA content increased over normal irrigation. But the increment was more significant under EDS comparing to LDS and proved that pre anthesis stage was more sensitive to oxidative stress than post-anthesis period.

2.6.7 Antioxidant defense system under drought

Gunes *et al.* (2007) stated that antioxidant activity varies with the stress intensity, stress duration, plant species, and cultivars. SOD and CAT activities decreased while APX activity increased in most of the chickpea cultivars compared to control. Mohammadi *et al*. (2011) studied that the content of SOD, CAT and GPX increased by 59, 73, and 49% under drought-stressed condition over normal irrigation in chickpea. The increased activities of the APX, CAT, POX and SOD are more significant at post-anthesis period comparing to pre-anthesis period of chickpea cultivars. According to Khan *et al.* (2019), the APX content increased by 93 and 92% under drought stress in drought-sensitive and drought-tolerant varieties, respectively, compared to control. In addition to that the CAT content also improved by 81% in drought-sensitive variety and 79% in drought-tolerant varieties in chickpea.

2.7 Adaptation mechanisms of chickpea to drought

Chickpea can tolerate drought stress through drought resistance traits of plants which are associated with important adaptive mechanisms viz. drought escape, avoidance and tolerance (Aslam *et al.*, 2015; Maqbool *et al*., 2017)

2.7.1 Drought escapes of chickpea

To tolerate drought-induced stress, drought escape is an important phenological characteristic which results in early phenology like early flowering and early podding with terminal drought (Maqbool *et al.,* 2017).

The early maturity of plant was brought by healthy growth, and technically escaped the terminal water stress as phenological development synchronized with water availability, which acts as special characters in screening of gerplasm during chickpea breeding (Sabaghpour *et al.*, 2003). With the aim to find early maturity trait in chickpea lines, Sabaghpour *et al.* (2006) found that ILC 1799 has produced the highest yield among ILC 3012, ILC 3283, and ILC 658 which clearly showed the tolerance capability concomitantly with highest adaptation and pre-maturity compared to the susceptible check (ILC 3279) which showed lowest productivity with late maturity.

Being characteristic features of drought escape, early maturing genotypes have higher WUE but lower leaf area index and lower yield potential (Aslam *et al.,* 2015). In another experiment, Hussain *et al.* (2015) showed that plants under rainfed condition needed 88-99 days to flower where normal irrigated plants needed more about 100-109 days to flower.

2.7.2 Drought avoidance of chickpea

Maintaining of water potential in plant tissue under water deficit condition is characterized as drought avoidance. The capacity of plants to reduce water loss along with the increment of water absorbing ability through dense root system can confer drought avoidance.

By observing root characteristics of chickpea at the rapid growth stage after sowing (35 DAS), Kashiwagi *et al. (*2006) established a significant association of root growth with the final grain yield under different terminal drought intensities. The density of layer wise root length of 15-30 cm depth was found relatively close to the crop production under various water stress levels. Under severe drought stress, the root zones nearly about 30-50 cm can be visualized to achieve importance (Kashiwagi *et al.,* 2015).

Besides, root can sense drought stress under water deficit condition and causes ABA production, which modulates stomatal closure, and signals to restrict water loss through transpiration (Saradadevi *et al.,* 2017).

The anatomy of root with the xylem of plants can be relied upon and having various capillary forces with least cavitation and these are useful for absorbation and transportation of soil water under water deficit soil (Li *et al*., 2009).

To relate the root anatomy with drought adaptive mechanism, Purushothaman *et al.* (2013) selected six major legume crops and resulted that in the midst of the studied crops, chickpea had a large (32) and small (44) numbers of metaxylem vessels (32), but in average condition the diameter of vessel (9.5 μm).

In drought-stressed plants, the WUE and water uptake patterns were different from that of well-watered plants (Zaman-Allah *et al.,* 2011). Twenty chickpea genotypes with similar phenological traits but differ in terminal drought tolerance index were used by Zaman-Allah *et al.* (2011) and showed that water extracted at the early vegetative stage was negatively correlated with water extracted during the reproductive phase. At the vegetative phase (28 DAS and to some extent 33-38 DAS) drought susceptible genotype (ICC 8058) uptaken more water than the tolerant (ICC 867) ones.

Tolerant genotypes of chickpea uptaken less water with a minimum index of stomatal conductance at the early growth stage than sensitive ones. On the contrary, tolerant genotypes extracted more water than sensitive genotypes after the anthesis period. But the experiment established that root growth components (depth, RLD, root dry weight) are complex and critical for the understanding of water management and adaptation to terminal drought (Zaman-Allah *et al.,* 2011).

In a field experiment conducted with chickpea at ICRISAT, stomatal conductance under water deficit condition was measured through the leaf canopy temperature where greater transpiration indicated a promising effect on greater reproductive growth and seed yield under terminal drought stress during pod formation stage (Kashiwagi *et al*., 2008).

Greater amount of water was reduced during the reproductive growth stage which was considered as an important factor for crop production under water stress (Kashiwagi *et al.,* 2015).

Ten genotypes of desi chickpea were chosen by Pang *et al*. (2017) and experimented that a conservation of water used techniques beneficial for chickpea seed production which revealed that the water scarcity at the period of premature podding. The study resulted that the genotype Neelam showed more WUE (0.44 g L^{-1}) than in CICA0912 (0.14 g L^{-1}). Neelam was utilized the minimum amount of water (10.4 L $plant^{-1}$) followed by DICC8218, and transpired less water per day at the early stage of water stressed (4-6 days after irrigation), when the opposite comparison was found the another stage of water stressed (12-17 days after irrigation). Thus, Neelam had the highest seed production comparison to other genotypes and proved that the conservative water use strategy is a key drought avoidance mechanism.

2.8 Genotypic variation of drought tolerance in chickpea

Considering the ongoing climate change as well as the gradual decline of available water resources, research efforts towards developing drought-tolerant germplasm is of paramount importance.

To identify the most critical traits that contribute to grain yield under drought, twelve chickpea genotypes with close phenology but good contrasts for root development, drought response and canopy temperature were chosen by Ramamoorthy *et al*. (2016) and proposed that partitioning and crop growth rate with the right phenology was the best selection strategy to enhance terminal drought tolerance in chickpea.

Salma *et al*. (2016) experimented that at the highest concentration of PEG among seven chickpea varieties, only Binachola-2 and Binachola-7 showed 66.67% germination at 3 days after sowing where others Binachola-3 and Binachola-8 did not grown under water stress and at low concentration of PEG they show reduced percentage of germination, fresh and dry weight of shoot, shoot and root length with RWC compared to Binachola-2 and Binachola-7. Thus, the experiment resulted that, Binachola-2 and Binachola-7 were the highest drought stress tolerant variety.

According to Johal and Hagroo (2019), among all the morpho-physiological characteristics RWC is the most important parameter for the screening of drought stress tolerance in chickpea genotypes and also showed that in rainfed condition, the highest reduction of RWC (15.12%) was observed in kabuli genotype and the lowest RWC (3.6%) was recorded in desi genotype ICC 4958 and proved that desi genotypes showed drought tolerance to drought stress.

Canci *et al*. (2009) showed that under drought stress seed weight of chickpea was the least affected traits and can be used in early breeding selection to develop drought resistant genotypes. To investigate the physiological basis of drought tolerance in desi and kabuli chickpea genotypes, Farooq *et al.* (2018) established that the desi genotypes (Bakhar-2011 and Bitall-2016) had better stand establishment and germination count under drought stress (50% WHC) than the kabuli genotypes (Noor-2013 and K-70005) along with the desi genotypes had more total chl, higher leaf CO_2 assimilation rate, and maximum PSII photochemical efficiency (Fv/Fm) than the kabuli genotypes under drought and well-watered condition.

Drought affected percentage of seed germination, seed water content, seed water absorbance, root and shoot development of seedling, but seedling vigor index is a suitable selection criterion for drought tolerance. To this respect, chickpea cultivars ‘Thiva’ and ‘Keryneia’, followed by ‘Gavdos’, exhibited superior performance in terms of drought tolerance (Koskosidis *et al*., 2020).

Saxena *et al*. (2003) confirmed that two genotypes of chickpea viz. ICC 4958 and ICC 8261 are extensively used to transfer the important drought adaptive root traits to improve the water stress tolerance in chickpea cultivars. Similarly, Ye *et al*. (2018) revealed that enhanced the length of root architecture with biomass production improved water and nutrient uptake in different legumes including chickpea and showed better performance in yield under moderate and severe drought stress.

Devasirvatham and Tan (2018) conducted an experiment to develop simple screening methods for determining the physiological and biochemical traits of different genotypes to identify drought stress tolerance in chickpea genotypes to the selected environment.

2.9 Brief strategies to induce drought tolerance in crops

Both, the exogenous application of natural and synthetic molecules in addition with selection, conventional breeding approaches can enhance drought tolerance in vulnerable crop species. For example, the exogenous application of osmoprotectants and plant hormones such as salicylic acid, Pro, ABA, glycinebetain, polyamines and gibberellic acid (GA3) have been found to induce drought stress tolerance, by elevating osmotic adjustment to increase turgor pressure, enhancing photosynthetic efficiency, and sustaining the membrane integrity under drought stress (Zhang *et al.*, 2004; Lamaoui *et al*., 2018).

Gong *et al.* (2005) concluded that in wheat plants, application of silicon (2.1 mM, Na_2SiO_3) improved the water potential and water content, photosynthetic pigments, total soluble proteins of water-stressed (50% of RWC) plants. However, silicon applications enhanced the CAT, SOD and GR content under drought stress.

An experiment was conducted with mung bean plant to show the effect of foliar application of micronutrients by Thalooth *et al.* (2006) and resulted that foliar application of zinc (0.85 mM, Zn-EDTA) increased the pod plant^{-1}, number of seed plant^{-1} and seed dry weight under drought stress (absence of one irrigation at vegetative, flowering and podding stages).

In wheat, selenium (Se) (0.1-0.2 mM, Na_2SeO_3) application improved RWC, LAI and CGR which resulted in the increment of grain yield under severe water stress at 50% flowering stage (Teimouri *et al.*, 2014). Furthermore, Newaz *et al.* (2015) studied that foliar application of selenium also enhanced leaf water potentiality, WUE, free amino acid, and total soluble sugar to induce drought tolerance in wheat.

2.10 Thiourea: A molecule with immense biological significance

To establish the effect of TU in breaking tuber dormancy potato tuber was immersed into 1% TU solution for 1 h by Germchi *et al.* (2011) and demonstrated that it reduced the sprouting time for 14 to 20 days. Besides, potato yield increased about up to 20%

if tubers soaked into 500-750 mM at the early stage of bud sprouting (Mani *et al.*, 2012).

The foliar application of TU (20 mM) at silking and milking stages in maize increased chl *a*, chl *b* and Car content 18.60 and 11.17%; 38.59 and 36.74%; 27.11, and 30.55%, respectively (Amin *et al.*, 2013). In another experiment conducted with maize, Sanaullah *et al*. (2016) proved that, medium supplementation with 400 µM TU in *in vitro* culture improved callusing induction (18%), direct shooting (22%), and direct rooting (36%) over control.

Application of TU in optimized concentrations can break innate or imposed bud dormancy and permit bud sprouting which greatly depends on plant genotypes (El-Keblawy *et al*., 2017). However, Chattha *et al*. (2017) established that wheat seeds primed with TU at 12 mM enhanced germination speed (reducing the time of 50% germination by 2.22 days), LAI and CGR and further improved yield and yield-related attributes. TU spray at pre-flowering and flowering stages also increased 20% grain yield in lentil over control (Singh and Singh, 2017).

Premaradhya *et al*. (2018) showed that foliar application of TU (13 mM) at reproductive (pre-flowering and pod initiation) stage has a positive effect in increasing shoot length (31%), total dry weight plant^{-1} (66%), primary branch no. (71%) under number of nodules plant^{-1} (12%) compared to control. Thus, the positive effect of TU was observed in lentil at harvesting period, when no. of pod plant^{-1}, no. of seed pod^{-1} and 100-seed yield was found to be increased by 58.02, 37.85 and 15.2%, respectively, over control. He also stated that TU foliar spray improved nitrogen (1.2 fold), phosphorus (2 fold), potassium (1.2 fold) and sulfur (3 fold) uptakement compared to plants with no spray.

Two foliar spray of TU at 13 mM during flowering (45 DAS) and seed setting (75 DAS) stages in chickpea recorded significantly higher plant height, LAI, number of primary branches plant^{-1}, chl content, number of pods plant^{-1}, 100-seed weight and seed yield plant^{-1} over control plants which was sprayed with water (Abhishek *et al.*, 2020).

2.10.1 Role of thiourea in conferring abiotic stress tolerance

Temperature stresses like cold, chilling and heat stress have strong impacts on critical stages of plant developments reducing plants optimal biochemical and physiological functioning.

Asthir *et al*. (2013) experimented with wheat under terminal heat stress and showed that using 6.6 mM TU increased chl content (8.43%) and reduced membrane injury compared to only heat-stressed one. Similarly, in four wheat cultivars, application of TU at the rate of 7 mM, enhanced root length, shoot length with root, shoot dry weight and improved reducing sugar content about 4-10% in roots and 9-10% in shoots under high temperature and TU application (Asthir *et al*., 2015).

Suryavanshi and Buttar (2016) reported that TU foliar spray (20 mM) in wheat plant was exposed to terminal heat stress enhanced grain yield (14%) over heat-stressed plant. In addition with, Waqas *et al*. (2017) found that TU (2.6 mM) foliar spray combined with moringa leaf extract at the six leaf stages reduced the length of phonological events of maize hybrid at reproductive stage and thus improved grain yield and quality of grain under chilling stress.

Conducting an experiment with maize Kaya *et al.* (2013) demonstrated that foliar spray of TU at 7 mM was able to ameliorate the oxidative damage caused by salinity stress (100 mM, NaCl) through increasing K^+ (21 and 36,4%) Ca^{2+} (39 and 55.5%), and P (42.6 and 37.5%) content and decreasing Na^+ (28.7 and 36.4%) content in leaf and root, respectively, over untreated salinity-stressed plants. Kaya *et al.* (2013) also demonstrated that TU showed a positive effect in increasing chl *a* and chl *b* content by 20.9 and 17.8%, and in decreasing EL, Pro and H_2O_2 content by 30.2, 32.6, and 41.6%, respectively, compared to non-treated salinity-stressed plants of maize. TU seed treatment (6.5 mM) and the exogenous foliar application of TU (5.6 mM) improved the leaf water potential and Pro content of salinity-stressed (100 mM, NaCl) maize plants with increasing fresh and dry weight of the biomass. However, photosynthetic efficiency also improved by the application of TU under salinity stress (Kaya *et al*., 2015). Medium supplementation of TU (400 µM) at seedling stage resulted in maximum improvement improvement of maize shoot and root dry weight and leaf area under no-stress 39.26, 46.77, and 38.64% and maximum improvement under 120 mM NaCl stress were 28.57, 36.0, and 52.63%, respectively (Sanaullah *et al*., 2016).

The effectiveness of TU has also been assessed by Talukdar (2014) and showed that shoot, root dry weight; and photosynthetic pigment declined with the exposure of mung bean on Arsenic (V) stress (25 µm) and TU (13 mM) treatment through the priming of seed and exogenous spray was more effective in alleviating As-stress through increasing root-shoot dry weight and reducing oxidative stress.

To investigate the effect of TU in mitigating boron (B) toxicity in two wheat cultivars (Bread wheat and Durum wheat), Kaya *et al*. (2019) demonstrated that after 4 weeks of B stress (0.2 mM, H_3BO_3) foliar spray of TU at the rate of 2.6 mM and 5.2 mM improved the shoot, root and total dry matter, RWC, Fv/Fm ratio, and suppressed the EL, MDA and H_2O_2 content, compared to stressed plants.

In maize, supplementation of TU (0.25 mM) at seedling stage improved root and shoot dry weight with total photosynthesis, and Car content by 11.78, 6.93, 13, and 15.65%, respectively, in Cd-stressed (1000 µM, $CdCl_2$) plants. Furthermore, TU significantly reduced the tissue Cd content and thus enhanced Cd-tolerance capacity in maize plants (Perveen *et al.*, 2015). In barley (*Hordeum vulgare* L.) , medium supplementation (*in vitro*) of 10 mM TU in 500 µM Cd-stressed plant, biomass and yield increased by 4.63 and 20.45%, respectively, compared to control (Ikram and Javed, 2015).

In an experiment conducted with mustard (*B. juncea* L.) by Pandey *et al.* (2012) stated that seedling fresh weight and dry weight were decreased by 31 and 53% under UV-B stress (280-320 nm) alone whereas in TU (6.5 mM) pretreated seedlings the reduction was 7 and 27%, respectively, over control. Similarly, chl content also declined under stressed seedlings (31-46%) but the reduction was lessening with the TU treatment (12-35%).

2.10.2 Thiourea-induced drought tolerance in crop

TU application can improve phloem translocation of photosynthates, leaf RWC; modulates stomatal conductance and balanced water uses, and thereby improves yield and thus induces drought resistance in field crop seeds (Bhunia *et al*., 2015; Srivastava *et al*., 2017; Singh and Singh, 2017).

To prove the beneficial effect of TU in mitigating drought stress Abdelkader *et al.* (2012) conducted a pot experiment and stated that foliar spray of TU at 5 mM reduced fast breakdown of total chl content in drought-stressed (40% FC) wheat (var. Gimaza 9) plants and resulted in the highest pigment content. Besides, TU increased P, K, Ca and Mg content in wheat leaves through enhancing nutrient availability to plant comparing with control and drought-stressed plants alone. He also reported that TU treated drought-stressed plants showed remarkable improvement in root length, shoot length, and root fresh and dry weight, shoot fresh and dry weight as well by 9, 25.3, 57.4, 78.2, 31.5, 24, and 31% compared to untreated drought-stressed plants. Yield and yield contributing parameters of wheat like 100-grain weight, grain no. plant^{-1}, grain weight plant^{-1}, spike length, and spike weight were also positively affected by TU supplementation under drought stress (Abdelkader *et al.,* 2012).

Hassanein *et al*. (2015) reported that seed treatment with TU (2.5 mM), SA (1 mM) and their combination effectively enhanced the wheat performance under water deficit condition (irrigation every 10 days interval) by increasing membrane stability, SOD and CAT activities with great reduction of POX and APX, comparing with normal irrigation. On the contrary, due to the application of TU on SA pretreated plants more than 30% reduction of putrescine, MDA and H_2O_2 contents in drought-stressed plants was observed comparing with normal irrigated plants.

Utilization of TU foliar spray at the rate of 10 mM at critical growth stages, under lower irrigation level increased water saving (14.1%) which was manifested by improved root growth supporting enhanced water uptake which resulted in improved biomass production (15.3%) and grain yield (20.6%), over control (no TU), which enhanced WUE under medium to severe drought condition (Wakchaure *et al.,* 2016).

In the following experiment conducted with onion (*Allium cepa*) by Wakchaure *et al.* (2018) demonstrated that TU treatment also improved bulb quality and production, reducing water consumption under water-scarce condition.

Conducting an experiment with rice (MR200), Mahadi *et al*. (2020) concluded that TU (2 mM) alone or in combination with GA (0.06 mM), SA (1 mM) and KCl (1 mM) increased germination percentage, germination index, seedling vigour, chl content, Pro, and protein content over untreared PEG-induced drought-stressed seedling (−1.2 Mpa). According to Yadav *et al*. (2020b), TU (6.5 mM) foliar application in drought-stressed (60% ET) plants resulted in noticeable increment of wheat grain yield by 11 and 10.5%, compared to control and drought-stressed plants only. In the same experiment TU (6.5 mM) increased grain yield of pearl-millet over drought-stressed plants significantly.

Chapter III

MATERIALS AND METHODS

This chapter describes the time, site, weather condition, planting materials, treatments, experimental design and layout, crop growing methods, fertilizer application, seed sowing technique, different cultural practices, data collection methods and statistical analysis of the experiment.

3.1 Location

The field experiment to study the morpho-physiological and yield attributes was conducted at the experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka (90º 77´ E longitude and 23º 77´ N latitude), Bangladesh, from December 2019 to March 2020. The chemical analysis for biochemical attributes were carried out at the Crop Science Laboratory at Sher-e-Bangla Agricultural University, Dhaka. Geographical location of the experimental area has been shown in Appendix III.

3.2 Weather condition of experimental site

The experiment was conducted in rabi season. Cold temperature and minimum rainfall is the main feature of the rabi season. The monthly maximum and minimum temperature with relative humidity during the study period (December 2019 to March 2020) has been collected from the Bangladesh Meteorological Department, Agargoan, Dhaka and has been shown in Appendix IV.

3.3 Materials

3.3.1 Plant materials

BARI Chola 7 has medium and light green colored leaflets with 55-60 cm plant height. Seed slightly round-shaped with smooth skin and bright brownish-yellow in color and its ripening time is about 125-130 days. 1000 seed weight is 170-180 g with 1.8-2.2 t ha^{-1} yields (Azad *et al.*, 2019).

Another variety BARI Chola 9 has large and deep green colored leaflets with 60-70 cm plant height. Seeds are dark brown colored with 125-130 days ripening time. 1000 seed weight is 180- 220 g with 2.3-2.7 t ha^{-1} yield (Azad *et al.*, 2019).

3.3.2 Soil preparation for pot experiment

Empty plastic pots with 18-inch depth and 14-inch diameters were used for the experiment. Twelve kilograms of sun-dried soil along with organic manures and fertilizers were put in each pot. After that, pots were prepared for seed sowing.

3.4 Treatments

The experiment consisted of the following treatment combinations:

V_1	D_0
	D_0+TU
	D_1
	D_1+TU
	D_2
	D_2+TU
	D_3
	D_3+TU

V_2	D_0
	D_0+TU
	D_1
	D_1+TU
	D_2
	D_2+TU
	D_3
	D_3+TU

Here,

V_1 = BARI Chola-7 and V_2 = BARI Chola-9,

D_0 = No drought stress (100% FC),

D_1 = Mild drought stress (25% depletion from FC),

D_2 = Moderate drought stress (50% depletion from FC),

D_3 = Severe drought stress (75% depletion from FC),

TU = Thiourea foliar spray (5 mM CH_4N_2S).

3.5 Design and layout of the experiment

The experiment was laid out in a completely randomized design (CRD) with three replications.

3.6 Seed collection

The varities of chickpea used for the experiment, BARI Chola-7 and BARI Chola-9 were collected from Pulse Crop Research Centre, Bangladesh Agriculture Research Institute (BARI), Joydebpur, Gazipur. The seeds were healthy, well matured and free from any extraneous materials.

3.7 Pot preparation

The collected soil was sun-dried, crushed and sieved. An appropriate amount of fertilizers and organic manure were mixed thoroughly with the soil before placing the soils in the pots. Pots were placed at the net house of Sher-e-Bangla Agricultural University, Bangladesh.

3.8 Fertilizers application

Fertilizers used in the experimental pots were organic manure, urea, triple superphosphate, muriate of potash and gypsum at the recommended rate. The whole amount of fertilizers was incorporated with soil before placing the soils in the pots.

3.9 Seed sowing technique

Fifteen healthy seeds were sown in each pot. After germination 6 plants were allowed to grow in each pot.

3.10 Treatments application

3.10.1 Maintaining drought condition

According to Northeast Region Certified Crop Advisor Study Resources (NRCCA) (2010), field capacity is relatable to the moisture content (MC) of soil. Soil moisture content of each treatment was measured by using a moisture meter (Model no: WH0291). The MC was measured from 25 DAS till pod maturation to maintain 40, 30, 20, and 10% for D_0, D_1, D_2, and D_3 treatments which indicates 100% FC, 75% depletion from FC, 50% depletion from FC and 75% depletion from FC.

3.10.2 Thiourea preparation and application

Foliar application of TU was applied at the rate of 5 mM and it was sprayed at 7 days interval from 30 DAS till pod maturation.

3.11 Crop husbandry

3.11.1 Thinning

After sowing seeds continuous observation was kept. It was observed that no single seed failed to germinate. So, there was need of thinning. Keen observation was made for thinning to maintain 6 seedlings. Thinning was done to maintain spacing of the plants.

3.11.2 Weeding

Sometimes there were some weeds observed in pots which were uprooted manually.

3.11.3 Plant protection measure

Root rot disease was observed at 10 days old seedling. Autostin® 50 WDG (Carbendazim) @ 1g L^{-1} was sprayed for two times at 3 days interval.

3.12 General observation of the experimental pots

Observations were made regularly and the plants looked normal green. The maximum flowering stage and pod initiation were not uniform.

3.13 Crop sampling and data collection

There were two stets of experimental pots. One set of pots was used for collecting growth, and yield parameters and another set was used for measuring physiological and biochemical parameters.

3.14 Harvesting and threshing

Crops of each treatment were harvested at different dates depending on their completion of 90% to 95% maturity when the whole plant became yellow and pods became brown to black in color. The seeds were separated from the plants. Dried seeds and stovers of each pot were weighed and collected as yield contributing data.

3.15 Collection of data

Growth, physiological and biochemical parameters were collected 45 DAS and 15 days after application of treatments at a time. The yield parameters were recorded at harvest.

3.15.1 Crop growth parameters

- Plant height
- Root length
- Root fresh weight
- Root dry weight
- Number of branches
- Above-ground fresh weight
- Above-ground dry weight

3.15.2 Physiological parameters

- Relative water content
- Chlorophyll *a* (chl *a*), chl *b* and chl (*a*+*b*) contents

3.15.3 Biochemical parameters

- Proline (Pro) content
- Lipid peroxidation
- H_2O_2 content
- Ascorbate and glutathione content

3.15.4 Yield and yield contributing parameters

- Weight of 100-seed
- Seed yield
- Stover yield
- Biological yield

3.16 Procedure of sampling for growth study during the crop growth period

3.16.1 Plant height

Plant heights were measured at 45, 60, 75, and 90 DAS from ground level (stem base) to the tip of the plant. Average plant height was calculated and expressed in cm.

3.16.2 Root length plant^{-1}

Four representative plants were uprooted from each pot randomly and washed them in water. Roots were cut and the length of each root was measured. The average value of the length was recorded as root length plant^{-1}.

3.16.3 Root fresh weight plant^{-1}

After measuring the root length of the uprooted plants, the roots were weighed in a balance and averaged them to have root fresh weight plant^{-1}.

3.16.4 Root dry weight plant^{-1}

After weighing for root fresh weight, the roots were dried in an electric oven maintaining 80 °C for 48 h. Then the plans were weighed in an electric balance and averaged them to have root dry weight plant^{-1}.

3.16.5 Number of branches plant^{-1}

The number of branches of those plants was counted and the average value was recorded as branch number plant^{-1}.

3.16.6 Above-ground fresh weight plant^{-1}

The above-ground portion of the uprooted sample plants were weighed in a balance and averaged them to have fresh weight plant^{-1}.

3.16.7 Above-ground dry weight plant^{-1}

After weighing for above-ground fresh weight, four sample plants were dried in an electric oven maintaining 80 °C for 48 h. Then the plans were weighed in an electric balance and averaged them to have dry weight plant^{-1}.

3.17 Procedure for sampling physiological parameters

3.17.1 Relative water content

According to Barrs and Weatherly (1962), RWC of leaves was calculated. Eight fresh leaves were taken and the fresh weight (FW) was determined and then floated on distilled water in Petri dishes. The Petri dishes must be kept in the dark place. After 24 h, the leaves were weighed again after removing excess water with soft tissue to determine turgid weight (TW). Finally, the leaves were oven dried at 80 ºC for 48 h to measure dry weight (DW).

Then, RWC was calculated as follows:

$$\text{RWC (\%)} = \frac{\text{FW - DW}}{\text{TW - DW}} \times 100$$

3.17.2 Photosynthetic pigments

Chlorophyll *a*, chl *b* and chl (*a*+*b*) were measured as photosynthetic pigments by following the method of Arnon (1949). The fresh leaves (0.5 g) were homogenized with 10 mL of acetone (80% v/v) using pre-cooled pestle and mortar and the supernatant was obtained by centrifuging at 5000×g for 10 min. After diluting the supernatant, the absorbance was measured with a UV-visible spectrophotometer at 663 and 645 nm for chl *a* and chl *b*, respectively. Chlorophyll (*a*+*b*) can be measured by adding chl *a* and chl *b*.

3.18 Procedure for measuring biochemical parameters

3.18.1 Measurement of proline content

Proline in leaf tissues was calculated following the protocol of Bates *et al*. (1973). Fresh leaf tissue (0.5 g) was homogenized well in 10 mL of 3% sulfosalicylic acid in pre-cooled pestle and mortar and the homogenate was centrifuged at 11,500×g for 15 min. Two mL of the filtrate was mixed with 2 mL of acid ninhydrin (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M phosphoric acid) and 2 mL of glacial acetic acid. Then the mixture was incubated in a 100 ºC in water bath for 1 h, then it was transferred into test tube and kept in ice to be cooled. After that toluene (4 mL) was added to the cooled mixture and mixed thoroughly by vortex mixture. After sometimes by transferring the upper aqueous layer the optical density of the chromophore containing toluene was read spectrophotometrically at 520 nm. Toluene was used as a blank. The Pro content was measured by comparison with a standard curve of known concentration of Pro and expressed as µm proline g^{-1} FW.

3.18.2 Measurement of lipid peroxidation

Lipid peroxidation was determined by measuring the malondialdehyde (MDA) content in 0.5 g leaf fresh weight, following the method of Heath and Packer (1968) with some modifications as described by Hasanuzzaman *et al*. (2012b). Leaf samples were homogenized in 3 mL 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 11,500×g for 15 min. The supernatant (1 mL) was mixed with a reagent named thiobarbituric acid (4 mL, TBA). The mixture was incubated at 95 ºC for 30 min and then cooled in an ice bath and centrifuging method repeated for 10 min. After that, at absorbance 532 nm the MDA content was measured and the measurements were corrected by subtracting the absorbance at 600 nm. The MDA content was measured by using the extinction coefficient 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

3.18.3 Determination of hydrogen peroxide content

Hydrogen peroxide was assayed according to the method described by Yu *et al.* (2003). Leaf tissue (0.5 g) was homogenized with 3 mL of 50 mM potassium-phosphate buffer (K-P) buffer (pH 6.5) at 4 ºC. The homogenate was centrifuged at 11,500×g for 15 min. Two mL of supernatant was mixed with 666.4 µL of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v) and kept in room temperature for 10 min. After that the mixture was again centrifuged at 11,500×g for 12 min. The optical absorption of the supernatant was determined spectrophotometrically at 410 nmto determine the H_2O_2 content (C= 0.28 μM^{-1} cm^{-1}) and expressed as nmol g^{-1} FW.

3.18.4 Extraction and measurement of ascorbate and glutathione

Ascorbate (AsA) and glutathione (GSH) content were estimated using the supernatant which was obtained by homogenizing 0.5 g fresh leaves in 3 mL ice-cold acedic extraction buffer (5% metaphosphoric acid containing 1 mM ethelenediaminetetraacetic acid-EDTA). The homogenate was then centrifuged at 11,500×g for 15 min at 4 ºC. This supernatant was collected to analyse the AsA and GSH content. AsA content was determined following the method of Huang *et al*. (2005), where supernatant was neutralized with 0.5 M K-P buffer (pH 7) and the oxidized fraction was reduced by 0.1 M dithiothretitol and then AsA assayed spectrophotometrically at 265 nm with 0.5 unit of ascorbate oxidase (AO) in 100 mM K-P buffer. Dehydroascorbate (DHA) can be measured by subtracting reduced AsA from total AsA. A specific standard curve of known concentration of AsA used dor quantification. And for measuring the GSH content, Yu *et al.* (2003) explained method was used with some modifications as described by Paradiso *et al.* (2008). The supernatant (0.2 mL) was neutralized with 0.3 mL of K-P buffer (0.5 M) (pH 7). After oxidizing GSH with 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) and reducing with nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of GR, the supernatant was used to measure total GSH content spectrophotometrically at 412 nm. Oxidized GSH (GSSG) content was evaluated after removing GSH by 2-vinylpyridine. The content of GSH was obtained by subtracting GSSG from total GSH. Two standard curves were made from the known concentration of GSH and GSSG for the final calculation.

3.19 Procedure of measuring yield and yield contributing parameters

3.19.1 Weight of 100-seed

Clean sun dried grains were counted and weighed by using an electronic balance. Then it was converted into 100-seed weight.

3.19.2 Seed yield plant^{-1}

The separated and cleaned seeds of each treatment were counted, weighed and averaged to have seed yield plant^{-1}.

3.19.3 Stover yield plant^{-1}

After separation of seeds from plant, the straw and shell were weighed and recorded as stover yield plant^{-1}.

3.19.4 Biological yield plant^{-1}

Biological yield was calculated by using the following formula:
Biological yield= Seed yield + stover yield

3.20 Statistical analysis

All obtained data were statistically analyzed following computer based software CoStat v.6.400 (CoStat, 2008) and mean separation was compared by LSD (Least Significant Difference) at 5% level of significance.

Chapter IV

RESULTS AND DISCUSSION

4.1 Growth parameters

4.1.1 Plant height

Plant height of both the chickpea varieties was remarkably decreased at all the levels of drought stresses (Table 1). Compared to control, plant height was reduced significantly under mild (17-21%), moderate (25-35%), and severe (40-47%) drought level at 45, 60, 75, and 90 DAS in BARI Chola-7. In BARI Chola-9 the reduction of plant height was 12-17, 21-33, and 30-46% under D_1, D_2, and D_3 drought stress conditions, respectively, over well-watered plants at different DAS, which was lower compared to previous variety. But the exogenous application of TU effectively enhanced the plant height compared to the drought stress alone. At 45 DAS plant height increased in TU treated plants of BARI Chola-7 under mild (13%) and moderate (11%) drought stress but TU showed no significant increment of plant height in the case of BARI Chola-9 at this period (Table 1). However, TU mitigated the negative effect of drought stress by increasing plant height under mild (7, 13, and 9%) and moderate (7, 10, and 9%) drought stress of BARI Chola-7 at 60, 75, and 90 DAS. Plant height of BARI Chola-9 increased by 6, 7, and 8% under TU sprayed mild stressed plants and 8, 12, and 13% under TU treated moderately stressed plants at 60, 75, and 90 DAS compared to plants exposed to untreated drought-stressed plants alone (Table 1).

Table 1. Plant height at 45 DAS, 60 DAS, 75 DAS, and 90 DAS of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress

Varieties	Drought levels	TU spray	Plant height (cm)			
			45 DAS	60 DAS	75 DAS	90 DAS
BARI Chola-7	D_0	–	23.12±0.82b	44.44±1.15c	50.19±2.28c	54.20±0.80bc
		+	23.06±1.38b	45.98±0.46c	50.19±2.22c	54.47±3.14bc
	D_1	–	19.02±0.81d	36.92±0.46f	40.43±1.36ef	42.71±1.70e
		+	21.42±0.88c	39.46±1.55e	45.49±1.21d	46.69±1.52d
	D_2	–	17.02±0.41e	33.43±1.21gh	34.79±2.29g	35.29±1.66fg
		+	18.93±1.19d	35.80±0.51fg	38.26±0.07f	38.61±2.14f
	D_3	–	13.86±0.56f	26.75±1.55i	27.11±2.31h	28.51±1.06h
		+	14.08±0.33f	26.72±1.56i	26.44±0.70h	29.63±1.76h
BARI Chola-9	D_0	–	24.80±0.18a	53.61±1.32a	59.46±1.54a	62.69±1.90a
		+	25.42±0.99a	52.85±2.72a	60.45±2.86a	63.11±2.18a
	D_1	–	21.82±0.62bc	46.40±1.01c	51.17±1.75c	52.30±1.84c
		+	22.38±0.81bc	50.00±0.50b	54.64±0.36b	55.68±3.30b
	D_2	–	19.61±0.83d	39.25±1.17e	41.79±2.00e	42.07±3.27e
		+	20.00±0.69d	42.20±1.70d	46.69±3.01d	47.15±1.17d
	D_3	–	17.50±0.64e	33.74±1.39h	33.48±1.40g	33.61±0.59g
		+	19.11±0.96d	32.70±1.76h	33.41±1.97g	34.33±1.49g
CV%			3.53	2.87	4.10	4.16

Data presented is mean (±SD) of three replicates for each treatment and different letters denote significant difference among the treatments at $P \leq 0.05$. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress.

The tallest plants were found in the control treatment and the shortest under drought conditions. Plant height was decreased with the increase of drought severity at any ages of the plants (Table 1). While, prolonged drought stress interrupted water flow from xylem to other elongating cells imposes both osmotic stress and ion toxicity, which results cell dehydration, reduction of cell turgor pressure and reduces cell division and cell expansion as well as normal cell functioning. Thus cell organ growth is restricted and plant height is reduced (Fathi and Tari *et al*., 2016; Abobatta, 2020). In chickpea growth parameters affected negatively under water deficit condition, including plant height (Hussain *et al*., 2015). Likewise, water stress has also been reported to reduce plant height in different field crops like mung bean (Ahmad *et al*., 2015), rice (Todaka *et al*., 2015), wheat (Yava and Unay, 2016), lentil (Gorim and Vandenberg, 2017), maize (Su *et al*., 2019) etc. However, through osmotic adjustment TU improved cell turgor pressure, RWC, photosynthetic rate and biomass production and increased plant height of drought-stressed plants (Table 1). Similarly, foliar application of TU increased plant height in clusterbean by increasing net photosynthesis and nitrogen metabolism and ameliorated the negative effect of drought stress (Garg *et al.*, 2006).

4.1.2 Root length

A notable increase of root length was observed with the increase of drought stress intensity in both the varieties (Figure 1). But under severe drought stress, notable decline of root length was found by 20 and 25% in BARI Chola-7 and BARI Chola-9 compared to control. However, the application of TU increased root length in D_1 (28%) and D_2 (10%) treatments of BARI Chola-9 and D_1 (34%) in BARI Chola-7 compared to control. On the contrary, a significant amount of root length was decreased under severe stress (10%) due to the application of TU in BARI Chola-7 compared to control, accordingly. The effect of TU in improving root length and drought tolerance capacity was more remarkable in the case of BARI Chopla-9 compared to BARI Chola-7 (Figure 1). But in the case of severe drought stress plants become unable to overcome the negative effect of stress and for this reason, root length decreased significantly at 25% FC while no further increment of root length happened by TU foliar application.

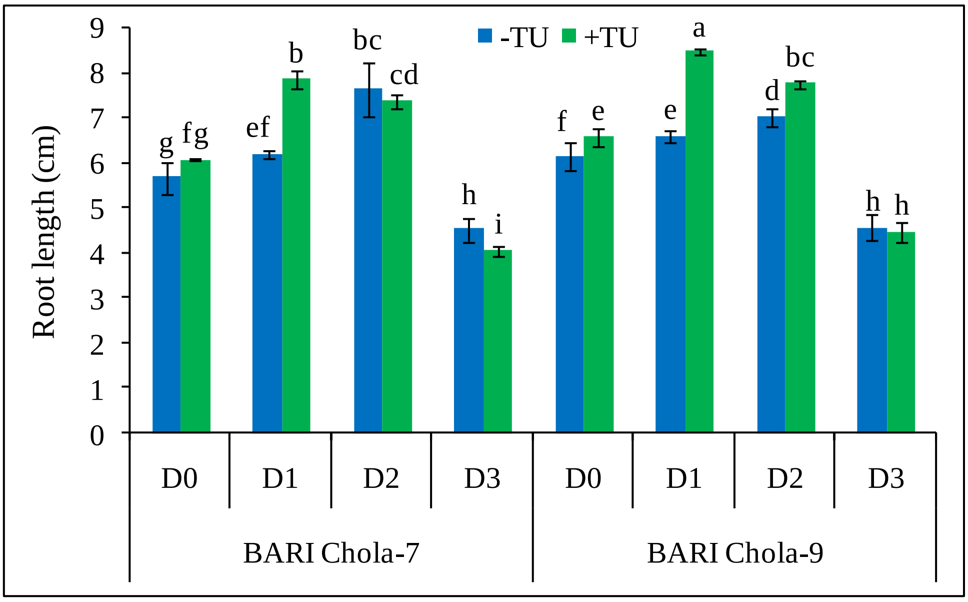

Figure 1. Root length of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. −TU= No TU and +TU= 5 mM TU foliar spray

Root length significantly increased, whereas root fresh weight and root dry weight decreased with the severity of drought stress. Drought stress resulted in the highest root length under moderately stressed plants of both varieties. Maximum extraction of the stored soil moisture is needed to make it available for transpiration in drought-stressed plants and that could be gained through an adaptation mechanism that is linked with root structure. The relation between deep root system and drought tolerance to chickpea was proved by Silim and Saxena (1993). According to Sangakkaran *et al*. (2000), under drought stress more carbon diverted to root and thus root length increased in mung bean, which helps to absorb stored water from a different depth. Under PEG-induced drought stress root fresh and dry weight decreased while root length increased in alfalfa (Zeid and Shedeed, 2006). In line with this, Alghabari and Ihsan (2018) revealed that lack of water supply resulted in the highest root length but lowest root fresh and dry weight in barley at 50% FC. Moreover, TU supplementation increased root length in mildly stressed plants of BARI Chola-7, and mild and moderately stressed plants of BARI Chola-9.

4.1.3 Root fresh weight

In response to different levels of drought stress root FW decreased sharply in contrast to control plants. Like FW, the severe drought stress showed the highest reduction in root DW in the case of both chickpea varieties. In BARI Chola-7 10, 38, and 50% lower root FW and in BARI Chola-9 19, 25 and 31% lower root FW were recorded inplants under mild, moderate, and severe drought stress levels, respectively, compared to control plants (Figure 2). On the other hand, the foliar application of TU remarkably increased root FW under moderate drought stress (13%) in BARI Chola-7, whereas in BARI Chola-9 the increment of root FW was found under mild (26%) and moderate (21%) drought stress compared to drought-stressed plants only (Figure 2).

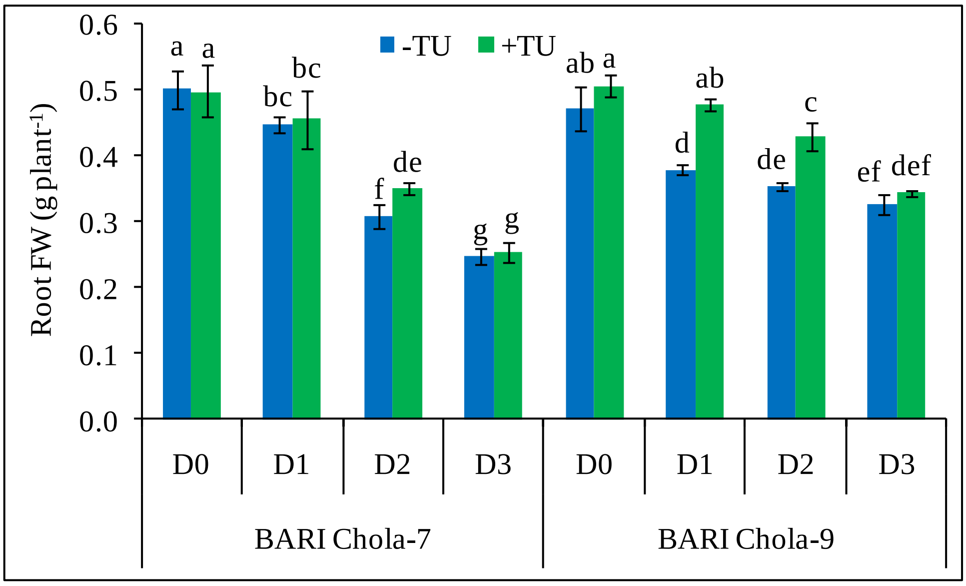

Figure 2. Root fresh weight of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

Water stress has always been considered to show harmful effects on the growth traits, including root FW of the plants. Reduction of root moisture percentage under water deficit condition due to reduced water potential, osmotic potential and turgor potential were found to be the main reasons behind the root FW reduction in chickpea varieties

(Yaqoob *et al.* 2013). Root FW of chickpea plants reduced with the increase of the drought stress severity (Sattar *et al*., 2017). Saha *et al.* (2019) established that root FW decreased by 24 to 45% in five rice varieties under drought stress compared to well-watered control plants. A similar result was observed in other crops like_mung bean (Prakash *et al.* 2017), rice (Larkunthod *et al*. 2018), rapeseed (Hasanuzzaman *et al*., 2018c), cotton (Iftikhar *et al.*, 2019), maize (Hussain *et al.*, 2019, Badr *et al.*, 2020) etc. In the contrary, the foliar application of TU improves root FW under drought stress of both varieties in this experiment (Figure 3). Creating osmotic balance in plant cell TU maintains cell volume in stressed plants (Singh, 2018). According to Akladious *et al.* (2013), TU improved the growth parameters by improving chl content and RWC of sunflower under heat stress.

4.1.4 Root dry weight

Upon exposure of both chickpea varieties to mild, moderate and severe drought stress, root DW decreased remarkably. Severe drought stress caused a maximum reduction in root DW. Root DW exhibited 10, 34, and 53% declined, in contrast, to control the condition under mild, moderate and severe drought stress, respectively, in BARI Chola-7. In the case of BARI Chola-9 the reduction was observed under mild, moderate, and severe drought stress by 19, 36, and 53% compared to well-watered plants (Figure 3). However, the exogenous application of TU increased the root DW sharply in both varieties. In BARI Chola-7 remarkable increment of root FW was observed with the combination of TU under mild (6%) and moderate (7%) drought stress. Under different drought stress levels TU treated plants of BARI Chola-9 showed 7, 18, and 16% increment in root DW in comparison to the non-treated stressed plants (Figure 3).

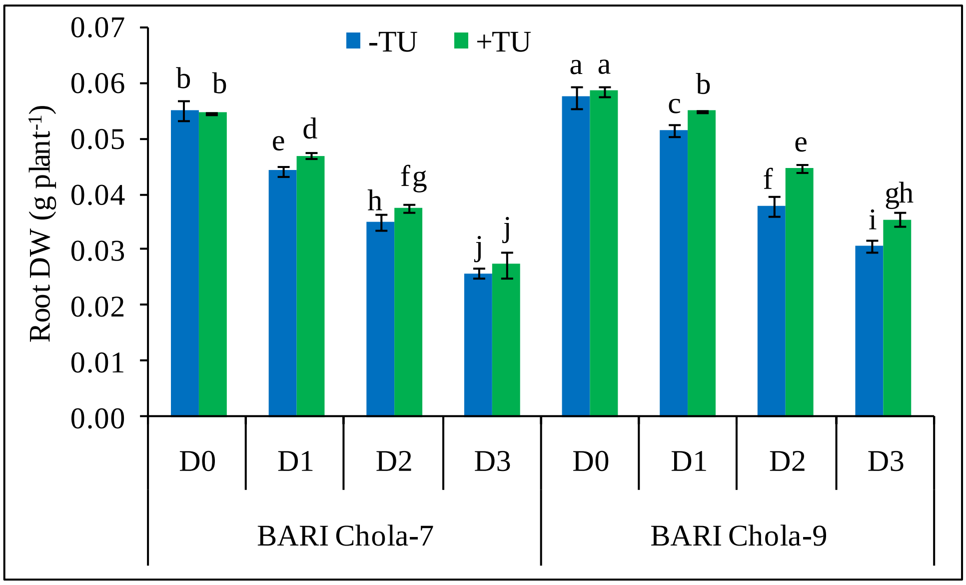

Figure 3. Root dry weight of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote a significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

The present study revealed that like root FW, root DW of the plants decreased under water deficit condition (Figure 3). Long term drought stress reduced the root DW of chickpea by reducing root density (Amede and Schubert, 2003). According to Hossinzadeh *et al.* (2012), a decrease of root DW occurs with the reduction of root length, root volume and root FW in chickpea cultivars. Similarly, Sattar *et al.* (2017) reported that root DW reduction was higher in chickpea plants exposed to 25% and 50% FC compared to control (100% FC). Drought stress limits nutrient and water uptake .by root and causes a reduction in root FW and root DW as well (Abdela *et al.*, 2020). Other studies also reported that water deficit condition resulted in reduced root DW in other crops such as, rice (Larkunthod *et al.*, 2018), maize (Hussain *et al.*, 2019; Badr *et al.*, 2020), rapeseed (Hasanuzzaman *et al.*, 2018c), mung bean (Prakash *et al.*, 2017), cotton (Iftikhar *et al*., 2019) etc. Moreover, TU supplemented drought-stressed plants showed a further increment in root DW in both varieties (Figure 3). Under combined salinity and high temperature stress TU increased length and dry weight of shoot and root and leaf area of wheat cultivar (Anjum *et al.*, 2011). In maize, Cd-toxicity was ameliorated by the exogenous TU application and showed that TU supplementation increased shoot and root DW (Perveen *et al.*, 2015). TU enhanced the accumulation of compatible solutes such as Pro and believed to enhance osmotic adjustment and decreases osmotic potentiality and thus promoted water use uptake into plant cell which resulted in improvement of growth and yield of different crops under various abiotic stresses (Burman *et al.*, 2004; Garg *et al*., 2006; Waqas *et al*., 2019).

4.1.5 Number of branches plant^{-1}

In the present study number of branches plant^{-1} of both varieties of chickpea was significantly reduced at all drought stress levels (Figure 4). The number of branches plant^{-1} was decreased by 26, 40, and 62% at 45 DAS and 33, 43, and 70% at 60 DAS under mild, moderate, and severe drought-stressed plants of BARI Chola-7, respectively, compared to control. Similarly, a sharp decrease in branch no. plant^{-1} was observed under mild, moderate and severe level of stress by 10, 19 and 39%, respectively at 45 DAS and 22, 39, and 45% at 60 DAS, respectively in the case of BARI Chola-9 (Figure 4). TU foliar spray significantly improved number of branches plant^{-1} of both varieties under well-watered control and drought-stressed plants as well. Number of branches plant^{-1} were increased by 28.5 and 13% under TU treated mild and moderately stressed plants of BARI Chola-7 and 12.3% under moderately stressed plants of BARI Chola-9 at 45 DAS, compared to plants without TU supplementation. TU foliar application increased number of branches plant^{-1} by 18 and 10% under mild and moderately stressed plants of BARI Chola-7 and by 10 and 18% under mild and moderately stressed plants of BARI Chola-9 at 60 DAS, compared to non-treated drought-stressed plants only. But the foliar application of TU did not show any significant result in mitigating severe drought stress (Figure 4).

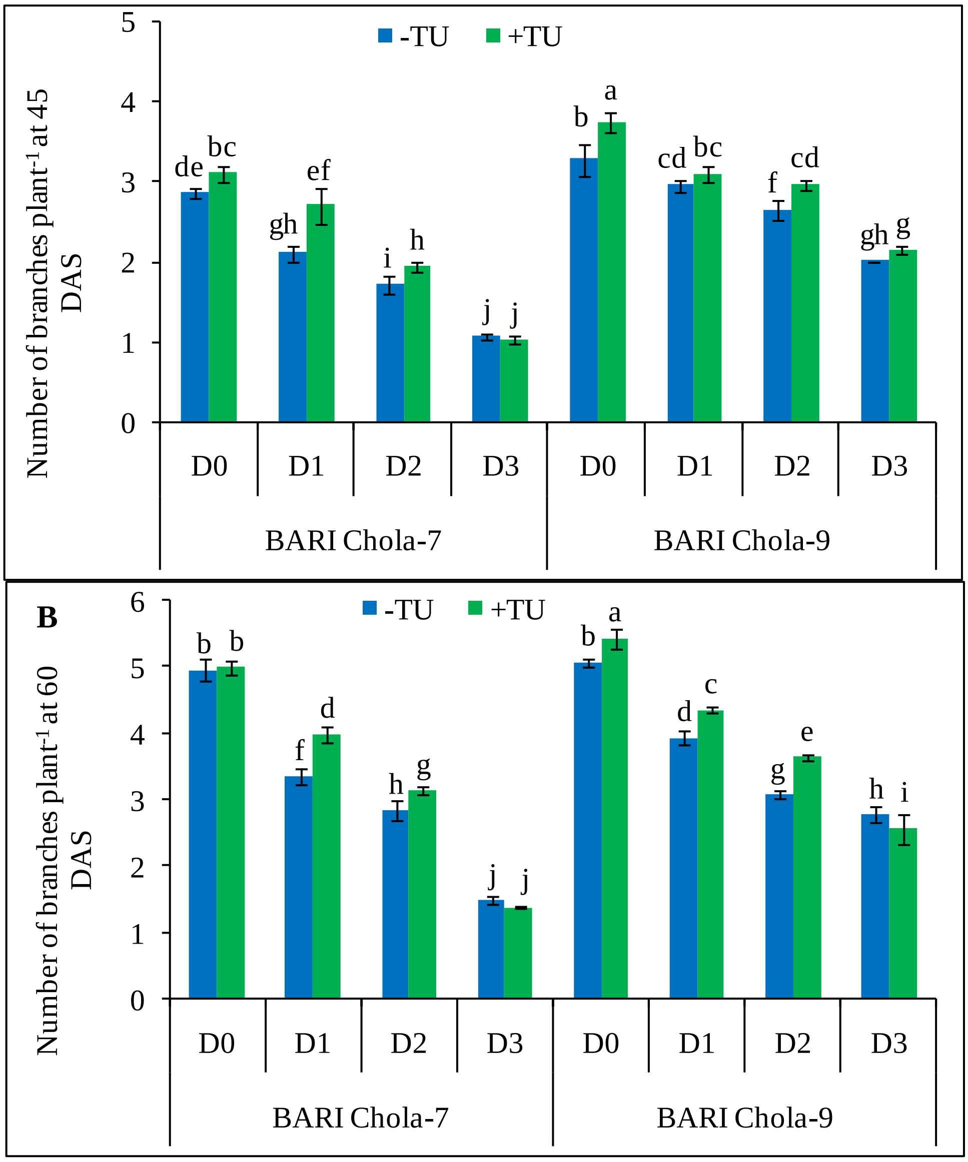

Figure 4. Number of branches plant^{-1} at 45 DAS (A) and 65 DAS (B) of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

According to Lima *et al.* (2011), water deficit condition reduced the tiller no. in sorghum due to the reduction of water and nutrient uptake through soil solution. Similarly, Ulemale *et al.* (2013) reported that in chickpea plant height and number of branches plant^{-1} declined due to the inhibition of cell elongation of plant by interruption of water flow from xylem to surrounding elongating cells. In the same way, drought stress at flowering and seed setting stage declined the number plant^{-1} by

13.7 and 21.1%, respectively in chickpea, compared to control (Abhishek *et al.*, 2020). Drought stress also hampers the growth of cells and reduces cell division, reducing carbohydrate supply to the growing cells (Abobatta, 2020). In agreement with this Mekonen (2020) reported that number of primary and secondary branches reduced notably in two chickpea varieties viz. Habru and Mastwal upon exposure to drought stress both at vegetative and seed filling stages. On the other hand, the application of TU increased the number of branches plant^{-1} (Figure 4). This result is supported by the findings of Burman *et al.* (2004) that the foliar application of 20 mM TU increased the plant height, number of brunches and other growth contributing parameters in clusterbean through improving nutrient uptake and nutrient metabolism. Application of TU at 13 mM alleviated the damaging effect of drought and increased branching number by 7.9 and 10% at vegetative and seed filling stages, respectively in chickpea (Abhishek *et al.*, 2020).

4.1.6 Above-ground fresh weight

Drought stress caused a profound decrease in above-ground FW in both chickpea varieties (BARI Chola-7 and BARI Chola-9). Upon exposure to mild, moderate and severe drought stress, above-ground FW declined by 36, 55, and 68% in BARI Chola-7 and 41, 55, and 63% in BARI Chola-9 compared to control. On the contrary, addition of TU foliar application at 5 mM improved above-ground FW by 26% in mild stressed plants compared to untreated drought-stressed plants of BARI Chola-7, but no further improvement of above-ground FW was found with TU application in moderate and severe stressed plants of BARI Chola-7 (Figure 5). However, TU application resulted in more remarkable improvement under mild (D_1) and moderately (D_2) drought-stressed plants by 16 and 47% compared to non-treated drought-stressed plants in BARI Chola-9. Furthermore, foliar application of TU did not show any significant improvement in shoot fresh weight under severe stressed plants (D_3) of BARI Chola-9 (Figure 5).

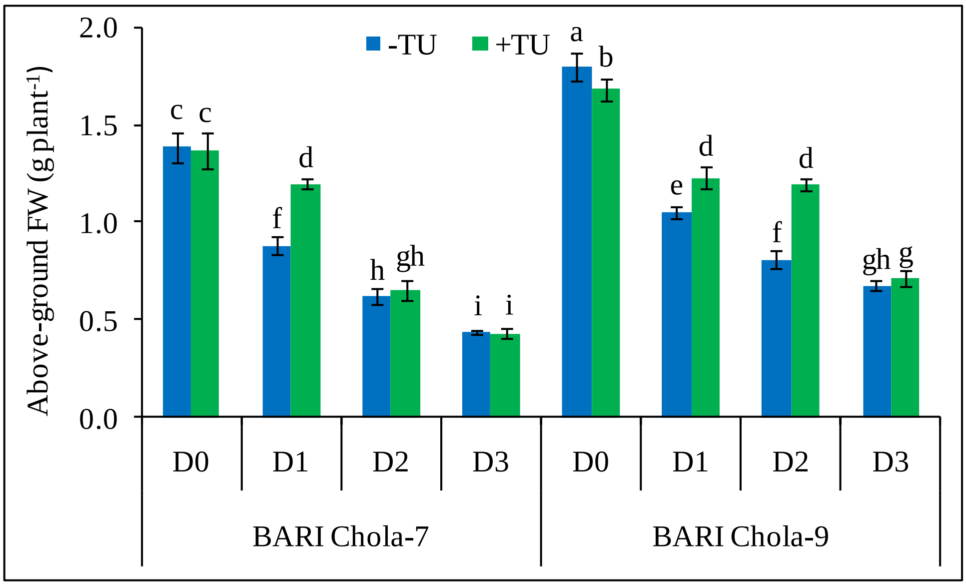

Figure 5. Above-ground fresh weight of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU =No TU and +TU= 5 mM TU foliar spray

Drought and osmotic stress reduce cell expansion by restricting the entrance of water, which subsequently affects cell division and growth (Dreesan *et al.*, 2012; Schmidt *et al.*, 2016; Hussain *et al.*, 2018). In this experiment, above-ground FW markedly decreased with the increase in drought stress intensity (Figure 5). Reduction of above-ground FW was observed by Amede and Schubert (2003) under mild drought stress in chickpea and common bean because of decreased dry matter accumulation and photosynthetic rate compared to control. Another reason behind the reduced above-ground FW under drought stress is lower moisture retention power in chickpea leaves compared to well-watered plants (Yaqoob *et al.*, 2012; Khadraji *et al.*, 2017). Moreover, in different studies, drought stress reduced the above-ground FW in some other crops like maize (Anjum *et al.*, 2017; Hussain *et al.*, 2019), rice (Saha *et al.*, 2019; Nasrin *et al.*, 2020), lentil (Sehgal *et al.*, 2017), mustard (Hasanuzzaman *et al.*, 2018c) etc. When drought stress supplemented with the exogenous TU, an improvement in above-ground FW was noted over untreated drought stress treatments alone. For instance, TU pre-sowing seed treatments or foliar applications also improved growth in clusterbean under water deficit conditions by improving net photosynthesis and dry matter accumulation (Garg *et al.*, 2006). Exogenous application of TU in combination with SA improved above-ground FW in wheat by alleviating drought symptoms such as inhibition of mitotic division and low turgor pressure from cell and tissue (Abdelkader *et al*., 2012). Waqas *et al.* (2019) demonstrated that TU treatment improved growth and development of different plants under different abiotic stress condition.

4.1.7 Above-ground dry weight

Like above-ground FW significant variation in plant above-ground DW was observed among stressed and control plantsof chickpea (Figure 6). The lowest above-ground DW was observed in severe drought stress in both chickpea varieties (Figure 6). Under mild, moderate and severe drought stress above-ground DW reduced by 38, 49, and 61%, respectively in BARI Chola-7 and 46, 50, and 70%, respectively in BARI Chola-9, compared to control condition. In the contrary, the exogenous application of TU increased the above-ground DW compared control. Both TU treated mild and moderately stressed plants of BARI Chola-7 and BARI Chola-9 showed improvement in above-ground DW. In contrast, TU foliar application significantly increased above-ground DW in mild and moderate drought stress in BARI Chola-7 by 46 and 19%, respectively and in BARI Chola-9 the increment was 51 and 18 %, respectively, compared to untreated drought-stressed plants (Figure 6).

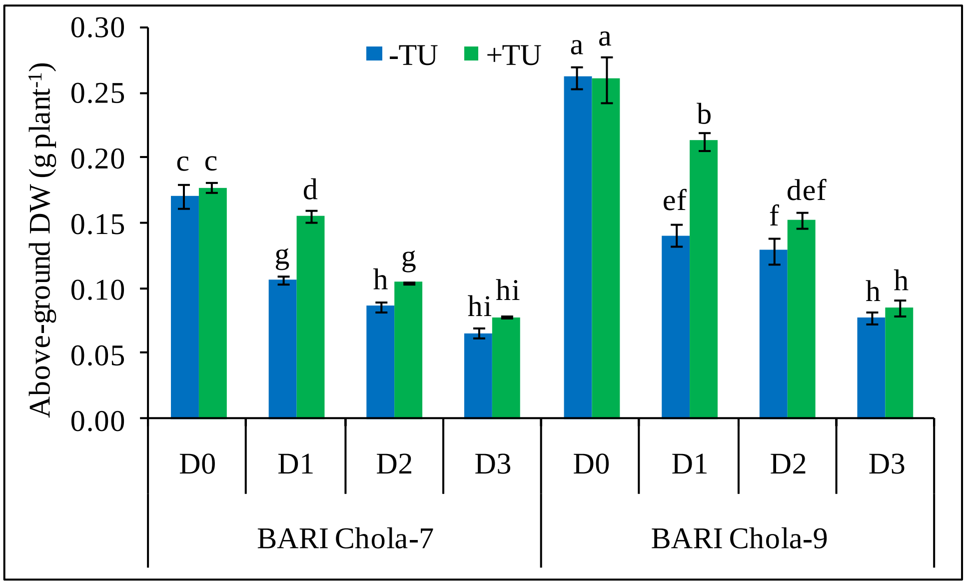

Figure 6. Above-ground dry weight of two varieties of chickpea is affected by thiourea under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU = No TU and +TU= 5 mM TU foliar spray

In this study, upon exposure to different levels of drought stresses, plants exhibited a reduction in above-ground DW (Figure 6). Randhawa *et al.* (2014) and Khadraji *et al.* (2017) described that drought stress reduced the above-ground DW along with above-ground FW and leaves DW in chickpea. Similar results were observed in rice (Saha *et al*., 2019; Nasrin *et al.*, 2020), maize (Anjum *et al.*, 2017; Hussain *et al.*, 2019), rapeseed (Hasanuzzaman *et al.*, 2018c), lentil (Sehgal *et al*., 2017) etc. According to Salma *et al.* (2016) and Abdela *et al.* (2020), reduction in above-ground length and above-ground FW occurred under drought in chickpea which ultimately leads to lower above-ground DW. Reduction in dry matter accumulation, plant growth and cell elongation occured due to drought stress (Nasrin *et al*., 2020). In contrast, TU foliar application increased the above-ground DW in this study (Figure 6). Application of TU increased the above-ground DW in chickpea by increasing above-ground FW (Garg *et al.*, 2006; Abdelkader *et al.*, 2012). TU acts as a stress-alleviating chemical and a growth regulator by altering several physiological and biochemical process like photosynthetic pigment synthesis, photosynthetic rate, carbohydrate accumulation etc. which leads to increment of above-ground DW of different crops like maize (Waqas *et al*., 2017), wheat (Wakchaure *et al*., 2016), mung bean (Mathur *et al*., 2006), clusterbean (Garg *et al*., 2006) etc.

4.2 Physiological parameters

4.2.1 Relative water content

Relative water content reduced under drought stress in both chickpea varieties (Figure 7). Plant exposed to mild, moderate, and severe drought stress showed decreasing RWC by 22, 37, and 48%, respectively, compared to well-watered control of BARI Chola-7 although no significant difference was found between 50% and 75% FC levels of drought stress in BARI Chola-7. In BARI Chola-9 the RWC was found to be decreased similarly by 16, 29, and 36% under mild, moderate, and severe drought stress, respectively, compared to control. However, the foliar application of TU improved this trait significantly in both varieties compared to drought-stressed plants alone. In BARI Chola-7 TU supplemented mild and moderately drought-stressed plants showed 22 and 18% increase in RWC, respectively, compared to the drought-stressed plants which were not sprayed with TU. Besides, TU improved the RWC by 13, 15 and 18% at mild, moderate, and severe stressed plants, respectively, compared to untreated plants exposed to different levels of drought stress, correspondingly in BARI Chola-9. But foliar spray of TU with severe stressed plants did not show any significant increment of RWC over D_3 plants in BARI Chola-7 (Figure 7).

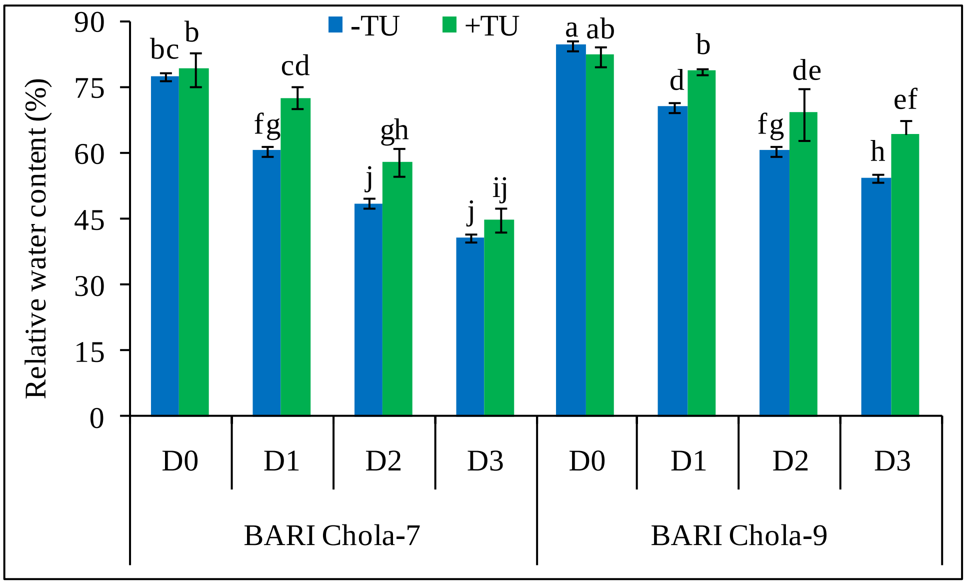

Figure 7. Relative water content of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU = No TU and +TU= 5 mM TU foliar spray

The most obvious effect of physiological drought is the loss of water from the tissue. Relative water content is inversely correlated with drought stress (Alam *et al.*, 2013). Water deficit condition affects plant-water relations, reduces water retention capacity and turgor of plant cell, causes osmotic stress, inhibits cell expansion and cell division, as well as the growth of plants as a whole (Hasanuzzaman *et al.*, 2018c; Bhuiyan *et al.*, 2019). In this study plant height, fresh and dry weight and RWC reduced when plants exposed to different levels of drought stress in both varieties (Figure 7). These results are corroborated to previous studies conducted with different crops under water deficit stress (Alam *et al.*, 2014a; Hasanuzzaman *et al.*, 2017b; Hasanuzzaman *et al.*, 2018c; Bhuiyan *et al.*, 2019). The reduction of RWC was found by Patel *et al*. (2011), when he exposed four chickpea varieties under drought stress. Additionally, Shariatmadari *et al*. (2017) reported that RWC decreased with the increase of drought stress levels of 70%, 50%, and 30% FC in chickpea cultivar. Awari *et al.* (2017) demonstrated that water deficit condition reduced RWC due to the reduction of cell turgor and stomatal conductance of leaves, which leads to the reduction of net photosynthesis and plant growth in two varieties of chickpea. In another experiment, Khan *et al.* (2019) reported that RWC reduced in both tolerant and sensitive genotypes of chickpea. Present findings revealed that the decrease of leaf RWC was more prominent in BARI Chola-7 compared to BARI Chola-9 (Figure 7). Upon exposure to water stress, the tolerant genotypes of chickpea maintained maximum RWC and thus showed higher amount of chl content and leaf FW as well compared to susceptible genotypes (Khan *et al.*, 2019). Although at any levels of drought stress RWC decreased significantly, the exogenous TU application in those plants helped in maintaining water status in the tissue (Figure 7), which is consistent with another study conducted with clusterbean (Burman *et al.*, 2004). The exogenous spray of 10 mM TU at different crucial growth and developmental stages of wheat significantly increased WUE by accumulating RWC, which helped plants to survive under medium and severe drought stress levels (Pasala *et al*., 2017). A recent study exhibited that in both wheat and pearl-millet TU reduced cell dehydration under salinity and drought stress conditions by the accumulation of osmolytes, which improved water uptake and increased RWC of tissues (Yadav *et al*., 2020b). However, RWC was restored by the exogenous application of TU under drought stress as evidenced by improved leaf RWC and fresh weight and dry weight of chickpea varieties (Figure 7).

4.2.2 Chlorophyll content

The present study revealed that drought stress caused a profound decrease in chl *a* content in both varieties (Table 2). Maximum chl *a* content was found in well-watered BARI Chola-9. Upon exposure of plants to mild, moderate, and severe drought stress resulted in a decline of chl *a* content by 23.6, 34.5, and 47.2%, respectively, compared to control in BARI Chola-7. Similarly, chl *a* content of BARI Chola-9 markedly reduced by 20, 32 and 50% under D_1, D_2, and D_3 levels of drought stress over control. The maximum reduction in chl content was observed under severe drought stress in both varieties, but in the case of mild and moderate drought stress BARI Chola-7 resulted in higher reduction of chl *a* content compared to BARI Chola-9. On the contrary, the foliar spray of exogenous TU with drought stress showed 11 and 22% higher chl *a* content under TU treated mild and moderately stressed plants of BARI Chola-7 compared to non-treated drought-stressed plants. However, in the case of BARI Chola-9 the promising effect of TU application in enhancing chl *a* content was observed in mild and moderately stressed plants by 11.7 and 21%, respectively, compared to drought-stressed plants alone. No further improvement of chl *a* content was found with TU spray in plants exposed to severe drought stress (D_3) of both varieties (Table 2).

Like chl *a* content, chl *b* content also significantly decreased under any level of drought stress. Plant exposed to D_1, D_2, and D_3 levels of drought stress resulted in a reduction of chl *b* content by 29, 51, and 70%, respectively, compared to control of BARI Chola-7. Upon exposure to mild, moderate and severe drought stress, chl *b* content reduced by 32, 53, and 70%, respectively in BARI Chola-9, over control. However, the foliar application of TU resulted in an improvement of chl *b* content in TU treated mild (21%), moderate (8%), and severe (28%) stressed plants of BARI Chola-7 compared to non-treated drought-stressed plants. Higher chl *b* content was found in TU treated control, mild, moderate and severe stressed plants by 5, 28, 20 and 33% over non-treated control and drought-stressed-plants alone of BARI Chola-9 (Table 2).

As the consequence of a significant reduction of chl *a* and chl *b* content under any level of drought stress chl (*a*+*b*) content also reduced in both varieties. Chlorophyll (*a*+*b*) content is reduced by 26, 42, and 57% in plants exposed to mild, moderate, and severe stressed plants, compared to control of BARI Chola-7 (Table 2). Besides, plants exposed to D_1, D_2, and D_3 levels of drought stress resulted in reduced chl (*a*+*b*) content by 26, 48, and 59%, respectively, compared to control of BARI Chola-9. In contrast, the addition of exogenous TU with drought stress resulted in significantly enhanced chl (*a*+*b*) content by 18.6, 15.2 and 11.6% in mild, moderate, and severe stressed plants of BARI Chola-7 compared to non-treated drought-stressed plants. However, in the case of BARI Chola-9 significant effect of TU in improving chl (*a*+*b*) content was observed under mild (19%), moderate (20.8%), and severe (12.8%) stressed plants over non-treated drought-stressed plants. In general, the chl *a*, chl *b* and chl (*a*+*b*) content decreased in both varieties under the drought condition as compared with control and decreased with the increase in the duration of drought stress. However, the percentage of decrease in the BARI Chola-9 was less than that in the BARI Chola-7 (Table 2).

Table 2. Chlorophyll *a* (chl *a*), chl *b*, and chl (*a*+*b*) content of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress

Varieties	Drought levels	TU spray	Chlorophyll content (mg g^{-1} FW)		
			chl *a*	chl *b*	chl (*a*+*b*)
BARI Chola-7	D_0	−	0.57±0.01b	0.46±0.011d	1.02±0.001c
		+	0.56±0.01b	0.48±0.006c	1.05±0.006b
	D_1	−	0.43±0.02e	0.33±0.004f	0.76±0.027f
		+	0.48±0.01d	0.40±0.015e	0.88±0.010d
	D_2	−	0.36±0.01f	0.22±0.005i	0.58±0.009h
		+	0.43±0.01e	0.23±0.005hi	0.66±0.012g
	D_3	−	0.29±0.01g	0.13±0.003l	0.43±0.007k
		+	0.30±0.01g	0.17±0.004k	0.48±0.004j
BARI Chola-9	D_0	−	0.64±0.01a	0.51±0.005b	1.15±0.004a
		+	0.62±0.03a	0.53±0.011a	0.16±0.018a
	D_1	−	0.51±0.03c	0.34±0.004f	0.86±0.015e
		+	0.56±0.02b	0.44±0.001d	1.00±0.022c
	D_2	−	0.42±0.01e	0.24±0.005h	0.66±0.003g
		+	0.48±0.02d	0.29±0.018g	0.77±0.014f
	D_3	−	0.29±0.01g	0.17±0.011jk	0.46±0.016j
		+	0.31±0.01g	0.19±0.003j	0.50±0.011i
CV%			3.22	2.59	1.59

Data presented is mean (±SD) of three replicates for each treatment and different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress.

The effect of drought stress on decreased chl level depends on the duration and severity of drought (Kpyoarissis *et al.*, 1995). ROS over-generation causes pigment photo-oxidation and damages photosynthetic apparatus which resulted in chl content reduction in wheat (Herbinger *et al.*, 2002) and resulted in a lowered capacity for light harvesting.The reason behind the reduction of chl content was established by Sepehri and Golparvar *et al.* (2011), who reported that with the increase of drought intensity, the content of clorophillase and peroxidase enzymes increased which resulted in remarkable reduction of chl content and alteration of chl content can drastically change photochemical reactions. Water deficit conditions caused a marked decline in photosynthetic pigments content and photosynthetic efficiency as well due to enzyme activation in the light-dependent stage of biosynthesis (Anjum *et al.*, 2011). Moreover, drought stress resulted in closing stomata, the altered metabolic pathway of plants which restricts $C0_2$ diffusion into the leaf, or due to the inhibition of rubisco, a non-stomatal factor (Rapacz *et al.*, 2010). Upon exposure to different stresses including drought stress, leaf chloroplasts are injured through compressed grana lamellae, disrupted stroma lamellae and distorted thylakoids and thus photosynthetic machinery negatively affected, which leads to disrupted photosynthesis and the leaves turned into pale yellow colour and causes sudden plant death (Farooq *et al.*, 2009; Jiang *et al*., 2017; Liu *et al*., 2018). Physiological responses and the ability to defense against drought as well as other abiotic stresses can be measured by chl content of plants (Anjum *et al.*, 2011). Findings of the present study are also apparent by consistent reduction of chl *a*, chl *b* and total chl *(a+b)* under mild, moderate and severe drought stress (Table 2). The results are in agreement with Nyachiro *et al.* (2001), who narrated a notable decrease of chl *a* and chl *b* caused by drought stress, in six wheat cultivars. Additionally, Manivannan *et al.* (2007) described that chl content declined in sunflower which exposed to water stress because of the extreme production of ROS, reduced nutrient uptake by the plants, and disturbance in enzyme activities at cellular levels. Present findings are corroborated with the results of previous studies on other stresses including drought stress (Alam *et al.*, 2014b; Hasanuzzaman *et al*. 2018ab; Jiang *et al*. 2017). Drought resulted in a sharp decline in the leaf chl content, plant photosynthetic efficiency as well as PSII efficiency in different chickpea cultivars (Macar and Ekmekçi *et al.*, 2008; Kumar *et al.*, 2012; Patel and Hemantaranjan, 2012; Sattar *et al.*, 2017; Khan *et al.*, 2019). Leaf chl content was higher in tolerant chickpea genotype over sensitive genotype under drought stress (Khan *et al.* 2019). The present study revealed that BARI Chola-9 showed significantly higher chl *a*, chl *b* and chl (*a*+*b*) content compared to BARI Chola-9 (Table 2). Moreover, water stress caused a reduction in photosynthetic pigments including chl, Car, anthocyanin etc. in various types of plants depending on the plant age and duration of the stress due to the oxidation of pigments, impaired pigment biosynthesis and so on (Anjum *et al.,* 2011; Saraswathi and Paliwal, 2011; Pandey *et al.,* 2012, Alam *et al.,* 2013; Nasrin *et al.,* 2020). In contrast, TU foliar application under different levels of drought stress increased the chl *a*, chl *b* and chl (*a*+*b*) content in both chickpea varieties compared to drought-stressed plants alone (Table 2). Exogenous application of TU was found to be effective in mitigating the harmful effects of water deficit conditions on photosynthetic capacity of plants by improving net photosynthetic rates and chl content of drought-stressed clusterbean compared to control plants at both vegetative and flowering stages (Burman *et al.,* 2007). It affects both carbohydrates and nitrogen metabolism which consecutively enhances plant performance (Khokhar *et al.,* 2016). TU improves phloem translocation of photosynthates towards active sink in crop plants such as cereals, pulses and oilseeds and enables them to enhance photosynthetic rates (Bhunia *et al.,* 2015; Singh and Singh, 2017; Verma, 2019; Waqas *et al.,* 2019).

4.3 Biochemical parameters

4.3.1 Proline content

Notable increase of Pro content was found to be increased with the increase of drought stress severity in both the varieties (Figure 10). Comparing with control, the highest amount of Pro accumulation was observed in D_3 drought treatment, which was 3 fold and 3.5 fold in BARI Chola-7 and BARI Chola-9, respectively. However, the application of TU showed increased content of Pro in D_2 (12%) and D_3 (7%) treatments, whereas reduced content was observed at D_1 (19%) compared to untreated plants of BARI Chola-7. On the contrary, a significant amount of Pro content was decreased in mild and moderate stress treatments by 21 and 22%, respectively, due to the application of TU in BARI Chola-9, compared to control, accordingly (Figure 8).

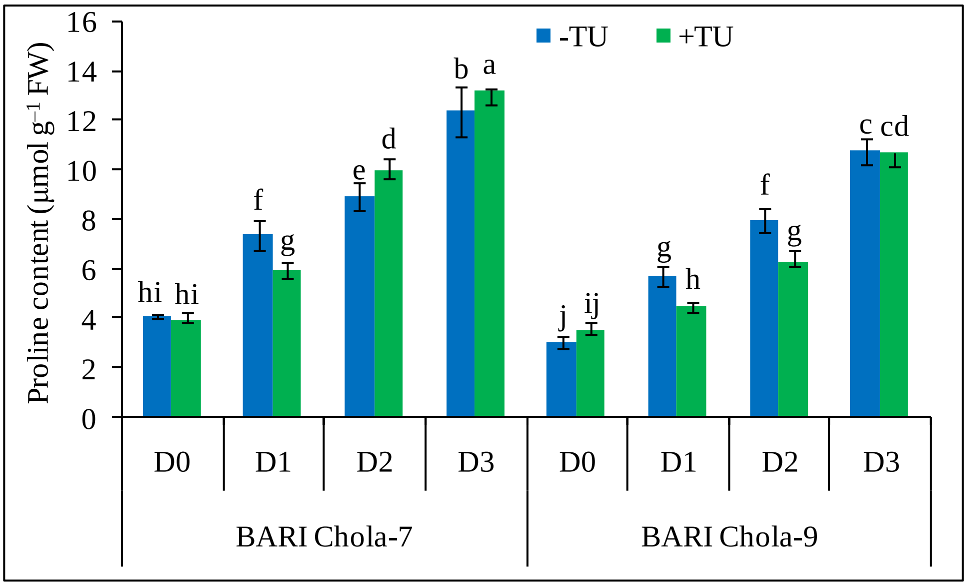

Figure 8. Proline content of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

According to Mafakheri *et al.* (2010), water stress increased the Pro content around ten fold compared to well-watered control plants of chickpea varieties under critical growth stages. Enhanced Pro content was found in both drought-tolerant and sensitive varieties of chickpea (Kaur *et al*., 2017; Khan *et al*., 2019). Increment of Pro level under several physiological stresses, including drought stress conditions was documented previously in different crops (Alam *et al*., 2013; Alam *et al*., 2014a; Singh *et al*., 2015; Nahar *et al*., 2016; Bhuiyan *et al*., 2019). Although Pro content profoundly increased under drought-stressed conditions, TU addition reduced the content under mildly stressed plants of BARI Chola-7 and mild and moderately stressed plants of BARI Chola-9 (Figure 8). This was due to the prevention of extra Pro biosynthesis under osmotic stress. These results are corroborated to previous studies (Alam *et al*., 2014b; Mwadzingeni *et al*., 2016; Nahar *et al*., 2017; Qayyum *et al*., 2013; Bhuiyan *et al*., 2019; Dien *et al*., 2019). However, further Pro content was found to be increased in moderate and severe stressed plants of BARI Chola-7 (Figure 8). This enhanced Pro content does not disturb normal functioning, but it's the accumulation in the cell cytoplasm increases water availability under drought stress and also reserve carbon and nitrogen for using in drought condition (Ashraf and

Foolad 2007; Singh *et al.*, 2015). In addition, it acts as a sign of stress induction and thus suggested to be played an adaptive role in plant stress tolerance (Farooq *et al.*, 2017b; Sharma *et al.*, 2019). Moreover, creating membrane integrity, osmotic balance and protein stability TU combination enhanced stress tolerance in a wide variety of plants like wheat, maize, mung bean under different abiotic stresses including drought (Kaya *et al*., 2015; Parveen *et al.*, 2016; Suryavanshi and Buttar, 2018; Waqas *et al.*, 2019).

4.3.2 MDA content

Plants exposed to different levels of drought stress resulted in a marked increment in the MDA content in both varieties of chickpea (Figure 9). In the case of BARI Chola-7 the values of MDA contents were 42, 55, and 94% higher in mild, moderate and severe (D_3) stressed plants, respectively, compared to control. Besides, MDA content was increased by 90, 125, and 166% upon exposure to mild, moderate and severe stressed plants compared to the control of BARI Chola-9. However, reduced MDA contents were recorded in stressed seedling combined with the exogenous TU application in BARI Chola-7 in TU treated mildly (10%) stressed plants compared to mildly drought-stressed plants alone. However, in the case of BARI Chola-9 the reduction of MDA contents were notable in TU treated drought-stressed plants under D_1 (21%) and D_2 (26%) compared to non-treated stressed plants. TU application did not show any significant reduction of MDA contents in plants exposed to severe drought stress compared to non-treated stressed plants of both BARI Chola-7 and BARI Chola-9 (Figure 9).

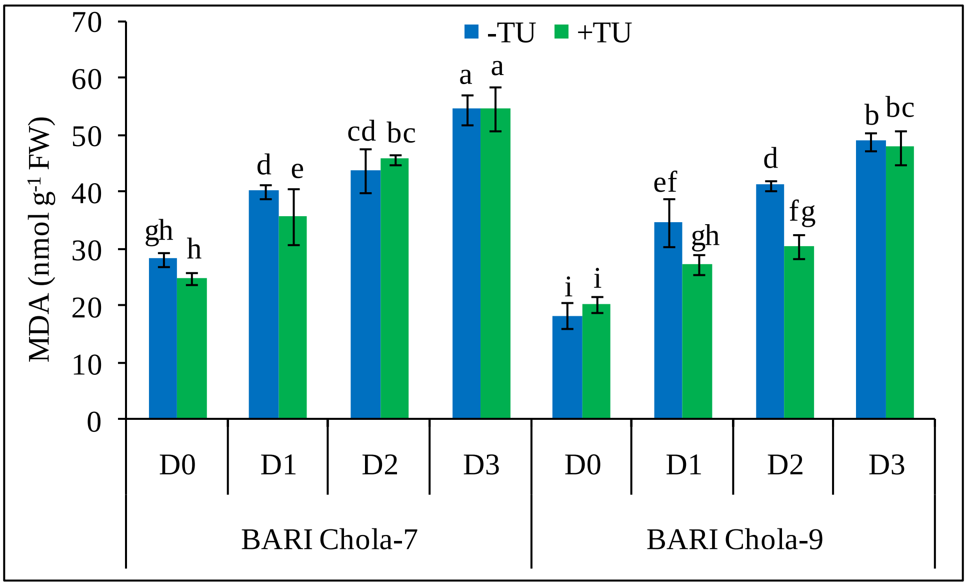

Figure 9. MDA content of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

4.3.3 H_2O_2 content

Plants exposed to different levels of drought stress resulted in a marked increment in the H_2O_2 content in both varieties of chickpea (Figure 10). In BARI Chola-7 the values of H_2O_2 content were 30, 64, and 78% higher in mild, moderate and severe stressed plants, respectively, compared to control. Likewise, H_2O_2 content was increased by 35, 49, and 81% upon exposure to mild, moderate and severe stressed plants compared to the control of BARI Chola-9. The increase of drought-induced H_2O_2 content was higher in BARI Chola-7 compared to BARI Chola-9. On the contrary, TU exogenous foliar spray in BARI Chola-7 declined H_2O_2 content under TU treated mildly (13%) stressed plants compared to non-treated drought-stressed plants alone. However, in the case of BARI Chola-9 the reduction of H_2O_2 content were observed in TU treated drought-stressed plants under D_1 (22%) over non-treated D_1 stressed plants alone. TU application did not show significant reduction of H_2O_2 content in moderate and severe drought stress compared to untreated drought-stressed plants of both BARI Chola-7 and BARI Chola-9 (Figure 10).

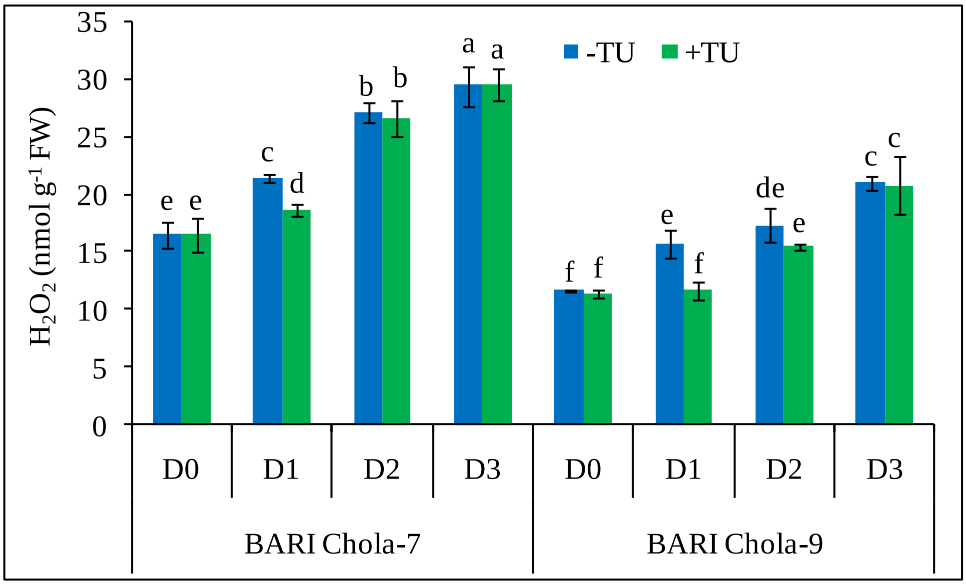

Figure 10. H_2O_2 content of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

Through the reduction of photosynthesis and enhancement of photorespiration drought stress is responsible for excessive production of ROS in cellular level which resulted in ruthless damage to biomolecules such as lipid, protein and nucleic acid leading to increased leakage, fast desiccation and cellular death (Hasanuzzaman and Fujita *et al*., 2011; Zhang *et al*., 2014; Zhang *et al*., 2019). Furthermore, under deficit water condition, cell components disturb membrane fluidity which resultd in the reduced structural integrity of cell components (Hasanuzzaman *et al.,* 2018c). Due to scavenging of superoxide radical and its higher concentration, H_2O_2 a toxic compound produced which is injurious to plant and cause membrane damage at higher concentration (Sharma *et al.*, 2012). Oxidative stress under drought is clearly indicated by the higher concentration of MDA and H_2O_2 which also occurred in present study where both the chickpea varieties exhibited remarkable increment of MDA and H_2O_2 value under different levels of drought stress (Figure 9 and 10). Similar results were observed in different crops under drought stress such as, wheat (Kosar *et al.* 2015), canola (Akram *et al.*, 2018), mung bean (Nahar *et al.*, 2017), mustard (Hasanuzzaman *et al.*, 2018), maize (Parveen *et al.*, 2019), rice (Sohag *et al.*, 2020). The increment was more pronounced in BARI Chola-7 compared to BARI

Chola-9 under mild, moderate and severe drought stress. According to Khan *et al.* (2019), drought-sensitive varieties resulted in higher reduction compared to tolerant varieties. Macar and Ekmekci (2009) observed higher MDA content in stressed plants corresponding to control. However, as a signal molecule, H_2O_2 implicated in signal transduction mechanisms for a number of processes in plants such as stomatal conductance, shoot growth and root growth (Neill *et al.*, 2002; Laloi *et al.*, 2004). Thus, levels of H_2O_2 are competently controlled to keep a balance between production and breakdown. In the present experiment, H_2O_2 accumulation increased during drought stress in tolerant and susceptible cultivars (Figure 10). There was a lower H_2O_2 level in tolerant cultivars, indicating greater antioxidant activity and also higher Pro and total phenol contents. Additionally, among four chickpea genotypes, Patel *et al.* (2012) demonstrated that the content of MDA and H_2O_2 were higher in drought-sensitive genotypes (JG 315 and DCP 92-3) compared to tolerant genotypes (Tyson and ICC 4958). Similarly, accumulation of higher H_2O_2 content was found in leaf tissue of sensitive genotype as compared to tolerant ones of maize (Kellos *et al.*, 2008) and chickpea (Oberoi *et al.*, 2014) under drought stress. The beneficial roles of TU application on chickpea plants were observed in terms of reduced oxidative stress as proved by decreased levels of MDA and H_2O_2 under mild and moderate drought stress (Figure 9 and 10). Therefore, it is speculated that TU might contribute to alleviate the drought-induced oxidative damage through the activation of ROS detoxification and improving antioxidant defense (Hassanein *et al*., 2015) that ultimately helps in membrane stability through stimulating the total antioxidant activity. Similar positive effect of TU application in reducing oxidative damage under drought stress were also proved in other studies with different plant species where TU reduced the MDA and H_2O_2 level for instance, chickpea (Mafakheri *et al.*, 2010; Vineeth *et al.*, 2016), wheat (Hassanein *et al.*, 2015), pearl-millet (Yadav *et al.*, 2020b) etc.

4.3.4 Ascorbate content

The AsA content of both chickpea varieties was markedly declined with the exposure of plants under different levels of drought stress (Figure 11). AsA content is reduced by 22, 50, and 64% in plants exposed to mild, moderate, and severe stressed plants compared to control of BARI Chola-7. Besides, plants exposed to mild, moderate, and severe drought stress reduced AsA content by 17, 28, and 51%, respectively, compared to control plants of BARI Chola-9. ASA reduction was higher in BARI Chola-7 under mild, moderate and severe stress compared to BARI Chola-9. The addition of the exogenous TU with drought stress resulted in significantly enhanced AsA content by 10% and 28% in D_1 and D_2 of BARI Chola-7 compared to non-treated drought-stressed plants. Furthermore, in BARI Chola-9 notable effect of TU in enhancing AsA content was observed only in mildly stressed plants which were 8% over untreated mild-stressed plants. No significant improvement of AsA content was found in plants exposed to D_3 of BARI Chola-7 and D_2 and D_3 of BARI Chola-9 (Figure 11).

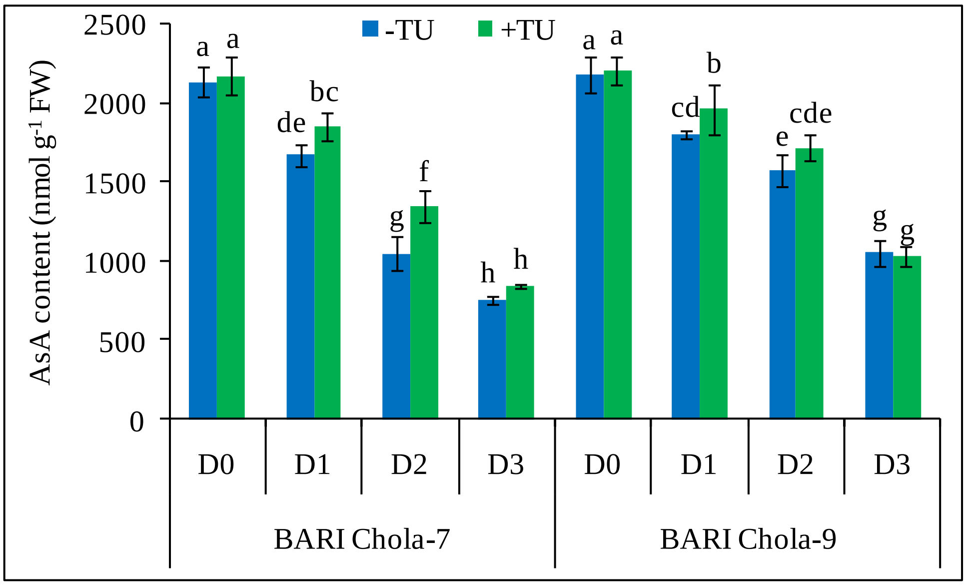

Figure 11. AsA content of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

4.3.5 Dehydroascorbate content

Upon exposure of plants to different levels of drought stress the DHA content was markedly increased in BARI Chola-7 by 47% (mild stress), 82% (moderate stress), and 106% (severe stress) compared to well-watered plants (Figure 12). The combination of the exogenous TU with drought stress resulted in a reduction of DHA content in TU treated control (16.5%), D_1 (21.8%), D_2 (15%), and D_3 (23.8%) in BARI Chola-7 compared to non-treated control and drought-stressed plants alone. However, in BARI Chola-9 plants exposed to mild, moderate and severe drought stress enhanced DHA content by 12, 41 and 82%, respectively, compared to control. However, the addition of TU to plants under drought stress reduced the DHA content in TU treated severely drought-stressed plants by 11% compared to severe stressed plants alone. In contrast, no significant reduction of DHA content was observed in the combination of TU with control and mildly stressed plants of BARI Chola-9 (Fig 12).

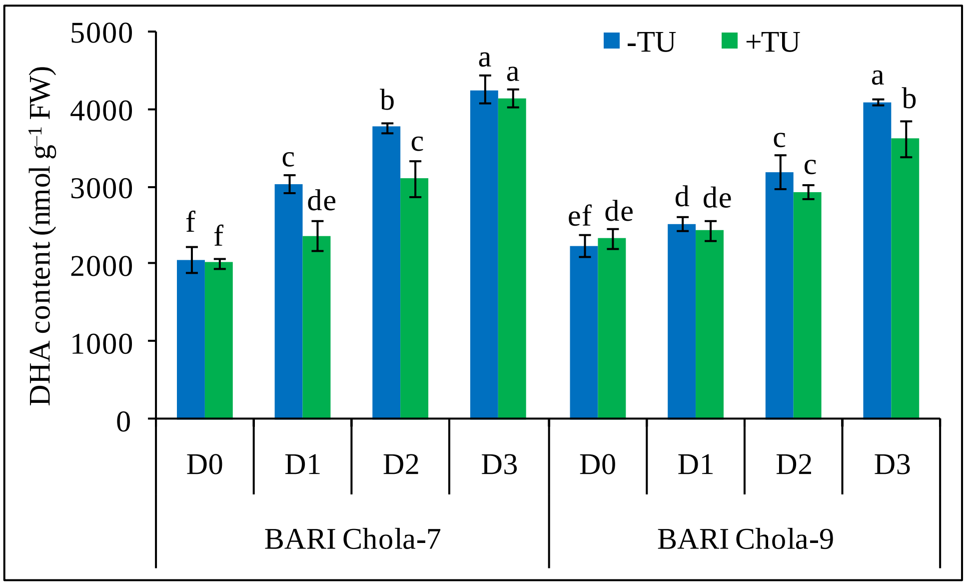

Figure 12. DHA content of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

4.3.6 AsA/DHA ratio

The AsA/DHA ratio of both chickpea varieties was markedly declined with the exposure of plants under different levels of drought stress (Figure 13). AsA/DHA ratio is reduced by 52, 73, and 82% in plants exposed to mild, moderate, and severe stressed plants compared to control of BARI Chola-7. Besides, plants exposed to D_1, D_2, and D_3 levels of drought stress reduced AsA/DHA ratio by 26, 49, and 73%, respectively, compared to AsA/DHA ratio of control of BARI Chola-9. However, the addition of exogenous TU with drought stress resulted in significantly enhanced AsA/DHA ratio in TU treated control (23%), mild (41%), moderate (57%), and severe (13.7%) stressed plants compared to untreated control and drought-stressed plants only. On the contrary, in the case of BARI Chola-9 no significant enhancement happened of AsA/DHA ratio in plants under different levels of drought stress with the application of TU (Figure 13).

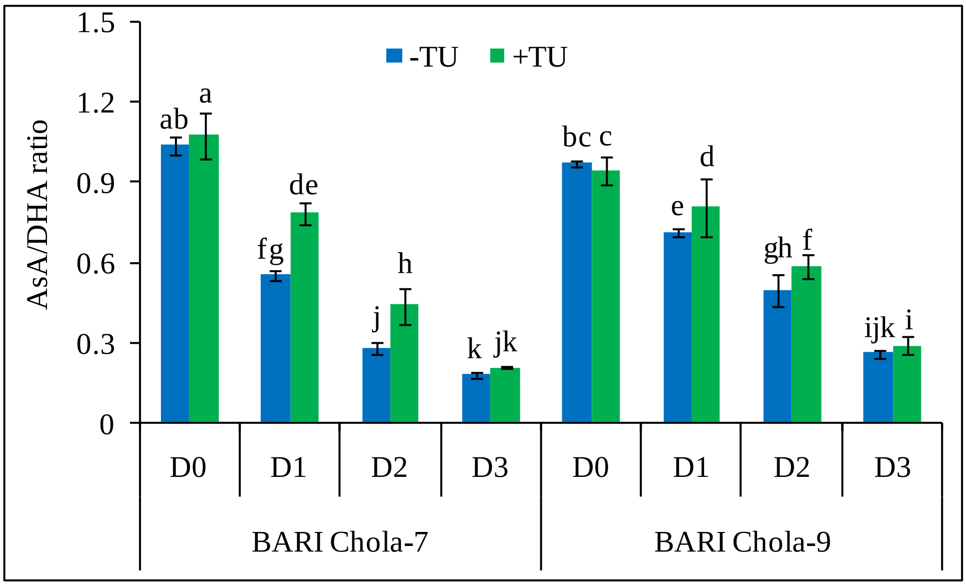

Figure 13. AsA/DHA ratio of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

Decrease of AsA content was recorded in plants subjected to drought stress, where the highest reduction was observed under severe stressed plants of drought-sensitive variety BARI Chola-7 (Figure 11). According to Alam *et al.* (2014a), AsA content decreased significantly in *B. campestris* under drought stress (15% PEG) condition. AsA is a water-soluble non-enzymatic antioxidant and acts as a ROS-scavanging molecule which is the main reason behind the notable reduction of its content under stress condition (Gill and Tuteja, 2010; Nahar *et al.*, 2017). In soybean, AsA production declined both in leaves and roots of drought-stressed plants over well-watered control plants due to the reduction of water potentiality and RWC in both the organs (Seminario *et al.*, 2017). In contrast, Hasanuzzaman *et al.* (2018c) studied that higher AsA content was observed under moderately stressed plants of *B. napus* (10% PEG) over control as plants have natural defense mechanism to survive under drought-stressed condition. He also found lower AsA content under severe stressed (20% PEG) plants compared to control. In chickpea, AsA content decreased by 38.7 and 55.1% significantly with the increase of Hg concentration 15μm and 30 μm, respectively, compared to control (Ahmad *et al.*, 2019). With the decrease of AsA content DHA content increased in the highest amount in severe stressed plants of both varieties compared to control (Figure 12).

When AsA is engaged in ROS scavenging, it is oxidized to dehydroascorbate (DHA), which results the decrease of AsA content and an increase of DHA content simultaneously to ameliorate the negative effect of stress (Dolatabadion *et al.* 2009). Drought stress (15% PEG) reduced DHA content in *B. napus* compared to control plants (Alam *et al.*, 2014b). Conducting an experiment with mung bean, Nahar *et al.*, (2017) stated that DHA content increased with the reduction of AsA under high temperature (40ºC) and drought stress (5% PEG) or in combined stress condition. AsA/DHA ratio decreased with the increase of drought stress severity in both varieties of chickpea (Figure 11). AsA is altered to DHA to mitigate ROS over-generation which increased the DHA content and decreased the AsA/DHA ratio (Gill and Tuteja, 2010; Nahar *et al.*, 2017). According to Alam *et al.* (2014a), notable decline of AsA content and AsA/DHA ratio was observed in *B. campestris* under 15% PEG compared to control. Likewise, drought stress (5% PEG) reduced AsA and AsA/DHA ratio in mung bean compared to control to ameliorate the effect of oxidative damage (Nahar *et al.*, 2017). After TU application, the drought-stressed chickpea plants showed the increment of AsA content compared to untreated drought-stressed plants (Figure 11). Several researches revealed that TU application improves the antioxidant defense mechanisms of cell which improved oxidative stress and drought stress tolerance through reducing ROS over-generation (Waqas *et al.*, 2019). Thus, foliar application of TU increased AsA content and the AsA/DHA ratio at mild and moderately stressed plants compared to untreated control (Figure 12 and 13). To ameliorate the damaging effect of arsenic toxicity AsA content restorted from DHA reducing DHA content and increasing AsA/DHA ratio through the foliar application of 6.5 mM and 13 mM TU under arsenic stress (30 µm, sodium arsenate) in two lentil genotypes (Talukdar, 2016).

4.3.7 Reduced glutathione content

Plants exposed to different levels of drought stress resulted in a marked increment in GSH content in both varieties of chickpea. In the case of BARI Chola-7 the values of GSH content were 26, 50, and 90% higher in mild, moderate and severe stressed plants, respectively, compared to GSH values of control. Similarly, GSH content were increased by 49, 67 and 90% upon exposure to D_1, D_2, and D_3 plants over the control of BARI Chola-9. The exogenous application of TU in BARI Chola-7 resulted in further increment of GSH content in TU treated mild (39%), moderate (31%) and severe (14%) drought-stressed plants compared to non-treated drought-stressed plants alone. Same trend of increases was observed in the case of TU treated control, mild, moderate and severe drought-stressed plants which were by 22, 21 and 7% compared to non-treated drought-stressed plants (Figure 14) of BARI Chola-9.

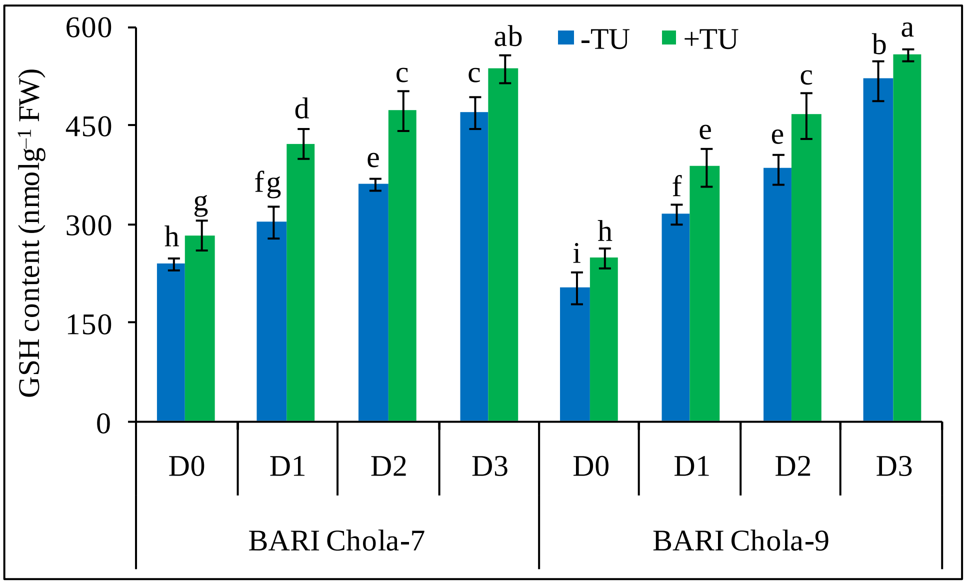

Figure 14. GSH content of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

4.3.8 Glutathione disulfide content

The GSSG content of both chickpea varieties was significantly increased with the exposure of plants under different levels of drought stress (Figure 15). The GSSG content was increased by 1.4 and 3.2 fold in D_1 and D_2 stressed plants while the greatest increase (5 fold) seen in D_3 stressed plants compared to control of BARI Chola-7. Besides, in the case of BARI Chola-9 the increment of GSSG content was found by 1.8, 3, and 3.8 fold in plants exposed to D_1, D_2, and D_3 levels of drought stress compared to GSSG content of the control. In contrast, the addition of exogenous TU under drought stress resulted in significant reduction of GSSG content by 17% in TU treated moderate and severe stressed plants of BARI Chola-7 compared to drought-stressed plants only. However, in BARI Chola-9 notable effect of TU in declining GSSG content was observed in moderate (24%) and severe (33%) stressed plants over non-treated drought-stressed plants. No significant reduction of GSSG content was found in plant exposed to mild drought stress of both BARI Chola-7 and BARI Chola-9 (Figure 15).

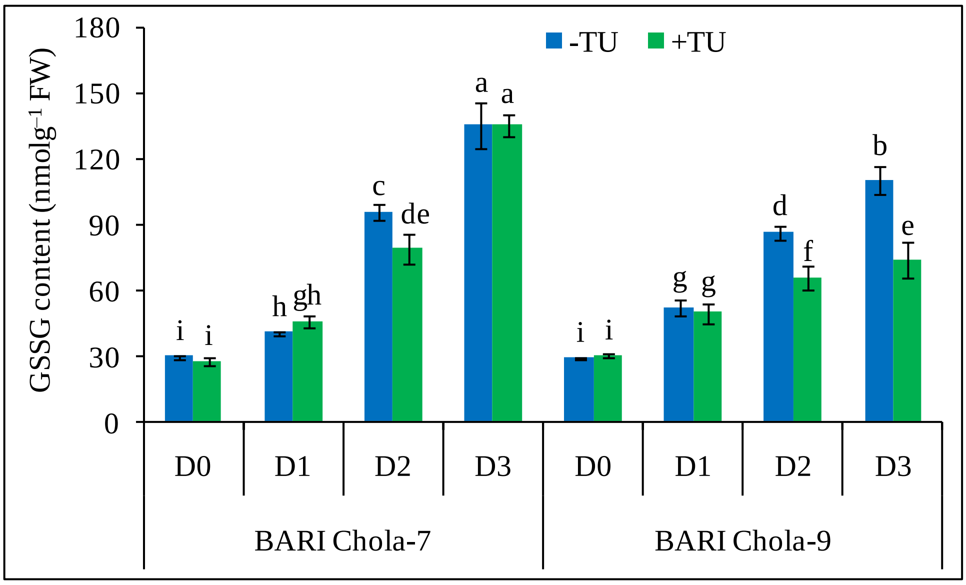

Figure 15. Glutathione disulfide (GSSG) of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

4.3.9 GSH/GSSG ratio

Upon exposure to different levels of drought stress the GSH/GSSG ratio in BARI Chola-7 decreased in D_2 and D_3 levels of drought stress by 53 and 57%, respectively, compared to control. With the application of exogenous TU a marked increment of GSH/GSSG ratio was observed in TU treated control, mild and moderate stressed plants (Fig 16). However, in the case of BARI Chola-9 the GSH/GSSG ratio was declined by 13, 37, and 33% upon exposure to mild, moderate, and severe drought-stressed plants over the control. With the combination of exogenous TU with drought stress resulted in an enhancement of GSH/GSSG ratio in TU treated drought stressed plants compared to non-treated stressed plants in BARI Chola-9 (Figure 16).

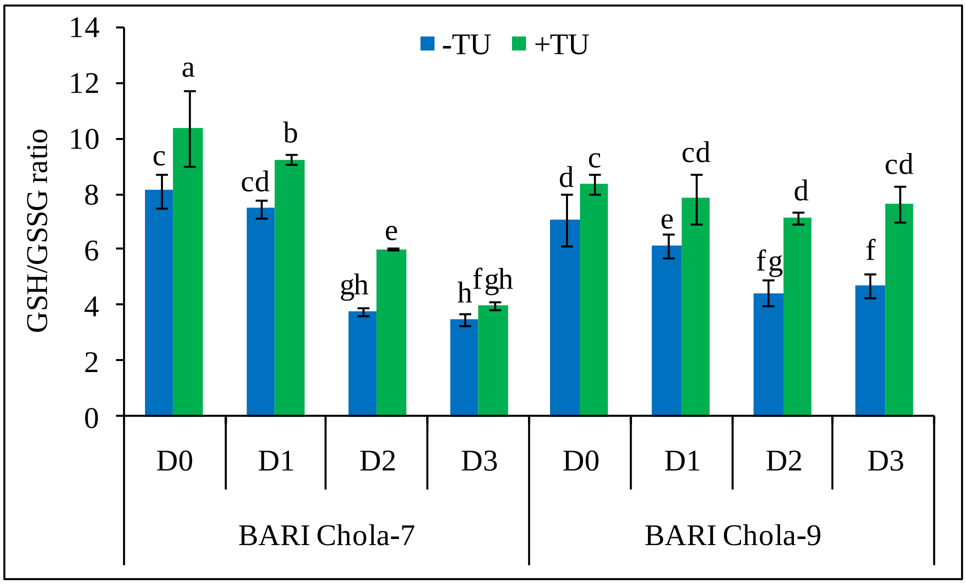

Figure 16. GSH/GSSG ratio of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

GSH is another non-enzymatic antioxidant which is able to detoxify ROS like H_2O_2 and minimize lipid peroxidation in the cell cytosol and chloroplasts. In this study GSH content increased under all stress levels remarkably, which is supported by the previous results (Hasanuzzaman and Fujita, 2011; Sharma *et al*., 2012; Alam *et al*., 2013, Alam *et al.*, 2014a). The GSH content was further increased by the application of TU in mild, moderate and severe stressed plants of both chickpea varieties as it has mitigated ROS over-accumulation and oxidative damage. After accumulation of excess ROS, GSH is converted to GSSG through oxidation (Hasanuzzaman *et al.*, 2019). In the present findings, the GSSG content increased significantly under mild, moderate and severe stressed plants of BARI Chola-7 and BARI Chola-9 over control (Fig 16), which indicates an adverse condition of the cell. Furthermore, TU foliar application decreased GSSG content in plants of BARI Chola-9 which were exposed to moderate and severely drought stress. Responses of TU was more prominent in BARI Chola-9 compared to BARI Chola-7 although after TU supplementation the GSSG content was declined in moderately stressed plants of BARI Chola-7. The ratio of GSH/GSSG expresses the redox status of the cell. In addition with that the GSH/GSSG ratio is more vital compared to the changes in individual GSH and GSSG content for tolerance to oxidative stress and stress-induced signaling process. With increased GSSG content GSH/GSSG ratio decreased which increased excess ROS generation and oxidative damage under drought-stressed chickpea plants. The same modulation of GSH, GSSG, and GSH/GSSG ratio was found in drought stressed plants of mung bean seedlings (Nahar *et al.*, 2017). However, the function of these non-enzymatic antioxidant compounds could not be overlooked in mitigating oxidative damage by scavenging more excess ROS under drought stress.

4.4 Yield parameters

Drought stress leads to a complete loss of cell integrity when levels of ROS production, antioxidant loss and lipid peroxidation reached to a certain threshold level, and finally, it causes plant death (Fathi and Tari, 2016). In this study, under severe drought stress BARI Chola-7 resulted in the highest MDA, H_2O_2 and Pro content with lowest leaf RWC and chl synthesis which caused the over-generation of ROS in plant cell. This over-accumulation of ROS leads to damage of important cellular ingredients including carbohydrates, proteins, lipids, DNA etc. Deterioration of these properties ultimately hampers the metabolic and physiological functions of cells and consequences plant death under severe drought stress. That's why in response to severe drought stress at 25% FC plant death occurred, after completing the vegetative satge in this study.

Plant can tolerate drought stress at vegetative stage and this results the earliness in reproductive maturity which may bring yield penalty (Maqbool *et al.*, 2017). In line with the statement Shah *et al.* (2020) stated thet better performance of chickpea genotypes at early growth stages is not guaranteeing the higher yield or drought tolerance at terminal growth stages. In this experiment, BARI Chola-7 has survived under severe drought stress at early growth period but all the studied plants of both TU treated and untreated plants have died within 95 DAS. For this reason, no yield-related data can be obtained from the treatments. However, BARI Chola-9 showed more tolerance to severe-drought stress compared to BARI Chola-7 though yield was very low compared to other treatments.

4.4.1 Weight of 100-seed

Weight of 100-seed two varieties of chickpea decreased significantly with the increase of drought stress intensity (Figure 17). Highest amount of weight of 100-seed was found in both TU untreated control and TU treated control plants of BARI Chola-9. The weight of 100-seed of BARI Chola-7 was lower than BARI Chola-9 in all the treatments. The reduction was 26 and 42% in mild and moderately stressed BARI Chola-7 and 18, 33 and 67% in BARI Chola-9 under mild, moderate and severe stressed plants, respectively, compared to control. However, application of TU showed increased weight of 100-seed in D_1 and D_2 treatments by 15 and 17%, respectively in BARI Chola-7 and at D_1 (10%) in BARI Chola-9 in comparison to drought-stressed plants only (Figure 17). The reduction of weight of 100-seed revealed that drought stress accelerated maturity and resulted in the development of small seeds. In dry beans (*Phaseolus vulgaris* L. cv. DBS 360) 19% reduction of weight of 100-seed was found in plants upon exposure to drought at 35 days after planting compared to well-watered control (Mathobo *et al.*, 2017). Another reason behind the reduction of 100-seed weight is the disruption of the supply of assimilates to the pods. Mekonnen (2020) stated that the weight of 100-seed of chickpea also reduced by 50% compared to control when plant subjected to drought stress at mid vegetative stage.

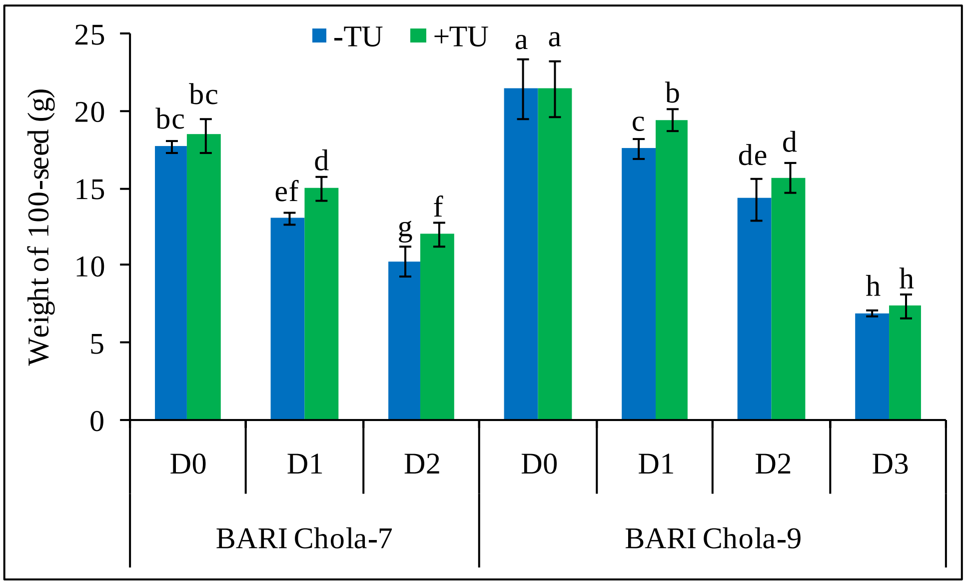

Figure 17. Weight of 100-seed of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

4.4.2 Seed yield plant^{-1}

Drought stress caused significant loss of seed yield compared to control of both chickpea varieties (Figure 18). Highest seed yield plant^{-1} was observed in TU supplemented control plants (9.53 g) of BARI Chola-9. The seed yield plant^{-1} declined by 8 and 21% under mild and moderate drought stress compared to control in BARI Chola-7. Besides in BARI Chola-9 the reduction was 16% (mild), 26% (moderate), and 67% (severe stress) over control. On the contrary, the foliar application of TU effectively increased seed yield plant^{-1} by reducing the negative effects of drought stress. Exogenous application of TU effectively increased the seed yield plant^{-1} by 8% under moderately stressed plants of BARI Chola-7 and mild (6%), moderate (8%), and severe stressed (13%) plants of BARI Chola-9 (Figure 18). In line with this, drought stress at vegetative and reproductive stages declined seed yield plant^{-1} significantly in chickpea due to the restriction of photosynthesis under drought stress (Mekonnen, 2020).

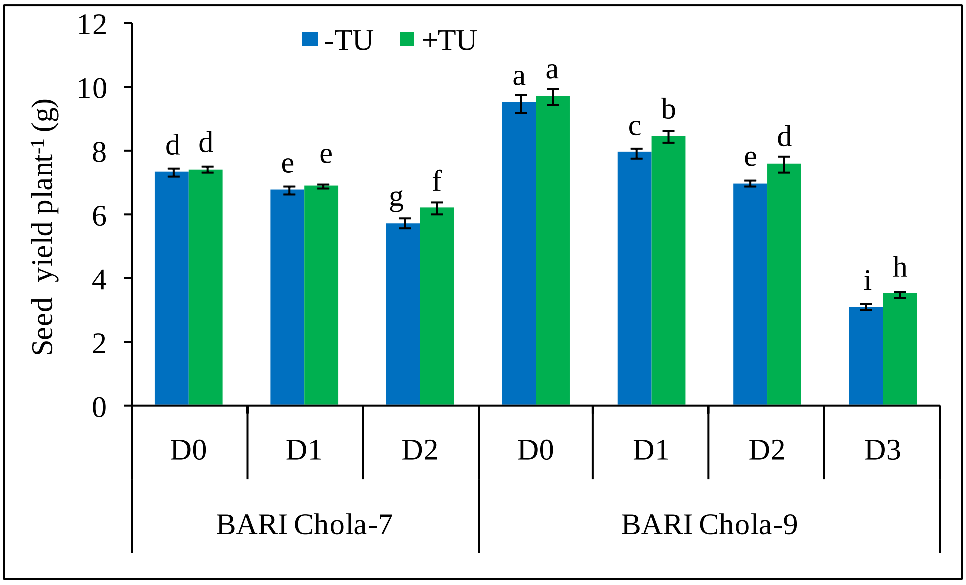

Figure 18. Seed yield plant^{-1} of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

4.4.3 Stover yield plant^{-1}

When plant exposed to drought stress stover yield plant^{-1} of BARI Chola-7 decreased under mild (5%) and moderate (14%) drought stress compared to control. Similarly, a noticeable decline of stover yield was observed by 24, 19 and 55% in mild, moderate, and severe stressed plants of BARI Chola-9, respectively. No further improvement of stover yield plant^{-1} was noticed in the case of TU treated drought-stressed plants of the varieties (Figure 19). Drought-induced oxidative damage impaired photosynthesis and disturbed in carbohydrate metabolism which resulted in the reduction of plant growth as well as yield parameters (Mansourifar *et al.*, 2011). In line with this, Alou *et al.* (2018) reported that like other yield parameters, stover yield plant^{-1} declined significantly under drought stress in rice (cv. Nerica 4®).

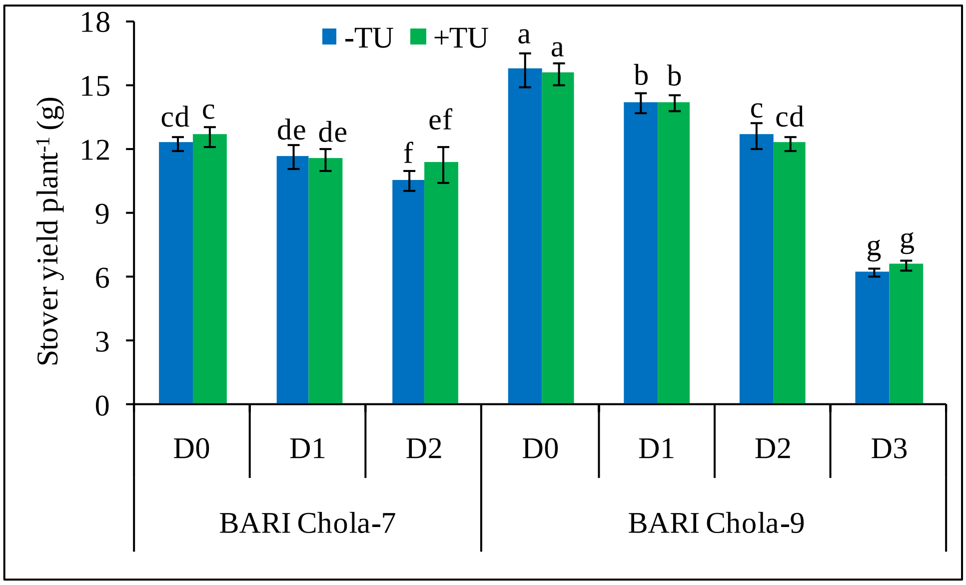

Figure 19. Stover yield plant^{-1} of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

4.4.4 Biological yield plant^{-1}

Biological yield plant^{-1} reduced significantly upon exposure to different levels of drought stress (Figure 20). The lowest biological yield plant^{-1} was recorded in severe stressed plants of BARI Chola-7 whereas the highest biological yield plant^{-1} was found in TU supplemented control plants of BARI Chola-9. In BARI Chola-7 drought stress resulted in noticeable reduction of biological yield plant^{-1} by 6% (mild stress) and 16.8% (moderate stress) compared to control. In the case of BARI Chola-9 the biological yield plant^{-1} declined by 12.4 and 22.3% under mild and moderately drought stress, respectively, while severe stress resulted in the highest reduction (142.69%). Foliar application of TU increased biological yield plant^{-1} at moderately stressed plants of BARI Chola-7 by 8% compared to control. But TU did not show any significant effect in increasing biological yield plant^{-1} in BARI Chola-9 under stressed plants (Figure 20). In the consequence of significant reduction of seed yield plant^{-1} and stover yield plant^{-1} under drought stress biological yield plant^{-1} declined notably which was supported by Shaban *et al.* (2012).

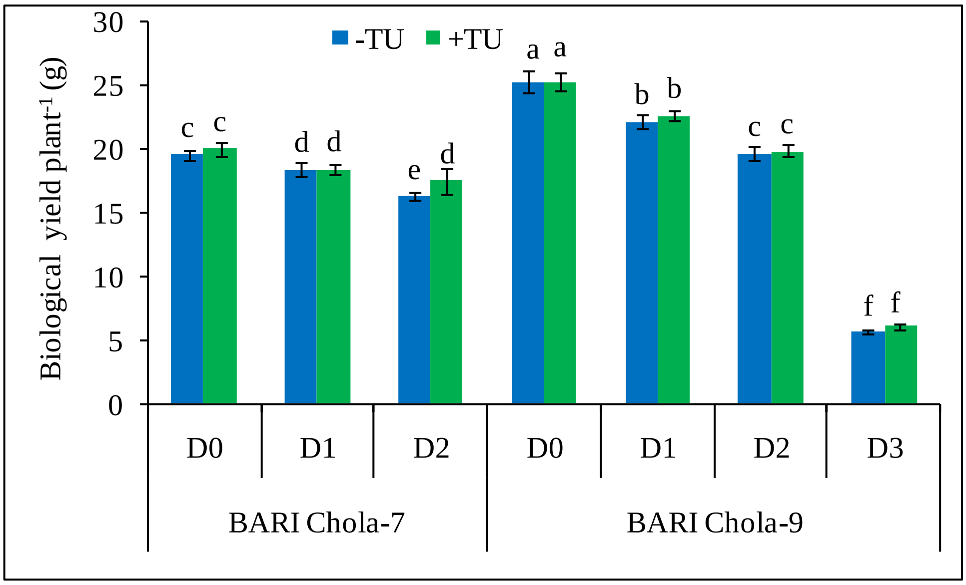

Figure 20. Biological yield plant^{-1} of two varieties of chickpea is affected by thiourea (TU) under different levels of drought stress. Data presented is mean (±SD) of three replicates for each treatment and bars with different letters denote significant difference among the treatments at $P \leq 0.05$ applying LSD test. Here, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. –TU= No TU and +TU= 5 mM TU foliar spray

The highest weight of 100-seed and seed yield plant^{-1} were observed in control (no moisture stress) and TU treated control plant of BARI Chola-9 compared to untreated drought-stressed plants (Figure 17 and 18). Moreover, BARI Chola-7 showed lower seed yield plant^{-1} and weight of 100-seed compared to BARI Chola-9. All the yield and yield components were remarkably higher in BARI Chola-9 under normal and different levels of stressed condition compared to BARI Chola-7. Seed yield plant^{-1}, weight of 100-seed, biological yield plant^{-1} and stover yield plant^{-1} decreased significantly with the increase of the drought stress intensity over control. Supporting this, Mansourifar *et al.* (2011) stated that seed yield plant^{-1}, weight of 100-seed, biomass yield declined noticeably under moderate drought stress compared to well-watered control. Another reason behind the reduced seed yield plant^{-1} was the reduced number of filled pods under stressed condition in chickpea. Early maturity under water stress condition increased leaf senescence, flower drop and pod abortion which resulted in decreased seed yield plant^{-1}in chickpea (Shaban *et al.*, 2012). Ulemale *et al.* (2013) demonstrated that chickpea reduction of photosynthate mobilization to the developing seed resulted in a significant decline in weight of 100-seed. In the present study, drought stress significantly reduced chl content and biomass production and resulted in the reduction of seed yield plant^{-1} and weight of 100-seed in stressed plants of both varieties. The present findings are supported by Ouji *et al.* (2016), who studied that up to 19% of weight of 100-seed reduced under water stress conditions over control plants of chickpea. In another experiment Abhishek *et al*. (2020) revealed that drought stress under flowering and seed setting stages reduced seed yield plant^{-1} and weight of 100-seed as well in chickpea. In line with this, conducting an experiment with two varieties of chickpea (Habru and Mastewal) Mekonnen *et al.* (2020) demonstrated that weight of 100-seed reduced notably under vegetative and seed filling drought stress by 17% and 40%, respectively, compared to control. In this study, notable increment of seed yield plant^{-1} and weight of 100-seed was observed under TU treated moderately drought-stressed plants of BARI Chola-7 compared to other TU treated and non-treated stressed plants. BARI Chola-9 showed more prominent responses to TU foliar application compared to BARI Chola-7 through increasing seed yield plant^{-1} and weight of 100-seed in mitigating the negative effect under all levels of drought stress (Figure 17 and 18). Drought stress-induced reduction in growth, dry matter production, photosynthesis and pod formation resulted in lessening seed yield and seed weight. However, the foliar application of TU improved the seed yield plant^{-1} and weight of 100-seed by modulating the negative effects of drought stress in this study. Supporting this, Bhadru *et al.* (2017) demonstrated that TU (6.5 mM) foliar spray in green gram increased seed yield through improving photosynthetic activities and source-sink relationships. In line with this, Abhishek *et al.* (2020) reported that in chickpea TU foliar application at 13 mM increased seed yield pant^{-1} and weight of 100-seed by 6% and 13%, respectively, over control and TU showed remarkable increment of yield and different yield component of chickpea under drought stress for instance, no. of pod plant^{-1}, weight of 100-seed, seed yield plant^{-1} and seed yield ha^{-1} as well.

Chapter V

SUMMARY AND CONCLUSION

The present study was conducted at the experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, to investigate the relative effectiveness of TU in alleviating the detrimental effects caused by three levels of drought stress on two chickpea varieties BARI Chola-7 and BARI Chola-9. The experiment was arranged in a completely randomized design (CRD) with three replications. Seedlings were grown in a controlled environment where drought stress was imposed by withhelding water at 75 (D_1, mild stress), 50 (D_2, moderate stress) and 25% FC (D_3, severe stress), which was measured with a moisture meter and was maintained regularly. The data were taken by sampling the leaves of 15 days stressed plants after 45 days of the normal growing period. There were about 12 seedlings maintained in each pot. The stress-alleviating chemical TU was applied as an exogenous foliar application at 7 days interval till pod filling stage under control or drought-stress conditions. Different data on morphology (plant height, root length plant^{-1}, root FW plant^{-1}, root DW plant^{-1}, shoot FW plant^{-1}, shoot DW plant^{-1} and number of branches plant^{-1}), physiology (leaf RWC, leaf chl content) and biochemical (Pro content, MDA content,, H_2O_2 content,, AsA content,, DHA content, AsA/DHA ratio, GSH content, GSSG content, GSH/GSSG ratio). At harvest, weight of 100-seed, seed yield plant^{-1}, stover yield plant^{-1}, and biological yield plant^{-1} were measured to compare the responses of the two contrasting varieties.

In the present study, plant height was reduced under mild, moderate and severe stressed plants of two of the varieties but the highest reduction was found in severe stressed BARI Chola-7 (40-47%) at 45, 60, 75 and 90 DAS. In contrast, TU mitigated the negative effect of drought stress by increasing plant height under mild (7, 13, 9%) and moderate (7, 10 and 9%) drought stress of BARI Chola-7 at 60, 75 and 90 DAS. Plant height of BARI Chola-9 increased by 6, 7 and 8% under TU sprayed mild stressed plants and 8, 12 and 13% under TU treated moderately stressed plants at 60, 75 and 90 DAS compared to plants exposed to untreated drought-stressed plants alone. Upon exposure to drought stress, above-ground FW and DW declined under severe stressed plants of two of the varieties, while the highest reduction was found in BARI Chola-7 compared to control and BARI Chola-9. On the contrary, TU application showed more remarkable improvement of above-ground FW and DW in both varieties over drought-stressed plants only. BARI Chola-9 showed a prominent response to TU in improving above-ground FW and DW.

Highest root length was observed in mild and moderately drought-stressed plants but under severe drought stress root length decreased notably. Further improvement of root length was found after TU supplementation in D_1 and D_2 treatments of BARI Chola-9. In BARI Chola-7 10, 38, and 50% lower root FW and in BARI Chola-9 19, 25, and 31% lower root FW were recorded in plants under mild, moderate and severe drought stress level, respectively, compared to control plants. On the other hand, the foliar application of TU remarkably increased root FW under moderate drought stress (13.2%) in BARI Chola-7 whereas in BARI Chola-9 the increment of root FW was found under mild (26%) and moderate (21%) drought stress compared to untreated stressed plants. Similar result was found in the case of root DW. Number of branches plant^{-1} was decreased by 26, 40, and 62% at 45 DAS and 33, 43, and 70% at 60 DAS under mild, moderate and severely drought-stressed plants of BARI Chola-7, respectively, compared to control. Similarly, sharp decrease of no. of branches plant^{-1} was observed under mild, moderate and severe level of stress by 10, 19, and 39%, respectively, at 45 DAS and 22, 39, and 45% at 60 DAS, respectively in the case of BARI Chola-9, compared to control. TU foliar spray significantly improved number of branches plant^{-1} of both varieties under well-watered control and drought-stressed plants as well. Number of branches plant^{-1} was increased by 28% and 13% under TU treated mild and moderately stressed plants of BARI Chola-7 and 12.3% under moderately stressed plants of BARI Chola-9 at 45 DAS, compared to plant without TU supplementation. TU foliar application increased number of branches plant^{-1} by 18 and 10% under mild and moderately stressed plants of BARI Chola-7 and by 10 and 18.3% under mild and moderately stressed plants of BARI Chola-9 at 60 DAS, compared to untreated stressed plants only. Considering all the growth parameters it can be stated that BARI Chola-7 showed more negative responses to drought stress compared to BARI Chola-9 and TU has remarkably mitigated the damaging effect of drought stress under mild and moderate stress levels but no notable improvement of growth parameters was found in the case of severe stress treatments of two of the varieties.

Relative water content decreased under mild to severe drought stress in both the studied varieties. The highest reduction was observed under severe drought stress in BARI Chola-7 (48%) and BARI Chola-9 (36%) compared to control. BARI Chola-9 showed higher RWC under stress compared to BARI Chola-7. However, TU supplementation under stress condition improved RWC under mild (22%) and moderately (18%) stressed BARI Chola-7 and mild (13%), moderate (15%) and severe (18%) stressed BARI Chola-9 significantly. Upon exposure of plants under mild, moderate and severe drought stress resulted in a decline of chl content by 23, 34 and 47%, respectively, compared to control in BARI Chola-7. Similarly, chl *a* content of BARI Chola-9 markedly reduced by 20, 32 and 50% under D_1, D_2 and D_3 levels of drought stress over control. On the contrary, the foliar spray of exogenous TU with drought stress showed 12 and 22% higher chl *a* content under TU treated mild and moderately stressed plants of BARI Chola-7 compared to non-treated drought-stressed plants. However, in the case of BARI Chola-9 the promising effect of TU application in enhancing chl *a* content was observed in mild and moderately stressed plants by 11% and 21%, respectively, compared to drought-stressed plants alone. Plant exposed to D_1, D_2 and D_3 levels of drought stress resulted in a reduction of chl *b* content by 29, 51 and 70.2%, respectively, compared to control of BARI Chola-7. Upon exposure to mild, moderate and severe drought stress, chl *b* content reduced by 32, 53 and 70.5%, respectively in BARI Chola-9, compared to control. However, the foliar application of TU resulted in an improvement of chl *b* content in TU treated mild (21%), moderate (8%) and severe (28%) stressed plants of BARI Chola-7 compared to non-treated drought-stressed plants. Higher chl *b* content was found in TU treated control, mild, moderate and severe stressed plants by 5, 28, 20, and 33% over non-treated control and drought-stressed plants alone of BARI Chola-9. In the consequence of significant reduction of chl *a* and chl *b* content under any level of drought stress chl (*a*+*b*) content also reduced in both varieties. However, the exogenous application of TU with drought stress resulted in enhanced chl (*a*+*b*) content by 18, 15 and 11% in mild, moderate and severe stressed plants of BARI Chola-7, compared to drought-stressed plants only. However, in the case of BARI Chola-9 significant effect of TU in improving chl (*a*+*b*) content was observed under mild (19%), moderate (20%) and severe (12%) stressed plants over non-treated drought-stressed plants.

Plants exposed to different levels of drought stress increased the MDA content and H_2O_2 content in both varieties of chickpea. MDA and H_2O_2 content were higher in BARI Chola-7 compared to BARI Chola-9. In the case of BARI Chola-7 the values of MDA contents were increased by 42, 55, and 94% and H_2O_2 contents were increased by 30, 64, and 78% higher in mild, moderate, and severe stressed plants, respectively, compared to control. Moreover, the MDA contents and H_2O_2 content were increased in mild (90 and 35%), moderate (125 and 49%), and severe (166 and 81%) stressed plants, respectively of BARI Chola-9, compared to control. However, mitigating the drought-induced oxidative damage TU foliar application has sharply reduced the MDA content in mild (21%) and moderate (26%) drought stress and H_2O_2 content in mildly (22%) stressed BARI Chola-9 compared to drought-stressed plants alone through improving water balance and osmotic potentiality under mild and moderately stressed plants which proved the ability to possess some adaptive mechanisms against drought. Increment of Pro content was observed under all levels of drought stress in both the studied varieties. It was observed that TU reduced the Pro content in mildly stressed BARI Chola-7 and mild and moderately stressed BARI Chola-9. Drought stress also found to be enhanced DHA, GSH and GSSG content and decreased AsA, AsA/DHA ratio and GSH/GSSG ratio under any levels of drought stress. The foliar application of TU mitigated the harmful effect of drought by balancing these non-enzymatic antioxidant compounds under mild and moderately stressed plants of both chickpea varieties.

BARI Chola-7 could not survive till maturity under severe drought stress at 25% FC and TU supplementation failed to mitigate oxidative damage under severe stress at terminal drought stress. So, there was an absence of two treatments ($V_1D_3TU_0$ and V_1D_3TU) in yield parameters. In the case of yield consideration, drought stress reduced all the yield and yield components (weight of 100-seed, seed yield plant^{-1}, stover yield plant^{-1} and biological yield plant^{-1}) at any level of drought stress. But the highest reduction of weight of 100-seed (67.8%), seed yield plant^{-1} (67.43%), stover yield plant^{-1} (55.2%) and biological yield plant^{-1} (142.69%) were found under severe stressed plants of BARI Chola-9. However, TU improved seed yield plant^{-1} and hundred seed weight notably through improving stress tolerance in both the varieties of chickpea except severe stressed plants of BARI Chola-7.

Considering all the above-mentioned observations it can be concluded that TU-induced improvement of growth and yield under mild and moderate drought stress was linked with increased chl content, RWC, non-enzymatic antioxidant defense system and reduced oxidative damages of the two chickpea varieties. The varieties showed different responses to different levels of drought stress while BARI Chola-7 was comparatively more sensitive to drought stress than BARI Chola-9. In addition, BARI Chola-9 showed more positive responses to TU compared to BARI Chola-7. The beneficial effect of TU as stress-alleviating molecule can not be ignored. Therefore, this study is requiring more progress in physiological and biochemical study to explain TU-mediated drought tolerance and the signaling pathway involved therein. These results in relation to effect of drought-induced oxidative stress on chickpea are in line with several previous research findings. However, the actual roles of exogenous TU in drought stress tolerance remain unknown. Even studies on the roles of TU in mitigating drought stress on chickpea are scarce in number. Moreover, factors involved in TU-induced drought stress tolerance and signaling effect of TU require advanced research.

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APPENDICES

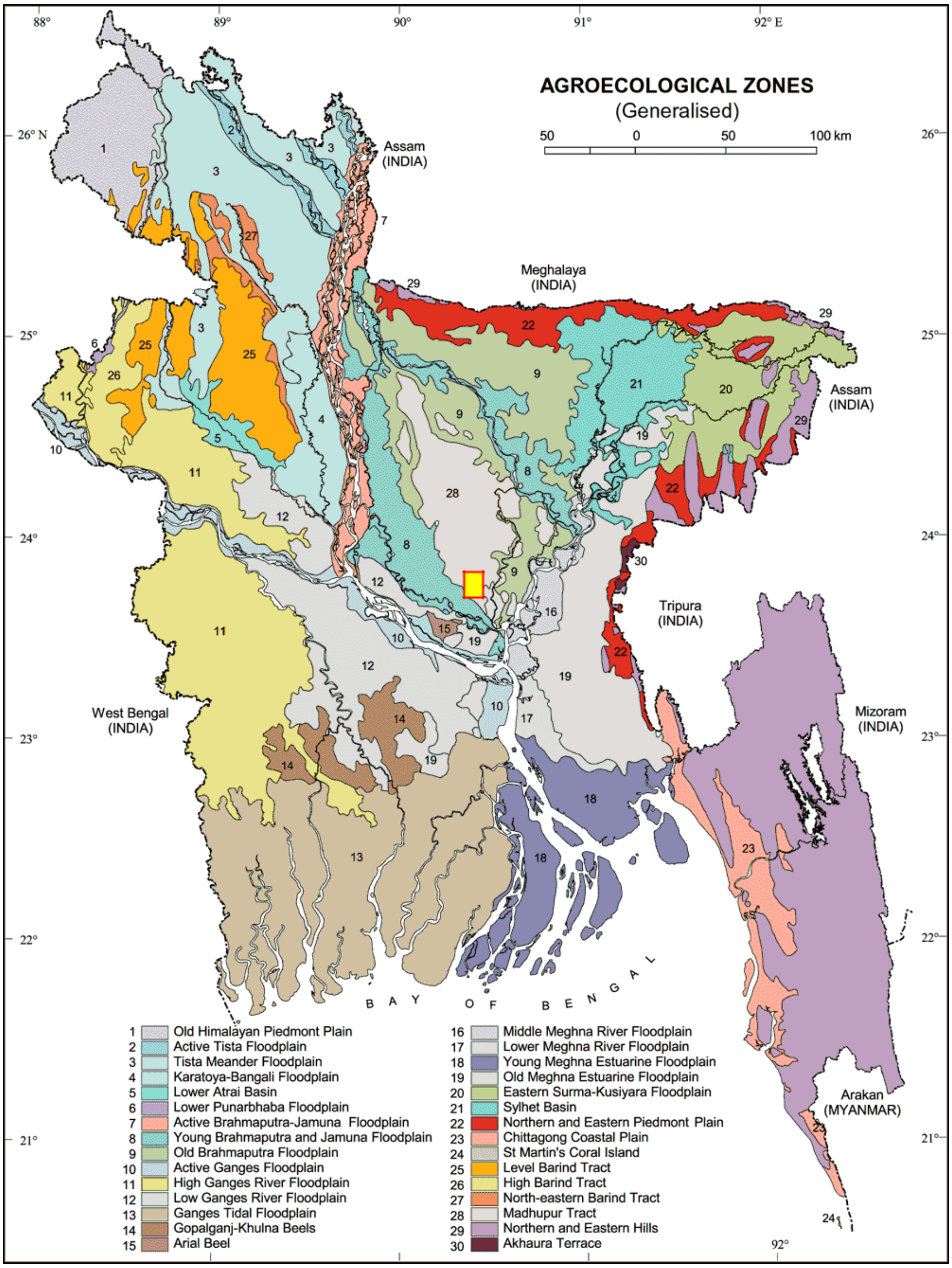
Appendix I. Phenotypic differences in BARI Chola-7 under different treatment combination

Here, V_1= BARI Chola-7, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. TU_0= No thiourea (TU) and TU= 5 mM TU foliar spray

Appendix II. Phenotypic differences in BARI Chola-9 under different treatment combination

Here, V_1= BARI Chola-9, D_0= no drought stress, D_1= mild, D_2= moderate, and D_3= severe drought stress. TU_0= No thiourea (TU) and TU= 5 mM TU foliar spray

Appendix III. Map showing the location of experiment



Appendix IV. Monthly average air temperature, rainfall, and relative humidity of the experiment site during the period from December 2019 to March 2020

Months	Air temperature (°C)		Relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum		
December, 2019	26.4	14.1	50	12.8
January, 2020	25.4	12.7	46	7.7
February, 2020	28.1	15.5	37	28.9
March, 2020	28.1	20.4	38	65.8

Appendix V. Mean square values and degree of freedom (DF) of plant height at 45, 60, 75 and 90 DAS of chickpea as influenced by TU foliar application under different levels of drought stress

Source of varience	DF	Mean square values			
		Plant height			
		45 DAS	60 DAS	75 DAS	90 DAS
Treatments	15	34.683	213.465	341.182	378.159
Error	32	0.660	1.613	3.569	4.034

Appendix VI. Mean square values and degree of freedom (DF) of root length, root FW, root DW, above-ground FW, and above-ground DW of chickpea as influenced by TU foliar application under different levels of drought stress

Source of varience	DF	Mean square values				
		Root length	Root FW	Root DW	Above ground FW	Above ground DW
Treatments	15	5.546	0.023	0.0001	0.534	0.012
Error	32	0.061	0.0001	0.0001	0.003	0.000

Appendix VII. Mean square values and degree of freedom (DF) of number of branches plant^{-1} at 45 and 60 DAS of chickpea as influenced by TU foliar application under different levels of drought stress

Source of varience	DF	Mean square values: Number of branches plant^{-1}	
		45 DAS	60 DAS
Treatments	15	1.819	4.494
Error	32	0.012	0.013

Appendix VIII. Mean square values and degree of freedom (DF) of RWC, chl *a*, chl *b*, chl (*a*+*b*), and Pro content of chickpea as influenced by TU foliar application under different levels of drought stress

Source of varience	DF	Mean square values				
		RWC	chl *a*	chl *b*	chl (*a*+*b*)	Pro
Treatments	15	555.192	0.043	0.054	0.188	32.103
Error	32	9.060	0.0001	0.0001	0.0001	0.207

Appendix IX. Mean square values and degree of freedom (DF) of MDA and H_2O_2 content of chickpea as influenced by TU foliar application under different levels of drought stress

Source of varience	DF	Mean square values	
		MDA	H_2O_2
Treatments	15	404.462	111.780
Error	32	6.832	1.452

Appendix X. Mean square values and degree of freedom (DF) of AsA, DHA. AsA/DHA, GSH, GSSG, and GSH/GSSG of chickpea as influenced by TU foliar application under different levels of drought stress

Source of varience	DF	Mean square values					
		AsA	DHA	AsA/ DHA	GSH	GSSG	GSH/ GSSG
Treatments	15	766089.29	1773103.398	0.289	32979.804	3999.433	13.784
Error	32	8108.051	22163.870	0.020	522.103	23.425	0.336

Appendix XI. Mean square values and degree of freedom (DF) of weight of 100-seed, seed yield plant^{-1}, stover yield plant^{-1}, and biological yield plant^{-1} of chickpea as influenced by TU foliar application under different levels of drought stress

Source of varience	DF	Mean square values			
		Weight of 100-seed	Seed yield plant^{-1}	Stover yield plant^{-1}	Biological yield plant^{-1}
Treatments	13	66.741	141.523	23.816	106.378
Error	28	1.050	0.794	0.261	0.320