EFFECT OF DROUGHT STRESS AND GIBBERELLIC ACID ON MORPHOPHYSIOLOGICAL PARAMETERS AND YIELD OF WHEAT

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CERTIFICATE

This is to certify that thesis entitled, "EFFECT OF DROUGHT STRESS AND GIBBERELLIC ACID ON MORPHOPHYSIOLOGICAL PARAMETERS AND YIELD OF WHEAT" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in AGRONOMY, embodies the result of a piece of *bona fide* research work carried out by Moumita, Registration No. 11-04556 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated:
Dhaka, Bangladesh

Dr. Parimal Kanti Biswas
Professor
Supervisor

DEDICATED TO MY BELOVED GRANDMOTHER AND MY PARENTS

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ABSTRACT

Drought stress is a major problem in wheat production but it could be managed by using various exogenous protectants. In the first experiment drought stress was imposed on various growth stages of wheat and to alleviate it GA was applied. Drought stress reduced plant height 16.42%, 22.06 % tiller and 36.10% biomass. The 28.31% spikelets spike^{-1}, 33.25% grains spike^{-1}, 35.17% 1000-grain weight, 45.40% grain yield, 36.43% biological yield and 13.14% harvest index was reduced due to drought stress but GA had played a role to reduce the damage of drought stress and in most of the cases it increased the growth and yield than no gibberellic acid. For growth CRI stage was most critical and for yield flowering and grain development stage was more sensitive to drought though GA treated plants showed less damage at CRI stage. In the second experiment, combined effect of GA and drought stress after 48h and 72h was studied. MDA, H_2O_2, proline content was increased and CAT, APX was reduced in drought stress. GA played a role to restore not only these but also AsA, GSH/GSSG ratio, MDHAR, DHAR activity was restored by GA. GA significantly worked on glyoxalase system. Under drought stress MG activity was increased but GA stimulated Gly I and Gly II activity to protect the wheat seedlings. So, the present study concluded that the severity of drought stress on wheat depends on the growth stage and it increased with the enhancement of duration of stress whereas GA helped wheat seedlings by upregulating of antioxidant defense mechanism and glyoxalase system.

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LIST OF ACCRONYMS AND ABBREVIATION

AGR	Absolute Growth Rate
APX	Ascorbate peroxidase
ANOVA	Analysis of Variance
AsA	Ascorbic acid/Ascorbate
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
CAT	Catalase
CGR	Crop Growth Rate
Chl	Chlorophyll
CRD	Completely Randomized Design
DAE	Department of Agricultural Extension
DAS	Days after sowing
DHAR	Dehydroascorbate reductase
DW	Dry weight
et al.	and others
FAO	Food and Agriculture Organization
FW	Fresh weight
GA	Gibberellic Acid
Gly I	Glyoxalase I
Gly II	Glyoxalase II
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced Glutathione
GSSG	Oxidized glutathione
GST	Glutathione S-tranferase

LIST OF ACCRONYMS AND ABBREVIATION (Contd.)

HI Harvest index

LSD Least Significant Difference

mM Milimolar

MDA Malondealdehyde

MDHA Monodehydroascorbate

MDHAR Monodehydroascorbate reductase

MG Methylglyoxal

$O_2^{\bullet -}$ Superoxide radical

$_1O^2$ Singlet Oxygen

$OH^{\bullet -}$ Hydroxyl radical

PEG Poly Ethylene Glycol

POD Peroxidase

POX Peroxidases

Pro Proline

ROS Reactive oxygen species

RuBisCo Ribulose-1,5-bisphosphate carboxylase/oxygenase

RWC Relative water content

SI Stress Intensity

SOD Superoxide dismutase

SPAD Soil Plant Analysis Development

SRDI Soil Resource Development Institute

μM Micromolar

Chapter 1

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the first cultivated food crops since 8000 years and still now it is used as one of the major cereal crops in Europe, West Asia and North Africa (Curtis, 2002). It belongs to the family Poaceae (Gramineae). The main cultivated species of wheat is *Triticum aestivum*. It is hexaploid and known as "common" or "bread" wheat (Shewry and Hey, 2015). Wheat is a widely adapted crop. It grows from temperate to cold region, irrigated to dry condition (Acevedo *et al.*, 2002).

Wheat serves a high amount of carbohydrate, and other nutrients like vitamins, minerals, lipid and some essential amino acids which may regard as a healthy diet. It is also a good source of dietary fiber. 100 g wheat serves 327 calories carbohydrate, 0.41 g sugar, 29 mg calcium, 3.19 mg iron, 126mg magnesium, 2.65 mg zinc, 3.99 mg manganese and other nutrients (USDA, 2016). The most important thing is it contains higher amount of protein (12.6 g) than rice (7.9 g) and maize (9.4 g) (USDA, 2016) and this protein is mostly gluten (75-80% of the protein in wheat) (Shewry, 2009 ; Shewry and Hey, 2015). Various food like bread, porridge, crackers, biscuits, Muesli, pancakes, pies, pastries, cakes are made from wheat flour. The outer husk is also used as feed for domestic animals and birds. Due to the diversified uses, its demand is increasing day by day.

Among the world cereal production wheat is now in the second position after maize. It is forecasted about 754.8 million tons wheat production in 2017-2018 year which is higher than rice (500.8 million tons), (FAO, 2018a). China, India, Russia, USA and Canada are the top 5 wheat producing countrys (FAO, 2018b). In Bangladesh the climate and soil conditions are suitable for wheat cultivation. Though wheat is produced in all over the country it grows well in Dinajpur, Rajshahi, and Rangpur districts (Karim *et al.*, 2010). Wheat covers about 4% of the total cultivated area and in Rabi season it covers 11% area**.** It supports 7% to the total supply of cereals crops (Anon. 2008) and it is in the third position among the cereal crops after rice and maize.

In our country, wheat consumption is about 28-30g/day/person that means the demand for wheat per year is about 4 million tons. Beside this demand is increasing due to the rapid use of bakery products as well as livestock and poultry feed. That's why to meet up this increased demand we have to import a significant amount of wheat per year (Karim *et al.*, 2010).

During the whole growing period plants have to face a lot of biotic stress like virus, bacteria , insect-pest infestation and abiotic stresses like drought, high temperature, chilling temperature, salinity, metal toxicity and many others. Among all, drought stress is more vulnerable abiotic stresses. As our useable water resource is limited, we have to fulfill the demand of vast population for agricultural commodities with this short amount of water (Somerville and Briscoe, 2001). Day to day its intensity is increasing and makes a threat to our food security.

Drought may cause due to shortage of rainfall or high amount of sunshine reflection (Paepe *et al.*, 1990). It is generally unpredictable. It depends on various factors like rainfall, temperature, soil moisture, crop type, crop duration and so on. When plant cannot uptake enough water due to various unfavorable conditions like deficit of soil moisture or high transpiration, plants face to drought stress. Though it may vary from plant to plant, drought stress negatively affects plant growth, yield and various biochemical and molecular functions of all plants. (Zhu, 2002; Chaitanya *et al.*, 2003 and Chaves *et al.*, 2003). Drought disrupt the normal physiological process in plants and causes various abnormal morphological changes in plants like stunted growth, leaf curling, reduced number of leaf, small leaf size etc (Rahdari and Hoseini, 2012). Growth and yield drastically reduced due to drought stress in rice (Lafitte *et al.*, 2007) and wheat (Rampino *et al.*, 2006). Same results also found in case of, maize (Kamara *et al.*, 2003) and barley (Samarah, 2005).

Drought stress causes damage to plants by excessive ROS production such as singlet oxygen ($_1O^{2-}$), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). ROS is responsible for cell damage, lipid per oxidation, breakdown of lipid and protein etc. and in severe cases it causes programmed cell death. To cope with this adverse situation, plants have to maintain some enzymatic (APX, DHAR, MDHAR, GR, GST, GPX, CAT) and non-enzymatic (AsA, GSH, Tocopherol, Phenolic compounds, alkaloids) metabolic system (Hasanuzzaman *et al.*, 2012a).

The severity of this problem is increasing and it is alarming that, within 2050 drought may damage more than 50% cultivable land (Vinocur and Altman, 2005; Kasim *et al.,* 2013). In Bangladesh, drought is a major problem for wheat production. About 32% wheat growing land is affected by drought. (Shamsi *et al.,* 2011).

When rainfall or soil moisture is inadequate for plant, irrigation is applied. It helps to grow crops, frost protection (Snyder *et al.,* 2005), soil erosion and weed control (Williams *et al.,* 1990). Due to urbanization and industrialization source of fresh water is decreasing and it affects every country in the world (World Bank, 2011). According to World Bank (2011) this problem is higher in developing countries. Besides, insufficient of irrigation water is already a major constraints for agriculture in many countries. That's why World Bank targets to water management because when farmers will get sufficient water they can cultivate successfully.

Irrigation demand may vary from crop to crops as well as it depends on crop growing stage. In maize enough irrigation water increase biological yield about 12% and grain yield 14.85% (Amin *et al.,* 2015). Many reports about potato (Yuan *et al.,* 2003), rice (Shao *et al.,* 2015), lentil (Kahraman *et al.,* 2016), chickpea (Thangwana and Ogola, 2012), wheat (Shirazi *et al.,* 2014) support that irrigation is required for higher crop growth and yield.

It was reported by Pal *et al*. (2000) that, 27.2% higher yield was found in wheat when 4 times irrigations were applied than 3 times irrigations besides he showed 64.5% more yield and higher growth than 2 irrigations in his same experiment. According to Khan *et al.* (2003) wheat growth and reduce drastically due to lack of water. Similar result was also found by Zhai *et al*. (2003), Malik *et al*. (2010) and Rahim *et al*. (2010).

Irrigation requirement varies with the growing stage of wheat. Water needs during crown root initiation (CRI) stage, tillering stage, booting stage, anthesis stage and grain development stage. According to Pal *et al*. (2000) tillering stage and milking stage needs more water but Mangan *et al.* (2008) said CRI stage was more critical. Mahmood and Ahmad (2005) also supported this theory. They said stress at CRI stage reduce 27% yield.

Plant can cope up with drought stress by physiological adaptation upto a certain stage but when it is severe plants need some external protectants. At very low concentration of plant growth regulators, plant can adapt with the adverse situation by creating some

physiological changes (Taiz and Zeiger, 2006). Now-a-days plant scientists work on various types of plant growth regulator like gibberellic acid, salicylic acid, jasmonic acid etc. as an exogenous protectant (Hasanuzzaman *et al.*, 2013a).

Gibberellic acid (GA) is known as Gibberellin A_3, GA, or GA_3. It is a hormone produce in plant and fungi. Its chemical formula is $C_{19}H_{22}O_6$ (Silva *et al.*, 2013). It was first identified in Japan in 1926 from a pathogen *Gibberella fujikuroi* which is responsible for rice bakanae disease (Camara, 2015).

In case of drought stress, plant height, fresh weight decreased. Gibberellic acid helps to plant by increasing water content and maintained protein damage (Taiz and Zeiger, 2006). GA helps plant in drought stress by increasing photosynthesis, maintaining stomatal conductance. It also helps to increase transpiration rate (Kumar *et al.*, 2001). Philipson (2003) reported that under drought stress, in *Picea sitchensis* GA helps to produce pollen and seed cone. It was also showed that exogenous GA helps to mitigate drought stress and increase yield in case of marigold (Sedghi *et al.*, 2012) and lentil (Milanesi *et al.*, 2008).

Considering the above circumstances the present study was undertaken with the following objectives:

i. To evaluate the effect of drought stress on growth and yield of wheat.
ii. To find out the most critical stage of wheat under drought stress.
iii. To investigate the role of gibberellic acid to mitigate drought stress in wheat.
iv. To measure the oxidative stress and antioxidant defense system in wheat under drought stress.

CHAPTER 2

REVIEW OF LITERATURE

In this chapter a brief review of various researches that were conducted about drought stress on wheat and GA have been included. These reviews are the short summary of research works conducted in Bangladesh and other countries in the world.

2.1 Wheat

In the world wheat covers more land area than any other food crops and it is about 220.4 million hectares (FAOSTAT, 2014). On an average wheat supplies 0.33 kilocalorie of energy also it contains beneficial vitamins and nutrients which are good for human diet (FAO, 2013). Wheat demand is increasing day by day due to its gluten protein which is responsible for many bakery products.

Steam of wheat is called culm and it is hollow in mature plant. It is cylinder shape and contains 3-6 nodes and internodes. Life duration is usually 100-120 days though it varies due to variety and weather condition. Wheat can grow under a wide range of climate and soil condition; however it grows well in clayey loam soils and requires dry weather and bright sunlight. In Bangladesh it is a rabi crop. It requires 40-110cm rainfall (Banglapedia, 2014).

Although wheat is an ancient domesticated crop, in Bengal it was started to cultivate in 1930-1931. It was regarded as a food crop around 1942-1943 (Banglapedia, 2014). About 4% of total cropped area is occupied by wheat and 11% cropping area is occupied during rabi season. After the liberation war in 1971, different natural hazards occurred in Bangladesh also population growth rate was higher (Hugo, 2006). In that circumstance, it was clear that only rice was not enough to meet the huge amount of food of the country (Banglapedia, 2006). Moreover, from 1971 to 1975 rice prize was higher in the world market (OECD, 2008) and in Bangladesh production was decreasing due to various kind of natural disasters (Index Mundi, 2012aAt that time, wheat gained its popularity as an alternative crop of cereals.

In Bangladesh, wheat is in second position among food crops. During 1970, local variety "KHERI'', IP-52, IP-125 were cultivated. After then "KALYANSONA'' and ''SONALIKA'' these two variety were imported from abroad. They were high yielding. Day to day its production is increasing. After 1998 (SOURAV) to present (BARI GOM-32), about 14 existing varieties are cultivated in our country. At present BARI GOM-25, BARI GOM-26, BARI GOM-27, BARI GOM-28, BARI GOM-29, BARI GOM-30, BARI GOM-31, BARI GOM-32 are widely cultivated varieties in Bangladesh.

Now-a-days wheat production is decreasing due to various kinds of natural disasters, pest and disease, competition with other Rabi crops etc. There are many reports about various types of biotic and abiotic stresses that are the main cause of wheat yield reduction. According to CIMMYT and ICARDA (2011) that about 20-30% yield reduction occurs due to increase of 2-3^{0}C temperature. Disease like leaf rust may cause 10-30% yield loss depending on plant susceptibility (Singh *et al.*, 2002). Due to severely wheat blast disease, yield may be reduced up to 100% as it needs to total wheat field burned (Islam *et al.,* 2016). Drought itself may cause more than 50% yield reduction as it has a detrimental effect on plant growth and development (Farooq *et al.,* 2009). However, researchers' efforts still continued to develop high-yielding varieties.

2.2 Abiotic stress

In whole growing period plants have to face a lot of unfavorable conditions due to the unstable surroundings. These stressful conditions consist of various kinds of pathogenic attack, virus and bacterial infections, insects-pest attack etc. which is known as biotic stress and abiotic stress includes the adverse effect of drought, salinity, high temperature or cold temperature, water-logging condition, nutrient deficiency or toxicity, heavy metals or arsenate toxicity and so on. Various kind of abiotic stresses affect plants growth and development. Though most of the abiotic stresses are related with the climate changes, it hampers plants geographical distribution and decreases plants productivity which ultimately makes threats to food security. It is reported by Rodríguez *et al.* (2005) and Acquaah (2007) that abiotic

stress is responsible for most of the damages of plant even it may be up to 50% (Hasanuzzaman *et al.*, 2012a).

The effect of these stresses is the production of ROS (Reactive Oxygen Species). The ROS included singlet oxygen ($_1O^2$), superoxide ($O^{2\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^\bullet$). At low concentration ROS works as a signaling molecules whereas at high concentration it makes damage to plant cell by lipid peroxidation, DNA and protein breakdown, and programmed cell death (PCD) (Hasanuzzaman *et al.*, 2012a).

As a sessile organism, plants have to deal with this stresses by adapting various enzymatic and non-enzymatic scavenging pathways. Enzymes include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione *S*-transferase (GST), glutathione peroxidase (GPX) and peroxidases (POX) as well as non-enzymatic compounds such as ascorbate (AsA), glutathione (GSH), carotenoids and tocopherols (Hasanuzzaman *et al.*, 2012a).

Plant itself has some mechanism to adjust with the adverse condition but when it is more than plant tolerance, plants need supports. Understanding the stress tolerance and its mechanism increase researcher's ability to develop resistant varieties or helps to find out the proper management system to supports plant.

2.3 Drought

When a region is affected by water shortage problem for an extend period of time due to the environmental phenomenon or human activity is defined as drought. According to FAO, (2013) drought can be explained by meteorological, agricultural, hydrological, and socio-economic perceptions.

In view of meteorological, when rainfall is absent or very little amount for a long time is known as drought. In agriculture, drought is defined as the shortage of soil moisture to fulfill the demand of a particular crop for a defined period of time.

In hydrological science, shortage of water in surface and subsurface area is drought.

In view of socio-economic condition, due to the lack of rainfall or when insufficient water supply hampers human activities is called drought.

The impact of drought stress is more detrimental than any others abiotic stresses and it is cleared by a report of FAO (2013). According to them, more than 11 million people have died and 2 billion people have been suffered from drought stress at worldwide since 1900.

The effect of drought stress on plant is very severe. It reduced plant growth and yield. It is responsible for poor germination (Harris *et al.,* 2002), it reduced plant fresh weight and dry weight (Zhao *et al.,* 2006), reduced cell division (Hussain *et al.,* 2008). Drought stress is also responsible for low water content, poor osmotic adjustment, decrease in CO_2 intake and poor photosynthesis (Cornic, 2000; Flexas *et al.,* 2004) and yield reduction (Benjamin and Nielsen, 2006; Praba *et al.,* 2009).

2.4 Plant Responses to Drought stress

2.4.1 Morphological responses

2.4.1.1 Germination and seedling establishment

The first effect of drought stress on plant starts with the germination process. According to Harris *et al.* (2002) drought has a detrimental effect on germination. Due to drought germination may be stopped or if seed germinates then the seedling growth will be very poor because water is very essential for germination process. The most important stage of seed germination is the imbibition process (water uptake) that helps to activate the hydrolytic enzymes. This enzyme is responsible for the breakdown of storage food materials into metabolically useful chemicals (Raven *et al.,* 2005). Bahrami *et al.* (2012) explained that sesame (*Sesamum indicum* L.) is a drought tolerant crop but its germination reduced extremely due to the drought stress. He showed that seedling establishment also hampered due to the drought stress. In an experiment Khayatnezhad *et al.* (2010) found that in maize, germination process reduced due to drought stress even in extreme condition it was zero germination rate. Same result was found in case of other crops like sorghum (Gill *et al.,* 2002),

sunflower (Mohammed *et al.,* 2002), rice (Harris *et al.,* 2002), lentil (Muscolo *et al.,* 2014), corn (Khodarahmpour, 2011) etc.

2.4.1.2 Cell division

Plant growth and development depends on optimum supply of water because it is related with cell division, cell enlargement, leaf area, nutrient uptake and supply to organ etc. (Rucker *et al.,* 1995). During the shortage of water, turgor pressure is reduced. Cell division also reduced due to the tower turger pressure. Plant growth will be optimum when cell division and cell enlargement is in optimum condition. in drought stress cell development is lower due to the shortage of water supply from xylem to surrounding new cell (Nonami, 1998). Impaired cell division, stunted growth of plant, loss of integrity of cell membrane is the result of drought stress (Hussain *et al.,* 2008).

2.4.1.3 Plant height

Plant height is inversely related with drought stress. With the increase of stress condition, plant height is decreased. Zubaer *et al.* (2007) showed in his experiment that plant height was reduced with the increase of water stress. He found 139.2 cm plant height in 100% field capacity where as at 40% field capacity it was 117.1 cm in rice. Mafakheri *et al.* (2010) described that in chickpea shoot length was reduced in stress condition. In vegetative growing stage plants height reduced if it faces drought stress. In an experiment Azarpanah *et al.* (2013) described that plant height decreased due to drought stress and he clarified that stress in vegetative stage, plant height remains 158.85 cm but when stress is in reproductive stage he found 169.07 cm plant height. He also considered the effect of drought. In I1 (Irrigation after 40, mm of cumulative evaporation, from Pan Class A) he got 170.29cm plant height but in I3 (Irrigation after 100, mm of cumulative evaporation, from Pan Class A) he got 157.29cm. Another theory is that stem growth and plant height is reduced in drought due to shrinkage in output changes in cellular water status (Prasad and Staggenborg, 2008). Ozkan and Kulak (2013) conducted an experiment to find out the effect of water shortage on sesame plant. They used two varieties and in both varieties they showed that moderate water stress had not any significant effect as sesame is a drought tolerant crops but when stress is severe, plant height decreased 43.30 % and 41.75 % than the control in Cunhuriyet and Özberk variety respectively.

2.4.1.4 Leaf

Due to drought stress soil water potentiality decrease that affects the leaf. It increases the leaf senescence and reduces the number of leaf. Also leaf area is decreased due to reduction of leaf expansion (Rucker *et al.,* 1995). Khan *et al.* (2001) conducted an experiment to find out the effect of drought stress on maize. Here he found that leaf number as well as leaf area decreased with the increase of drought stress. Same result was also found in berseem (Lazaridou and Koutroubas, 2004) and groundnut (Craufurad *et al.,* 2000). In case of *Vigna unguiculata* lower leaf number per plant and more leaf senescence was found in lower soil moisture (Manivannan *et al.,* 2007a). DaMatta (2004) also found the same result. He showed that with the increase of drought stress, leaf shedding was also increased. In maize leaf number per plant, leaf area index, leaf length was decreased due to drought stress (Azarpanah *et al.,* 2013).

2.4.1.5 Fresh weight and dry weight

Fresh weight or dry weight depends on plants growth and development. When soil moisture is not sufficient to fulfill the demand of plants, plants regular activities are disrupted and plants growth also reduced ultimately plant carries lower weight. Siddique *et al.* (2001) supported the theory as he explained that in drought stress plants are unable to uptake sufficient amount of nutrient and cannot produce enough food for their growth that's why dry weight is lower in shoot. A little bit different result was found by Jørgensen *et al.* (2011). He conducted his experiment with four variety of groundnut. In his experiment he noticed that only in Ramayana variety shoot dry weight decreased significantly whereas in other three varieties it was non-significant although in case of corn, Zubaer *et al.* (2007) found lower fresh weight as well as dry weight in his experiment. He assumed in drought stress dry matter production is decreased due to the lower photosynthesis. Azarpanah *et al.* (2013) described that in maize, he found about 7% higher fresh matter and dry mater in I1 (Irrigation after 40 mm of cumulative evaporation, from Pan Class A) than I3 (Irrigation after 100 mm of cumulative evaporation, from Pan Class A).

2.4.1.6 Reproductive development

Reproductive development is a critical phenomenon for yield determination. At reproductive stage, drought stress causes the flowers and fruit dropping. Gan *et al.* (2004) amd Sinaki *et al.* (2007) supported this and stated that the prominent effect of drought stress is observed in this stage is abortion of fruits or pods and decrease the yield. Damage may vary from crops to crops but in all crops water is essential for the reproductive development. Due to lack of moisture pollen grain becomes dry and inactive, pollen tube cannot fully developed, photosynthates production and supply is disrupted and so on. All these causes are responsible for unfilled grains and it is supported by Hossain (2001). Plants suffer from water shortage at reproductive stage bears higher number of unfilled grains. Zubaer et al. (2007) also found higher number of unfilled grains in water shortage condition. Leport *et al.* (2006) stated that there is a relation with the size of the seed and its abortion in stress condition. Large seeded varieties are more vulnerable to drought stress and pod dropping is higher when it faces the drought stress in early pod development stage.

2.4.1.7 Effect on yield

Yield depends on the whole growing condition of the plant. All the morphological, physiological and biochemical functions are responsible for the determination of yield. Number of branches, panicle length, pod numbers, spikelets/spike etc. are direct yield contributing parameters though these are vary from crops to crops like Number of tillers, no. of grains, panicle length for rice, spike length for wheat, no. of pods for legume, and so on. Photosynthesis, chlorophyll content, stomatal conductance and others physiological process are also responsible for yield determination especially for the cereal crops (Gutie´rrez-Rodrı´guez *et al.*, 2000; Labuschagne *et al.*, 2008). Dubey (2005) stated that photosynthesis directly determines the yield and in drought stress yield is decreased due to lower photosynthates (Hossain, 2001).

Gonzalez *et al.* (2010) also found a significant correlation between the physiological traits and yield. He explained that terminal drought stress reduce the yield in barley. He found about 27% mean yield reduction in his experiment due to the stress.

Zubaer *et al.* (2007) showed in rice that higher grain yield was found when plant is in optimum condition even grain size also reduced due to the water stress. He found largest grain size and yield in 100% FC than the 70% FC and the lowest yield and grain size was found in 40% FC. Din *et al.* (2011) conducted an experiment with five

drought tolerant canola varieties and he found that due to water shortage depending on variety more or less 40-60% yield loss occurred.

In chickpea number of pods plant^{-1} and yield reduced in stress condition (Mafakheri *et al.*, 2010). Jørgensen *et al.* (2011) also found the same finding that depending on the variety 31%-75% yield reduction was visible in groundnut.
Azarpanah *et al.* (2013) conducted his experiment in field condition and used three irrigation levels. In maize he showed that total grain yield, ear length, ear weight, kernel number row^{-1}, Cob diameter, Cob weight, 1000 - kernel weight, harvesting index all the yield contributing parameters decreased during stress condition but in case of three irrigation levels he got maximum yield.

Drought stress is harmful for all types of crops though it depends on various factors. It was described by Ozkan and Kulak (2013) that in sesame when soil moisture is in FC or ½ FC most of the yield contributing parameters were significantly similar but in case of ¼ FC yield is significantly reduced. They used two variety, Cumhuriyet cv. and Özberk cv. In Cumhuriyet cv. Yield/pot (58.15%), yield/plant (60.27%), capsule no./plant(36.43%) are higher in ½ FC than ¼ FC though yield in FC and ½ FC was almost similar. In case of Özberk cv. in FC, it gave higher yields. Yield/pot, yield/plant, capsule no./plant was 43.34%, 38.66%, 47.88% higher respectively than ¼ FC although 1000 seed weight was higher in FC (18.33%) in Cumhuriyet cv. and in Özberk cv. it was higher in ½ FC (30.18%) than ¼ FC.

In a greenhouse experiment Samarah (2005) used 12 seeds of barley cultiver to find out the effect of drought stress on barley and found that yield/plant reduced 56.87%, grain number plant^{-1} reduced 54.63%, 50% reduction in fertile seed, and tiller number was reduced about 25%.

Gholinezhad *et al.* (2009) conducted an experiment on sunflower. He used three different levels of irrigations including optimum irrigation, mooderate stress and severe stress. Biological yield, seeds/head, 1000 grain weight etc. are decreased with the incease of stress. Maximum yield was gained by optimum moisture condition and it was 4200 kg ha^{-1}. He concluded that grain yield decreased about 44% than the maximum yield due to the severe stress.

Pervez *et al.* (2009) imposed drought on tomato at different growth stage to determine the effect of drought stress. In his greenhouse experiment he showed that lack of water in any stage of the growing period reduced the total yield, number of fruits and so on.

The adverse effect of drought stress was also found in case of rice (Guan *et al.*, 2010), tuber yield in potato (Ramírez *et al.*, 2014), lentil (Kumar *et al.*, 2012), soybean (Liu *et al.*, 2004) and so on.

Akter (2014) conducted an experiment in Sher-e-Bangla agricultural university field about the effect of irrigation and nitrogen fertilizer. She applied four different level of irrigation. In her experiment she got higher number of siliqua plant^{-1}, higher siliqua length as well as higher no. of seeds siliqua^{-1} in full irrigation condition. 1000 seed weight, seed yield, stover yield, and harvest index were also significantly higher at three irrigations level. The highest seed yield (1589 kg ha^{-1}) was obtained at three irrigations level while control treatment (no irrigation) gave the lowest yield (1095 kg ha^{-1}).

A field experiment was done in Sher-e-Bangla agricultural university by Ferdous (2014). She used four level of irrigation treatments including one is no irrigation. She found that in Soybean, yield and yield contributing parameters were reduced in drought stress condition and Three times irrigation gave the highest (1.63 t ha^{-1}) seed yield.

In case of BRRI dhan28 Momin (2014) found an interesting result. He noticed that BRRI dhan28 gave higher yield when I_2 (irrigation was applied at 2 days after field drying) than I_1 (all time available water) although the lowest yield was found at I_5 (irrigation at 8 days after field drying). In this experiment, Momin (2014) got 16.27% higher yield in I_2 than the stress condition, I_5.

Kibria (2013) also found the similar result in case of mustard (SAU Sarisha-3). He found that, he got higher siliquae per plant (138.8), seeds per silliqua (20.06) and seed yield (1.98 t ha^{-1}) from I_2. Shortest plant (98.49 cm), minimum branches per plant (7.17), siliquae per plant (111.9), seeds per silliqua (19.37) and seed yield (1.34 t/ha) was produced by no irrigations.

Tallest plant (101.00 cm), maximum branches per plant (7.70), siliquae per plant (138.8), seeds per silliqua (20.06) and highest seed yield (1.98 t/ha) was produced by two irrigations.

In case of lentil, drought stress reduces the seed yield, harvest index and so on. In a field experiment conducted by Zakaria (2010) used three varieties and four level of irrigations. He showed that when soil moisture is in optimum condition it gives higher yield. pods plant^{-1}, seeds pod^{-1}, plant dry weight, 1000-seed weight, seed yield, stover yield, biological yield and harvest index and were found significantly variable with irrigation levels.

2.4.1.8 Root

In drought stress root is also affected. Sometimes root plays a crucial role in stress adaptation but there are many controversial theory arise about it because in some cases root length and weight increase to adapt with drought stress and in some case due to drought stress it decreases (Anjum *et al.*, 2011). In *Catharanthus roseus* during drought stress root growth was increased to uptake more water (Jaleel *et al.*, 2008) whereas in corn root length decrease about 60% (Khodarahmpour, 2011). According to Khodarahmpour (2011) root length is a special criterion to count the stress damage because in drought stress there is a relation between the root growth and root-shoot ratio. Wu and Cosgrove (2000) showed that in water shortage, root-shoot ratio increases because according to them roots are less responsive than shoot to drought. Though Turner *et al.* (2001) gave opposite opinion to it. According to him in water shortage root become more expansive to uptake water from lower subsurface area of soil. Zeid and Shedeed (2006) found that in alfalfa plants root length was increased in drought stress and same result was found in rice by Kamoshita *et al.* (2002).

2.4.2 Physiological responses under drought stress

Drought stress damages the plant in various ways like reduced growth, disruption in phoytosynthesis and other physiological functions ultimately poor yield. Anjum *et al.* (2011) described the physiological damage done by drought stress. he showed drought stress in maize led to the considerable decline in net photosynthesis (33.22%), transpiration rate (37.84%), stomatal conductance (25.54%), water use efficiency (50.87%), intrinsic water use efficiency (11.58%) and intercellular CO_2 (5.86%) as compared to well water control.

2.4.2.1 Photosynthesis

Drought stress have a direct effect on plant photosynthesis by disrupting the thylakoid electron transport, the carbon reduction cycle and the stomatal control of the CO_2 supply and like this (Allen and Ort, 2001). In drought stress leaf area, leaf number, chlorophyll contents above all total photosynthesis area is decreased ultimately photosynthesis is decreased. It is proved that, photosynthesis is related with stomatal activity. Under drought stress, stomata become closed and CO_2 absorbability is reduced. Because of it photosynthetic carbon assimilation is decreased and the result is reduced photosynthesis (Del-Blanco *et al.,* 2000; Samarah *et al.,* 2009).

Photosynthesis is also disrupted due to decline of in rubisco activity (Bota *et al.,* 2004) Dehydration of cell (Hoekstra *et al.,* 2001), presence of tight-binding inhibitors (Parry *et al.,* 2002), decrease of some photosynthetic enzymes and so on. According to Bota *et al.* (2004) in severe drought stress condition, Rubisco activity is decreased and it may be due to the reaction of CO_2 and Mg^{2+}. Decrease of photosynthesis under drought was happened because of decreased of ribulose-1, 5-bisphosphate carboxylation by Rubisco, speed of ribulose-1, 5-bisphosphate regeneration, Rubisco and stromal fructose bis-phosphatase activities, and the quantum efficiency of photosystem II in higher plants (Reddy *et al.,* 2004; Zhau *et al.,* 2007).

It is also reported that some tight-binding inhibitors can decrease Rubisco activity under drought stress as well as Parry *et al.* (2002) also found the same findings that in tobacco (*Nicotiana tabacum*), Rubisco activity decreased under drought stress due to the presence of tight-binding inhibitors.

Hoekstra *et al.,* (2001) said dehidration of cell is another reason for photosynthesis reduction. It causes cell shrinkage, decrease in cell volume aggregation of protein and denaturation of it. Increased concentration of cell components due to water shortage, leading to increased viscosity of the cytoplasm, and it make inactive of enzymes which are related to photosynthesis (Hoekstra *et al.,* 2001).

2.4.2.2 Chlorophyll content

Reduction of chlorophyll content is another effect of drought stress. The decrease in total chlorophyll content is the result of pigment photo-oxidation and chlorophyll degradation (Farooq *et al.,* 2009). Chlorophyll content is also reduced due to the loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation, and the appearance of lipid droplets (Kaiser *et al.,* 1981). Reduction of chlorophyll content is reported by various researchers. It was observed that total chlorophyll content is reduced due to the drought stress in case of different sunflower varieties (Manivannan *et al.,* 2007b), barley (Gonzalez *et al.,* 2010), canola (Din *et al.,* 2011), chickpea (Mafakheri *et al.,* 2010) and so on.

2.4.2.3 Water relation

Relative water content (RWC) is widely used to measure the plant water status, parameters for counting metabolic activity and an index for dehydration tolerance. RWC is affected by the interaction of severity, duration of the drought stress and plant species. It is also related with the water availability in soil, water uptake and transpiration (Anjum *et al.,* 2011). Anjum *et al.* (2011) said that RWC is higher in young leaves and decrease with the increase of dry matter accumulation and plant maturity. Decreasing RWC in response to drought stress had been reported in a wide variety of plants by Nayyar and Gupta (2006). Plants in drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, due to increase of leaf temperature (Siddique *et al.,* 2001). It was reported that RWC decreased in Wheat (Keyvan, 2010), potato (Soltys-Kalina *et al.,* 2016), peanut (Shivakrishna *et al.,* 2017) and many other crops.

Water use efficiency means the ratio of dry matter production and total water consumed by plant. In case of drought stress plants get water at very low amount

that's why plant use this water very effectively and it try to produce its maximum dry matter with little amount of water. Abbate *et al.* (2004) found water use efficiency is higher in stressed plant than the well watered plant. They thought it may be occurred due to the closure of stomata to reduce transpiration.

Lazaridou and Koutroubas (2004) concluded that water use efficiency increased in drought stress due to the lowered loss of water because in water shortage condition, leaf number and leaf area is reduced transpiration rate is also reduced. Lazaridou *et al.* (2003) supported this and showed that leucern (*Medicago sativa*) grown under water stress had greater water-use efficiency than the irrigated Plant but an opposite theory was given by De-Lucia and Heckathorn (1989) that in *Pinus ponderosa* and *Artemisia tridentata,* drought stress did not reduce the water-use efficiency because a rapid decrease happened in stomatal conductance with increasing of stress.

2.4.2.4 Nutrient uptake

Drought stress reduces plants growth and yield and one of the reasons of it is unavailability of nutrient. Also it may be happened that nutrient is available in root zone but plant cannot uptake it or cannot transport it from root to other parts of plants due to lack of water. Garg (2003) and McWilliams (2003) stated that due to reduction of transpirational flow, difficulty in nutrient uptake and the unloading mechanism inorganic nutrients uptake is reduced. However, nutrient uptake and transportation varies from plants to plants and density of drought stress. Usually, drought increases the N and reduces the P but no definite effects on K (Garg, 2003). Grossman and Takahashi (2001) said that drought stress reduced the availability of energy that is need for assimilation of different ions like NO_3^- /NH_4^+, PO_4^{3-} and SO_4^{2-}.

It was descrived by McWilliams (2003) that in cotton N and K uptake was reduced in drought. P and PO_4^{3-} contents in the plant also reduced in drought because of lowered PO^{3-}_4 mobility due to lack of moisture (Peuke and Rennenberg, 2004). So it may be concluded that drought stress reduces the availability, uptake, translocation and metabolism of nutrients. A reduced transpiration rate due to water deficit reduces the nutrient absorption and efficiency of their utilization.

2.4.2.5 Root signaling

Under drought stress root makes a signal that transport through xylem to make adaptation by physiological changes of plants in stress condition. Abscisic acid (ABA), cytokinins, ethylene, malate and other unidentified factors is responsible for this root–shoot signaling (Anjum *et al.*, 2011).

ABA is a dominate signal in controlling growth and transpiration. ABA helps to increase efflux of potassium ion (K^+) in the guard cell. For this reason turgor pressure is decreased and stomata closed. If plant is in dehydrated condition, turgor pressure of plant is lost drastically. At this situation ABA level may increase upto 50-fold (Guerrero and Mullet, 1986). The role of ABA to transfer signal from root to shoot was challenged because in some experiments it was found that concentration of ABA in xylem sap of stressed plant was lower than the exogenous ABA required to close stomata in detached leaves (Munns and King, 1988).

Cytokinin is also responsible for signal transporting from root to shoot. Schachtman and Shin (2007) explained that cytokinin is very responsive when plant is in nutrient shortage and cytokinins are mainly produced in roots. So it may be assumed that it may helps in drought mitigating. In recent experiments response of cytokinins found lower under drought stress and it may vary from crops to crops (Dodd, 2003). Although researchers also found that exogenous application of cytokinins may help to reduce the effect of drought stress.

2.4.3 Oxidative stress in plants under drought stress

Production of reactive oxygen species (ROS) is one of the most important biochemical responses in drought stress. The production of ROS in plants is known as oxidative stress. Oxygen plays an important role in normal metabolism and in cell signaling but during drought stress, reactive oxygen species (O_2^-, $^1O_2$, H_2O_2, OH^-) are produced which are dangerous for plants (Peltzer *et al.,* 2002; Gill and Tuteja, 2010).
ROS levels increase drastically resulting in oxidative damage to proteins, DNA and lipids (Apel and Hirt, 2004). ROS causes damage plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death (Hasanuzzaman *et al.,* 2013a).

In drought stress stomata become close and CO_2 is reduced. That's why carbon fixation is disrupted and excessive excitation energy is produced in chloroplast (Mittler, 2002; De Carvalho, 2008 and Hasanuzzaman *et al.,* 2013b). In severe stress condition, excited pigments in thylakoid membranes may interact with O_2 and form O_2^- or O_1^2 (Niyogi, 1999; Reddy *et al.,* 2004) and more downstream reactions produce other reactive oxygen species such as H_2O_2 and OH^-. In mitochondria, reaction of O_2 with other reduced components of the electron transport chain can produce ROS (Möller, 2001) and in peroxisomes, during photorespiration H_2O_2 may produce (Fazeli *et al.,* 2007).
Malondialdehyde (MDA), hydrogen peroxide (H_2O_2), methyl glyoxal (MG) etc. are used as oxidative stress indicators. Nahar *et al.* (2015) showed that under drought stress hydrogen peroxide (H_2O_2), lipid peroxidation, reactive oxygen species like H_2O_2 content and $O_2^{\cdot-}$ generation rate, MG level are increased. Alam *et al.* (2014) also found the same result in case of drought stress.

2.4.4 Antioxidant enzymes

When plant is in stress condition, plants try to create a defensive system which helps plant to avoid injury and allow to continue its normal function. This defensive system creates a balance between ROS production and activities of antioxidative system to determine whether plants survive or damaged by ROS (Moller, 2001). To minimize the effect of this damage, plants have to maintain an enzymatic and non-enzymatic antioxidant system, such as low-molecular mass antioxidants (glutathione, ascorbate,

carotenoids) and ROS-scavenging enzymes (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) (Apel and Hirt, 2004) and non-enzymatic (AsA, GSH, Tocopherol, Phenolic compounds, alkaloids) metabolic system (Hasanuzzaman *et al.*, 2012a). Ahmadizadeh *et al.* (2011b) showed that during drought stress in wheat, RWC decreased, MDA, AsA and GSH contents increased. Again, SOD and CAT density increase 35.6 and 3.1%, respectively comapare to control.

Cai *et al.* (2015) demonstrated an experiment with wild type variety and *OsABA8ox3* RNAi gene containing variety of rice. They found that *OsABA8ox3* RNAi plants had higher superoxide dismutase (SOD) and catalase (CAT) activities and less malondialdehyde (MDA) content than the wild type variety in drought condition which gave a less membrane damage in cell.

Siddiqui *et al.* (2015) conducted an experiment to find out the responses of different variety of faba bean plants in drought stress condition. He found "C5" and "Zafar 1" as resistant genotypes where relatively antioxidant enzymes activities like CAT, POD and SOD were higher and Total Chl, and leaf RWC was also higher on the other hand, genotypes "G853" and "C4" were found as sensitive due to lower antioxidant enzymes activities.

Alam *et al.* (2014) used trehalose to increase the drought tolerant in different brassica species. He showed that antioxidant enzymes activities were increased due to applying trehalose but in *B. juncea* he found higher activity catalase (CAT), glutathione S-transferase (GST), glyoxalase I (Gly I) activities; reduced MDA, H2O2 contents and LOX activity than other varieties. He concluded that *B. juncea* is naturally more tolerant to drought stress.

2.5 Effect of drought on wheat

2.5.1 Growth

From germination to seed maturity, drought may occured in any stage and causes a significant damage to plants.

2.5.1.1 Seed germination and plant height

Timmusk *et al.* (2014) explained that in his experiment he found in irrigated condition seed germination was 72% whereas in drought stress it was reduced upto 50%. Taheri *et al.* (2011) explained that there was a relation with the plant height and seed yield. In his experiment he found decreased plant height due to drought stress. Kilic and Yagbasanlar (2010) conducted an experiment with different variety of wheat. They showed the result of drought stressed plant and well watered plant. There they found that mean plant height was 7.7% reduced in drought stress condition. Malik and Hassan (2002) and Khanzada-Barkat *et al.* (2001) found the same result that in different wheat genotypes plant height was significantly reduced under drought stress.

2.5.1.2 Tiller number

Kabir *et al.* (2009) found that tiller no. was 9.81% higher in watered condition than the drought stress. Akram (2011) also found that when drought stress was severe, tiller number plant^{-1} reduced significantly. Subhani and Chowdhry (2000) also found the same result.

Malik *et al*. (2010) showed that when single irrigation was given, tiller production was very poor. Maximum tillers m^{-2} were produced when five irrigations was applied.

Kabir *et al*. (2009) conducted an experiment on four levels of irrigations. He found when two irrigations were given maximum tiller number was gained and lowest tiller number was found from no irrigation.

2.5.1.3 Dry matter

In heat dry matter reduced drastically due to the drought stress. Zhang *et al.* (2006) found the lowest dry matter content from no irrigation level. Kilic and Yagbasanlar (2010) reported that dry matter depends on plant growth and due to drought stress plant growth was reduced as well as dry matter production was also reduced. Chaudhary and Dahatonde (2000) also found the same result.

2.5.1.4 Other growth parameters

Crop growth rate, Leaf area index was decreased due to drought stress (Akram, 2011). He found 30.21% reduction of net assimilation rate due to drought.

Again, Subhani and Chowdhry (2000) noted that plant growth has a linear relationship with yield also it has a relationship with the photosynthesis. They mentioned that in drought stress flag leaf area, plant height, biomass per plant was reduced. Kabir *et al.* (2009) reported that under drought stress chlorophyll content, fresh weight, leaf number was also reduced.

2.5.2 Effect of drought stress on Physiology and metabolism

Kilic and Yagbasanlar (2010) found in their experiment that well watered plant requires more days to be matured and flowering, number of days to maturity was 3.8% higher in well watered plant, grain filling period was 10.2% higher than drought stressed plant.

Keyvan (2010) conducted his experiment with different level of irrigations and described that in drought stress chlorophyll a, chlorophyll b as well as total chlorophyll content decreased. He found 53.29% chl a, 53.73% chl b, and total chlorophyll content reduced upto 53.56% in drought stress. He also found that proline content increased and relative water content of flag leaf was decreased in drought stress treatment.

Tatar and Gevrek (2008) stated that in case of drought stress various physiological changes have been occured. They found that Relative water content was decreased where as proline content was increased to protect stressed plant.

Reddy *et al.* (2004) mentioned that in drought stress stomata become close and CO_2 uptake was reduced. He stated that it is an initial response to drought stress and due to this phenomena, photosynthesis of wheat is decreased.

Clarke *et al.* (1991) conducted two experiments in a glass house and in a summary he described that total water use by wheat was higher in low-stress treatment. He found about 96% water was used by the low-stress treatment whereas 62% was used by wheat in high-stress treatment but water use efficiency and mean residual

transpiration was indifferent for those treatments. RWC of flag leaf, osmotic potential and stomatal conductance was higher in low stressed plant.

Ali *et al.* (2013) found in his experiment that in drought stress physiological changes was happened but it vary from variet to varity which determine either the variety is resistant or susceptible to drought stress. In his experiment he used twelve wheat varieties with four irrigation level at fifteen days interval. He showed that electrolytes leakage was increased and other physiological characteristic such as turgidity, relative leaf water contents was decreased. In his study, lowest mean values of electrolytes leakage was 9.22% after irrigation of 60 DAS which increased to 13.5% and 15.5% after irrigation of 95 DAS and 120 DAS respectively. It was reported that under drought stress, plants maintain a relation between its physiological responses and tolerance mechanism like membrane stability (Datta *et al.* 2011), pigment content stability, relative water content etc. (Ghobadi *et al.* 2011).

2.5.3 Oxidative stress in wheat under drought

plants in drought condition is considered to promote antioxidants defense systems to face the increased levels of ROS, that is responsible for membrane damage by increasing lipid per oxidation, and it is one of the main reasons for cell damage (Shao *et al*., 2005). Badawi *et al*. (2004) mentioned the same opinion as he said when plant is in stressful condition, ROS is the important damaging factor.

Tatar and Gevrek (2008) reported that in case of drought stress wheat was affected by various oxidative stresses. They showed that in drought stress lipid per oxidation or MDA content was increased Dong et al., (2018) also mentioned that in drought stress malondialdehyde (MDA), and hydrogen-per-oxide (H_2O_2) was increased.

Sairam and Saxena (2000) stated that under drought stress oxidative stress causes a metabolic damage to plants. It increases lipid per oxidation which causes severe cell membrane damage to plants. Abedi and Pakniyat (2010) reported that in stress condition, growth and yield was reduced due to the increase of oxidative stress because accumulation of ROS, particularly O_2 and H_2O_2 in chloroplasts, mitochondria, and peroxisomes increased.

2.5.4 Antioxidant enzyme activities of wheat

Under stress condition, free radicles are developed. Various type of enzymatic and non-enzymatic antioxidant plays a crucial role to mitigate the stress. Tatar and Gevrek (2008) explained that proline is an osmo-protectants and helps plant to survive by reducing the ROS. Due to lack of soil moisture regular water uptake is hampered. In this case proline appeared in cell to increase the water uptake.

Ahmadizadeh *et al.* (2011a) compared 37 wheat verities performances under drought stress. He showed in his experiment that magnitude of mean performance for SOD and CAT increased and index of damage (ID) was decreased in drought stress. Average value of SOD increased 35.6% and CAT density increased 3.1% under stress condition. Bakalova *et al*. (2004) and Shao *et al*. (2005) also supported Ahmadizadeh *et al.* (2011a) said that SOD, CAT and other antioxidant enzyme activities increased in drought stress and it is higher in resistant variety than the susceptible one.

Very recent Dong *et al.* (2018) conducted an experiment to show the effects of drought stress on some physiological and biochemical Indexes of wheat seedlings. He found that in drought stress the activities of antioxidant enzymes like peroxidase (POD), proline (Pro), glutathione (GSH) content was increased in wheat seedlings.

Shabbir *et al.* (2016) showed that under drought stress catalase activity, proline content, and peroxidase activity was increased. He found proline increased 66%, 19% increase of catalase and 8% increase of peroxidase.

Varoius kind of management, use of external application of protectant like plant hormone, salicylic acid, gibberellin, ascorbic acid, nutrient like potassium (Wei *et al.*, 2013), zinc and salicylic acid (Yavas and Unay, 2016), boron (Abdel-Motagally and El-Zohri, 2016) helps to mitigate the drastic effect of drought stress (Hasanuzzaman *et al.*, 2018).

2.5.5 Yield and yield contributing parameters

Drought stress has a negative effect on yield of wheat. By reducing assimilates, tillers number, number of spike, no. of spikelet per spike, grain size, grain weight all are reduced due to the stress and ultimate result is poor yield (Hasanuzzaman *et al.,* 2013c, Hasanuzzaman *et al.,* 2018). Effect of drought stress is vary from genotype to genotype and in severe cases yield may reduced upto 50% (Hasanuzzaman *et al.,* 2018).

Chaudhary and Dahatonde (2000) conducted an experiment, where he found that grain yield didn't vary with the irrigation frequency but when wheat got sufficient amount of water according to its requirement, it gives higher yield.

Kilic and Yagbasanlar (2010) found 61% yield reduction in stressed condition as well as number of grain per plant, spike length, pedicle length, 1000 grain weight was higher in well irrigated condition.

A field study was conducted by Waraich *et al.* (2007) in two consecutive years 2002-2003 and 2003-2004 to find out the drought stress effect on wheat. He applied four levels of irrigations. Grain yield and others yield components was decreased linearly in response to drought stress. When he applied four irrigation level, mean grain yield was increased was 47% than the single irrigation. It was 23% and 9% for three irrigation and two irrigation level respectively during 2002-03 and in 2003-04 it was 91, 84 and 23% respectively. Water deficit reduced spikes m^{-2} and grains $spike^{-1}$ in both year.

Kabir *et al.* (2009) conducted an experiment with four level of irrigation to show the effect of yield and yield performance of wheat cv. Gourab under different level of irrigation. He found that yield was increased 46.36% than the drought stressed condition, straw yield was increased 26.89%, HI 44.67%, and spikelets $spike^{-1}$ was increased about 11.59%.

In a field experiment Akram (2011) applied four level of stress. He found spike length (11.57 cm); number of spikelets/spike (17.83) was higher where no stress was applied again it was lower where stress applied during stem elongation and anthesis period. He found about 22%% yield reduction due to drought stress.

Taheri *et al.* (2011) explained that severity of drought stress vary from crops to crops even in one crop it may vary variety to variety. That's why he conducted his experiment with 17 wheat lines with three level of drought condition. He determined

7 agronomical traits. He showed that grain yield, 1000 grain weight, biomass, main spike length and awn length reduced due to the drought stress.

This is also supported by the findings of Chander and Singh (2008). They found in his experiment that numbers of grains per spike were decreased under drought stress. Drought stress reduced the yield components, like number of grains per spike, number of spikes per plant, harvest index and so on. But they did not found any significant effect of drought stress on 1000 grain weight.

Ozturk and Aydin (2004) observed highest yield (4.4 t/ha) in full irrigated condition and the lowest yield was 1.5 t ha^{-1} found in continuous stress condition. They applied full irrigation (FI), rainfed (R), early water stress (EWS), late water stress (LWS) and continuous water stress (CWS) condition. 1000 grain weight was highest in FI and the reduction of grain number per unit area was in EWS 44.4 %, LWS 13.9% and CWS 54.9 % than FI.

2.5.6 Effect of drought stress on quality

The quality of strong gluten wheat was reduced with irrigation increasing (JI *et al.*, 2006). Kilic and Yagbasanlar (2010) conducted his experiment and showed that protein content increased in drought stressed condition.

A field experiment was conducted by Ozturk and Aydin (2004) to show the effect drought stress on winter wheat. They observed various yield contributing character as well as some qualitative characters too. They applied five level of stress - fully irrigated (FI), rainfed (R), early water stress (EWS), late water stress (LWS) and continuous water stress (CWS). They found that CWS increased grain protein content by 18.1 %, sedimentation volume by 16.5 %, wet gluten content by 21.9% whereas LWS caused an increase of 8.3 % in grain protein content, 8.7 % in sedimentation volume, 10.8 % in wet gluten content compared with FI treatment.

2.5.7 EFFECT OF DROUGHT STRESS ON DIFFERENT GROWTH STAGE OF WHEAT

Bukhat (2005) stated that drought stress is not vulnerable for all growth stage of crops. Some stages are susceptible that causes great losses to the crops and some are resistant to drought and has no effect on growth and yield. Drought stress reduces the plant biomass, reduce tiller number, grains number and so on and it may happen in any stage.

Chaudhary and Dahatonde (2000) found in his experiment that when irrigation was applied at CRI (crown root initiation) stage, jointing, flowering and milk stages and at tillering stage, it gave higher yield than any other stages. Water use efficiency was higher in this irrigation level.

Kabir *et al.* (2009) conducted his experiment with four levels of irrigations namely (i) no irrigation i.e. control, (ii) one irrigation given at Crown root initiation (CRI) stage, (iii) two irrigations given at CRI and Panicle initiation stages and (iv) three irrigation given at CRI, panicle initiation and grain filling stages. He found higher plant height and higher yield when irrigation was applied in CRI stage than the other two levels or three levels of irrigations. Plant height, seed yield, straw yield, spike length was higher when irrigation was applied at CRI stage.

Keyvan (2010) showed that when he applied four levels of irrigations like I_1- drought stress at the start of stem elongation stage, I_2 - drought stress at the start of boot stage, I_3 - drought stress at the start of grain filling stage and I_4 - full irrigation, he found that stem elongation stage is more critical than others. Chlorophyll content, relative water content is sensitive in this stage. When drought stress is in reproductive stage it may cause a reduction in grain number and weight (Gupta *et al.*, 2001).

Akram (2011) used four drought treatments i.e., T_0 control (No stress imposed), T_1 (drought stress was imposed at stem elongation stage, T_2 (Water stress imposed at anthesis stage), T_3 (Water stress imposed at stem elongation and anthesis stage) on wheat. He found LAI (leaf area index) was decreased in T_3 treatment though number of tillers, fertile tillers, spike length and number of spikelets/spike reduction was statistically similar with T_1 and T_3. He found that drought stress hampered plant in mostly when it imposed at stem elongation and anthesis stage though the damage for T_1 and T_3 is statistically similar.

Ashraf (1998) said anthesis stage is more critical for drought stress in wheat because if drought stress is imposed in this stage, pollination is reduced and grain number is also reduced. Zhang and Oweis (1999) also said that if drought stress is happened in anthesis or after anthesis, it reduces the yield because at this stage soil moisture allows plants to increase p0hotosynthesi and translocation of carbohydrate to grains also increased.

Taheri *et al.* (2011) treated the wheat plant in his experiment with three different level of drought stress. 1st one was normal irrigation, where the plots were irrigated with approximately 10 day intervals throughout the growing season, 2nd one was moderate stress (after anthesis drought stress condition, where stress was after the heading of the wheat) and 3rd one was no irrigation, no irrigation after germination (intensive stress). He found that most of the yield contributing parameters was highly affected by drought stress during anthesis period. Taheri *et al.* (2011) stated that grain filling stage is a sensitive stage for wheat because in this stage if plant is in water shortage, carbon source, reservation or assimilates become limited for the grain development. Schneekloth *et al.* (2009) said that yield reduced mostly when drought stress is in heading/flowering or dough condition. In this stage drought may cause 10% yield reduction but moderate stress in early vegetative stage has no significant effect.

2.6 Gibberellic acid

Gibberellic acid is also known as Gibberellin A3, GA, and GA_3). It is a hormone that founds in both plant and fungi. Chemical formula of GA is $C_{19}H_{22}O_6$. Pure GA is white to pale yellow color and solid in nature (Silva *et al.*, 2013). Its IUPAC name is 3S,3aS,4S,4aS,7S,9aR,9bR,12S)-7, 12-dihydroxy-3-methyl-6-methylene-2oxoperhydro -4a,7-methano-9b,3-propenoazuleno[1,2 b]furan-4-carboxylic acid, molar mass is 346.37 g mol^{-1}, melting point 233-235^{0}C and solubility in water is 5 g L^{-1} at 20^{0}C.

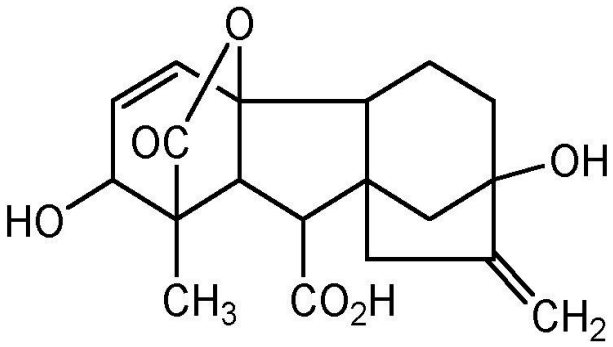


Figure 1. Chemical formula of gibberellic acid

GA_3 was first identified in Japan in 1930, from a plant pathogen named *Gibberella fujikuroi* which is responsible for a rice disease called BAKANAE *disease* ("foolish seedling"). It makes plant so much taller than normal seedlings that they cannot support themselves resulting in lodging and died (Gupta and Chakrabarty, 2013). Gibberellic acid (GA) is used in mainly forstimulating plant growth and development as it is a hormone. It helps to plant in seed germination, shoot growth, flowering, determining sex expression, and grain development. It has also an interaction with different environmental factors.

2.6.1 EFFECT OF GIBBERELLIC ACID ON GROWTH

2.6.1.1 Seed germination

Gibberellic acid helps to seed germination by breaking the seed dormancy. Seed dormancy or germination depends on some factors like light, temperature, moisture, and some hormone and enzymes. Gibberellic acid and abscisic acid (ABA) is one of the most important growth regulating hormones. GA stimulates the seed germination whereas, ABA is involved in the establishment and maintenance of dormancy (Debeaujon and Koornneef, 2000 and Gupta and Chakrabarty, 2013).

GA plays role in germination by two ways. Firstly it increases the growth potential of embryo and then it induces the function of hydrolytic enzymes (Ogawa *et al.,* 2003, Kucera *et al.,* 2005). Another thing is GA biosynthesize and shows its response in embryo and in aleurone layer and helps to develop shoot cell division or elongation by upregulating the expression of α-amylase gene. This α-amylase gene is synthesized in aleurone layer (Gubler *et al.,* 2002).

2.6.1.2 Stem elongation

It is stated that GA plays an important role in stem elongation and it is proved by the physiologist (Ross *et al.,* 1997). It cell division and expansion in response to light or dark that allow plant to internode elongation (Alabadí *et al.,* 2008; Feng *et al.,* 2008). The GA biosynthetic pathway is a complex pathway (Gallego-Bartolomé *et al.,* 2011) and it is very hard to understand the actual site of GA biosynthesis in plants. Very little is known about this and still we have to understand the actual signal transduction pathway that is associated with the stem elongation of plants (Gupta and Chakrabarty, 2013). Sakamoto *et al.* (2001) stated that GA biosynthesis has a relationship with cell fate determination. A protein named NTH15 is present at the corpus region of the shoot apical meristem and when its activity is under controlled GA starts its function to stimulate cell division and enlargement. It was reported that in *Arabidopsis* GA synthesis occurred in growing tissue (Silverstone *et al.,* 1997). Li and He (2013) stated that GA release DELLA protein which helps plant to cell elongation.

2.6.1.3 GA in the Flowering and Sex Expression

GA has a role in floral development and it can determine the male flower or female flower. The development of floral part or flower inducing mostly depends on its concentration (Gupta and Chakrabarty, 2013). Goto and Pharis (1999) said that in *Arabidopsis* plant GA requires higher concentration to develop stamens than any other floral part. Griffiths *et al.* (2006) explained that GA is required for the flower initiation and flower fertility. If GA is deficient in tomato or *Arabidopsis* stamen development become abnormal (Chhun *et al.,* 2007 and Hu *et al.,* 2008) and in extreme deficiency condition female flower remains in sterile condition. GA deficiency may increase the non-viable pollen (Nester and Zeevaart, 1988), undeveloped floral parts like sepals, petals, and pistils (Goto and Pharis, 1999) and sometimes flower abortion (Chhun *et al.*, 2007)

In case of rice same result was also reported. GA plays a role in pollen germination and pollen tube development and it is mediated in early anther development (Chhun *et al.*, 2007).

GA helps in sex expression in plants but it may vary from crops to crops and with concentration. In cucumber repeated use of GA induce male flower where as in lower concentration it produce female flower in bitter gourd and improves fruit quality (Banarjee and Basu, 1992). It was reported that GA induced pistillate flower in castor bean, corn and hyoscyamus (Gupta and Chakrabarty, 2013).

2.6.1.4 Effect of GA on yield

GA has a role in increasing yield as we know it helps to initiate flowering (Gupta and Chakrabarty, 2013).

Abdel and AL-Rawi, (2012) showed that 200mg/L GA application increase the total yield and yield components of lentil. In the experiment three verities of lentil was used. In all cases GA gave higher yield and seed yield per plant increased 7.85% and harvest index was 14.94% higher than no application of GA though 1000 seed weight was not significantly changed.

GA has a positive effect on chickpea. It helps to increase the branch number that is associated with yield increase (Iqbal *et al.*, 2001; Hasanuzzaman *et al.*, 2007).
Emongor *et al.* (2007) found the same result by spraying GA on cowpea. GA increased the nodulation number, leaf area index, 1000 seed weight, total yield and harvest index. Takahashi and Kobayashi (1991) described that GA plays a positive role on rice. In dwarf variety it is not visible but in case of normal genotypes GA increased growh and yield.

GA has an important role in growth and yield. It helps to promote growth, flowering and yield, and it is proved in case of mustard (Khan *et al.*, 2002), mungbean (Mohammed, 2007), Potato (Sharma *et al*., 1998).

Uddain *et al.* (2009) conducted an experiment at Horticulture Farm in Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. In his experiment, he used different growth regulators including GA. He found higher number of fruit clusters, fruits plant^{-1} and individual fruit weight due to the application of GA. He found 11.24% higher yield than no application of plant growth regulators.

2.6.2 Effect of GA on stress mitigation

Under drought condition, plant growth regulators improved nutrient uptke, physiology, and metabolic activities of plant. Sang-Mo *et al.* (2014) stated that in drought condition GA mitigated the adverse effect of drought and improved plant growth in soybean. Cohen *et al.* (2009) supported this and said that in maize gibberellin producing *Azospirillum lipoferum* alleviate the drought stress.

Gibberellic acid works as a protectant in stress condition. It has potentiality to scavenge the ROS. Aktas *et al.* (2008) stated that in drought stress GA helps plants with their more negative water potentiality and maintaining photochemical efficiency of PSII. All these features of GA help plant to survive in drought condition.

It is assumed that GA helps plant in stress condition by increasing the nutrient uptake. GA increases the nitrogen use efficiency in stress condition that helps plant to adjust with the adverse condition (Iqbal *et al.*, 2001).

Singh *et al.* (2005) gave the same opinion an also said that GA increasd the chlorophyll content and mineral nutrients uptake that also helped in stress mitigation. In wheat, under saline stress condition GA helped plant by modulation of ions uptake, root-shoot partitioning and hormones homeostasis (Iqbal and Ashraf, 2013).

In case of rice plant growth regulators like NAA-Na, GA_3 or 6-BA improve the photosynthetic ability, and decrease the leaf senescence and helps to increasing the seed-setting in different environmental condition (Li *et al.*, 2010). Pan *et al.* (2013) conducted an experiment and showed that different growth hormones like gibberellic acid (GA_3), paclobutrazol (PBZ), 6-Benzylaminopurine (6-BA) played a role as an Antioxidant enzymes aid that helped in deleting ROS. He showed that after GA applying SOD, POD activities were increased and MDA content decrease. Achard *et al.* (2008) stated the same thing that GA helps in regulating the ROS level.

Day to day role of GA in response to abiotic stress is increasing but role of GA incase of drought stress has been relatively little published (Colebrook *et al.*, 2014).

CHAPTER 3

MATERIALS AND METHODS

This chapter deals with a brief description on experimental site, climate and soil, land preparation, layout, experimental design, intercultural operations, data recording and their analyses.

EXPERIMENT NO. 1

3A.1 Site description

This experiment was conducted at the Sher-e-Bangla Agricultural University farm, Dhaka, under the Agro-ecological zone of Modhupur Tract, AEZ-28 during the November, 2016 to February, 2017. The land area is situated at 23°41′N latitude and 90°22′E longitude at an altitude of 8.6 meter above sea level. The experimental site is shown in the AEZ Map of Bangladesh in Appendix I.

3A.2 Climate

Sher-e-Bangla Agricultural University farm, Dhaka is under the sub-tropical climate with high temperature and high humidity and heavy rainfall. Occasional gusty winds in kharif season (April-September) and less rainfall associated with moderately low temperature during the Rabi season (October-March) is visible. The weather data of the experimental site recorded by the meteorology center, Dhaka for the the study period is shown in Appendix II.

3A.3 Soil

The farm belongs to the General soil type, Shallow Red Brown Terrace Soils under Tejgaon Series. Top soils were clay loam in texture, olive-gray with common fine to medium distinct dark yellowish brown mottles. The experimental area was flat having available irrigation and drainage system. The land was above flood level and sufficient sunshine was available during the experimental period. Soil samples from 0-15 cm depths were collected from experimental field. The analyses were done by Soil Resources and Development Institute (SRDI), Dhaka. The physicochemical properties of the soil are presented in Appendix III.

3A.4 Materials

3A.4.1 Plant materials

In experiment-1 BARI Gom-30 was used. It was released in 2014. It was developed by crossing BAW-677 and Bijoy (BARI Gom-23).

Characteristics of BARI Gom-30:

Plant height: 95-100 cm.

Duration: 100-105 days.

Grain no. spike^{-1}: 45-50.

1000-seed weight: 44-48 g.

Seed: white, shiny and medium in size.

Yield: 4.5-5.5 t ha^{-1}.

This variety is short duration, resistant to high temperature, leaf spot and rust, and blast disease of wheat. It takes 57-62 days to spike initiation.

3A.4.2 Fertilizer doses

Fertilizer	Doses (kg/ha)
Urea	250
TSP	140
MOP	100
Gypsum	110
Zinc sulphet	10
Boric acid	0.5

Source: Krishi Projukti Hatboi, BARI, Joydebpur, Gazipur, 2016

3A.5 Treatments

Factor A (Main plot): Gibberellic acid (2)

a. No gibberellic acid **(G_0)**

b. 100 ppm gibberellic acid **(G_1)**

Factor B (Sub-plot): Water stress (8)

a. Full stress condition **(T_0)**
b. No stress **(T_1)**
c. Stress at CRI stage **(T_2)**
d. Stress at flowering stage **(T_3)**
e. Stress at grain development stag (**T_4)**
f. Stress at CRI and flowering stage **(T_5)**
g. Stress at CRI and grain development stage **(T_6)**
h. Stress at flowering and grain development stage **(T_7)**

3A.6 Design and layout of the experiment

Experimental Design : Split- plot design
No. of Replications : 3
Total No. of Plots : 48
Plot Size : (2.70 *2) m^2
Time of Experiment : November, 2016 – February, 2017
Seed rate : 120 kg/ha

3A.7 Seed collection

Seeds of BARI Gom–30 were collected from Wheat Research Center at Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3A.8 Preparation of experimental land

The land was first ploughed on 7 November, 2016 by tractor. The land was then harrowed again on 12 and 13 November to bring the soil in a good tilth condition. The final land preparation was done on 14 November, 2016. The land was leveling and laddering, weeds and stubbles were removed from the field. The experiment was laid out on 15 November, 2016 according to the experimental design.

3A.9 Sowing of seeds

Seeds were sown on 15 November, 2016 by hand and it was in line sowing method. When land is in proper "Joe" condition, furrows were made and watering was done in the line and wheat seeds were sown. Seeds were then covered properly with soil by hand. The line to line distance for wheat was 20 cm and plant to plant distance was 4 - 5 cm.

3A.10 Intercultural operations

Seed germination was started after 3 days of sowing. After seed germination various kinds of intercultural operations were done like thinning, weeding, irrigation, mulching and most importantly taking plant protection measures.

3A.10.1 Application of fertilizers and manure

All fertilizer except urea was applied at basal dose during final land preparation. Urea fertilizer was applied at three installments. First portion was applied at basal dose with other fertilizers, second one was given during CRI stage and third one was given before flowering stage.

The doses of fertilizer per plot are given below:

Fertilizer	Doses (g/plot)	application method
Urea	220*3	1st dose at basal application, 2nd dose at CRI stage, 3rd one was before flowering
TSP	115	at basal application
MOP	85	at basal application
Gypsum	95	at basal application
Zinc sulphet	10	at basal application

Source: Krishi Projukti Hatboi, BARI, Joydebpur, Gazipur, 2016

3A.10.2 Gap filling and thinning

As germination was vigorous no need to gap filling but I had to pull up some seedlings to maintain proper population in a row. Thinning out was done at two times. One is after 12 days of germination and second one was after 19 days of germination.

3A.10.3 Weeding

There were so many weeds prominent in the research field like kakpaya ghash (*Dactyloctenium aegyptium* L.), Shama (*Echinocloa crussgalli*), Durba (*Cynodon dactylon*), Mutha (*Cyperus rotundus* L.) Bathua (*Chenopodium album*) Shaknatey (*Amaranthus viridis*), Foska begun (*Physalis beterophylls*), Titabegun (*Solanum torvum*), and so on. Weeding was done in three times. First one was 19 DAS (day after sowing) with thinning operation, second one was at 30 DAS and last one was 55 DAS.

3A.10.4 Mulching

Mulching was done by soil during weeding time. As weeding was done by hoe, soil was uplifted between the two lines by making a furrow.

3A.10.5 Plant protection measures

While the plant was in seedling condition, severe root rot disease was occurred. At that time fungicide was applied three times with a seven days interval.

3A.10.6 Irrigation and Gibberellic acid spraying

As my treatments were irrigation related, irrigation was applied very carefully. First irrigation was given at 20 DAS or CRI stage, second irrigation was given at 45 DAS or at flowering stage and third irrigation was given at grain development stage or 80 DAS. First irrigation was applied to T_1, T_3, T_4 and T_7. Second irrigation was applied to T_1, T_2, T_4, T_6 and third irrigation was applied to T_1, T_2, T_3 and T_5. As T_0 was full stress condition, no irrigation was applied to it except the germination time. Irrigation was applied by using an 8 L watering can. In each plot it was applied 8*4= 32 L water. GA was spraying @ 150 mg L^{-1} dose. It was spraying very carefully to ensure the foliar application. As all we know CRI stage is critical stage for seedling establishment and growth that's why GA was applied after irrigation at 20 DAS.

3A.10.7 General observation of the experimental field

Except the regular intercultural operations, I observed my research plot time to time to find out the visual difference among the treatments and to protect plant from different types of insects, pests and diseases.

3A.11 Harvesting and post harvest operation

Maturity of wheat was determined when 90% of the plants became golden yellow color. Harvesting was done at 23 February, 2017. Middle four lines was carefully harvested and separated. They were properly tagged and brought to the threshing floor for recording data. Before threshing plants were dried properly and then threshed by using pedal thresher. The grains were cleaned and sun dried to a moisture content of 12%. Straw was also sun dried properly.

3A.12 Data collection

Grain yield and straw yield was collected from middle four lines from the plot. Four lines were collected separately and bundled. Then this yield was converted to ton/ha. Growth parameters were collected from 25cm length of second line of left side. Ten plants were marked by binding red rope to identify the same plants for data collection. Destructive harvest data were collected from 25 cm area of second line of right side.

3A.12.1 Crop growth characters

i. Plant height (cm)
ii. Number of tillers plant^{-1}
iii. Leaves number plant^{-1}
iv. Dry matter plant^{-1} (above ground portion) (g)
v. SPAD value
vi. Days of 50% flowering
vii. Translocation factor (%)
viii. Absolute growth rate (%)

3A.12.2 Yield and yield components

i. Length of spike (cm)
ii. Number of spikelets spike^{-1}
iii. Number of grains spike^{-1}
iv. Pedicel length
v. Weight of 1000 grains (g)
vi. Grain yield (t ha^{-1})
vii. Straw yield (t ha^{-1})
viii. Husk yield (t ha^{-1})
ix. Harvest index (%)
x. Biological yield (t ha^{-1})

3A.12.3 Stress determining parameters

i. Stress intensity %

3A.13 Procedures of recording data

An outline of data recording procedure is given below:

Data was collected very carefully, growth parameters, destructive harvest was done with a regular interval. Crop growth characters were measured total four times; 20 DAS, 45 DAS, 70 DAS and finally during harvest. Destructive harvest was done at CRI stage, flowering stage and during harvest time

3A.13.1 Crop growth characters

3A.13.1.1 Plant height

Plant height was measured at 25 days interval starting from 20 days after sowing (DAS) and continued up to harvest. The height of the plant was determined by measuring the distance from the soil surface to the tip of the leaf before heading, and to the tip of spike after heading. The collected data were finally averaged.

3A.13.1.2 Number of tillers plant^{-1}

It was very tough to identify the main plant and the tiller in case of wheat. To identify the accurate number of tiller firstly it was selected 25 cm area of second row in each plot. Then the plant number in that area was counted at 20 DAS. During harvest time the plant number was again counted. Then the tiller number was calculated by this equation:

$$\text{Tiller numbers per plant} = \frac{(\text{plant no. during hurvest} - \text{plant no. at CRI stage})}{\text{total number of plant in 25 cm area}}$$

3A.13.1.3 Number of leaves plant^{-1}

The leaves of each plant were counted during the data collection procedure. Leaf number was counted for ten plants then it was calculated as average number leaves plant^{-1}.

3A.13.1.4 Dry weight plant^{-1} (above ground part)

Ten plants at different stage i.e. at CRI stage, flowering stage and during harvest time were collected and dried at air for one day then it was oven dried at 70° C for 36 hours. The dried samples were then weighed and averaged.

3A.13.1.5 SPAD value

SPAD value was measured with the help of spadometer instruments. The top, middle, and bottom of each leaf blade were measured with this instrument. Then it was averaged and counted as chlorophyll content.

3A.13.1.6 Absolute growth rate (AGR)

Absolute growth rate (AGR) was calculated by following formula:

$$\text{Absolute growth rate (AGR)} = \frac{\text{plant height at harvest} - \text{plant height at 20DAS}}{\text{Harvesting days} - 20\ \text{DAS}}$$

3A.13.2 Procedure of measuring yield and yield contributing parameter

3A.13.2.1 Spike length

Spike length was counted from ten plants from basal node of the rachis to apex of each spike and then averaged. It was measured at harvesting time.

3A.13.2.2 Number of spikelets spike^{-1}

Number of spikelets were counted from 10 spikes and averaged to determine the number spikelets spike^{-1}.

3A.13.2.3 Number of grains spike^{-1}

The number of grains spike^{-1} was counted from 10 spike and number of grains spike^{-1} was measured by the following formula:

$$\text{No. of grains per spike} = \frac{\text{Total number of grains}}{\text{Number of spike}}$$

3A.13.2.4 Weight of 1000 grains

Spikes were dried and thrashed then 1000-seed grains were cleaned and counted. Then these seed was measured with an digital electric balance.

3A.13.2.5 Grain yield

Grain yield was determined from the central four line of each plot. They were dried and thrashed and grain straw and husk was separated. Then the grain was weighted and expressed as t ha^{-1}.

3A.13.2.6 Straw yield

Grain yield was determined from the central four line of each plot. They were dried and thrashed and grain straw and husk was separated. Then the straw was weighted and expressed as t ha^{-1}.

3A.13.2.7 Biological yield

Biological yield was calculated by using the following formula:

$$\text{Biological yield} = \text{Grain yield} + \text{straw yield}$$

3A.13.2.8 Harvest index

Harvest index is the ratio of economic yield to biological yield and was calculated following the formula:

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Biological yield}} * 100$$

3A.13.3 Stress determining parameters

3A.13.3.1 Stress intensity %

Stress intensity % was measured by following formula:

$$\text{Stress intensity \%} = 1 - \frac{\text{yield at stress condition}}{\text{yield at non-stress condition}} * 100$$

Materials and methods for experiment: 2

3B.1 Experimental location

The experiment was conducted at Laboratory of Plant stress responses, Kagawa University; Kagawa, Japan during the period from March 2017 to August, 2017.

3B.2 Plant materials

In experiment-2 BARI Gom-21 was used. It was released in 2000.

Characteristics of BARI Gom-21:

Plant height: 90-100 cm.

Grains no. spike^{-1}: 40-45

1000-seed weight: 46-48 g.

Seed: white, shiny and large in size.

Crop duration: 105 days

Yield: 3.6-5 t ha^{-1}.

This variety is resistant to high temperature, leaf spot and rust.

3B.3 Plant materials, growing condition and stress treatments

Healthy uniform Wheat (*Triticum aestivum* cv. BARI Gom-21) seeds were selected and thoroughly washed with distilled water after sterilization by 70% ethanol. Seeds were then sown in Petri dishes (9 cm) lined with six layers of filter paper moistened with 10 ml of distilled water and kept in dark at germinator for 48 hours. All Petri dishes contained morphologically uniformed 40 germinated seedlings. Then seedlings were grown in growth chamber (IWAKI, Asahi Techno Glass, Japan) under controlled conditions (light: 350 µmol photons $m^{-1}s^{-2}$; temperature: 25±2°C; relative humidity: 65–70%) by using 50% Hogland solution as a nutrient. Full strength nutrient solution contained 8% N, 6.43% P, 20.94% K, 11.8% Ca, 3.08% Mg, 0.07% B, 0.24% Fe, 0.03% Mn, 0.0014% Mo, 0.008% Zn, and 0.003% Cu.

Seven days old seedlings were subjected to drought stress by using 12% of polyethylene glycol (PEG-6000) in Hogland solution and grown under the above conditions for 48 h and 72 h. GA (100ppm) and water was spraying while the drought stress was imposed.

After 48 h of treatment, seedlings were collected and used for the study of various growth and physiological parameters and after 72 h same procedure was followed to take growth and physiological parameters. The study was carried out following a Completely Randomized Design (CRD) with twelve treatments and repeated three times under similar condition.

3B.4 Experimental treatments:

1. Control, 48 h
2. Control, 72 h
3. Control, 48h+ water spray
4. Control, 72 h+ water spray
5. Control, 48 h+ GA (100 ppm)
6. Control, 72h + GA (100 ppm)
7. 12% PEG, 48h
8. 12% PEG, 72 h

9. 12% PEG, 48h + water spray
10. 12% PEG, 72h +water spray
11. 12% PEG, 48h + GA (100 ppm)
12. 12% PEG, 72h + GA (100 ppm)

3B.5 Collection of data:

3B.5.1 Crop growth parameter

i. Plant height
ii. Fresh weight plant^{-1}
iii. Dry weight plant^{-1}

3B.5.2 Physiological parameters

i. Relative water content (RWC)
ii. Photosynthetic pigment

3B.5.3 Oxidative stress indicators:

i. Lipid peroxidation
ii. H_2O_2 content
iii. Proline content
iv. Methylglyoxal content
v. Ascorbate content
vi. Glutathione content
vii. Activities of antioxidant enzymes (CAT, APX, MDHAR, DHAR, GR, GPX, Gly I and Gly II)

3B.5.4 Fresh weight and dry weight of seedling

For fresh weight and dry weight measurement, 10 seedlings from each treatment were selected. These selected seedlings were uprooted carefully, weighed in a digital balance (except the root portion); data were recorded and considered as fresh weight (FW). Dry weight (DW) was determined after drying the seedlings at 80°C for 48 h.

3B.5.5 Estimation of lipid peroxidation

To estimate lipid peroxidation, malondialdehyde (MDA) content was measured according to Heath and Packer (1968) with a slight modification by Hasanuzzaman *et al.* (2012b). Leaf samples (0.5 g) were grounded in 5% (w/v) trichloroacetic acid (TCA) and the homogenates were centrifuged at 11,500×*g* for 15 min. The supernatant was then mixed with thiobarbituric acid (TBA) and heated at 95°C for 30 min in a water bath. After cooling the supernatant, absorbance was read at 532 nm. MDA content was calculated by using extinction coefficient 155 $mM^{-1}cm^{-1}$ and expressed as n mol of MDA g^{-1} FW.

3B.5.6 Determination of Methylglyoxal Content

Methylglyoxal was measured according to the method of Wild *et al.* (2012). 5% perchloric acid was used to homogeniz the leaf. They were centrifuged at 4 °C for 10 min at 11,000×g.

Charcoal was added to decolorize the supernatant a saturated solution of sodium carbonate was added to neutralize it at room temperature. MG was estimated from this supernatant by adding sodium dihydrogen phosphate and N-acetyl-L-cysteine to a final volume of 1 ml. Formation of the product N-α-acetyl-S-(1-hydroxy-2-oxoprop-1-yl) cysteine was recorded after 10 min at a wavelength of 288 nm, and the MG content was calculated using a standard curve of known concentration.

3B.5.7 Measurement of H_2O_2

H_2O_2 was assayed according to the method described by Yu *et al.* (2003). H_2O_2 was extracted by homogenizing 0.5 g of leaf samples with 3 ml of 50 mM potassium-phosphate (K-P) buffer (pH 6.5) at 4°C. The homogenate was centrifuged at 11,500× g for 15 min. Three ml of supernatant was mixed with 1 ml 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 measured spectrophotometrically at 410 nm to determine the H_2O_2 content using extinction coefficient 0.28 $\mu M^{-1}cm^{-1}$ and expressed as $nmolg^{-1}$ fresh weight.

3B.5.8 Determination of Leaf Relative Water Content

Relative water content (RWC) was measured according to Barrs and Weatherly (1962). Leaf laminas from randomly chosen plants were taken. Leaves were weighed as FW and then immediately floated on distilled water in a petri plate for 8 h in the dark. Turgid weights (TW) of leaves were obtained after removing excess surface water with paper towels. Dry weights (DW) of leaves were measured after drying at 80°C for 48 h. Then, RWC was calculated using the following formula

$$RWC\ (\%) = [(FW-DW)/\ (TW-DW)] \times 100$$

3B.5.9 Determination of Proline (Pro) Content

Free Pro in leaf tissues was measured following the protocol of Bates *et al.* (1973). Fresh leaf tissue (0.25 g) was homogenized well in 5 ml of 3% sulfo-salicylic acid on an ice cooled morted on ice. The homogenate was centrifuged at 11,500×g for 15 min. 2 ml of the supernatent was than mixed with 1 ml of acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) and 1 ml of glacial acetic acid. The mixture was placed at 100°C in water bath for 1 h, then transferred in to test tube and kept in ice to be cooled, after a while when it was cooled, 2 ml of toluene was added 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 using toluene as a blank. The amount of Pro was calculated from the standard curve using laboratory grad Pro.

3B.5.10 Determination of Chlorophyll Content

A fresh leaf sample of 0.25g was taken from randomly selected seedlings to measure the Chlorophyll (Chl) content. The samples were homogenized with 10 ml of acetone (80% v/v) using pre-cooled pestle and mortar and the homogenate was centrifuged at 10,000 × g for 10 min. The absorbance of the supernatants was measured with a UV-visible spectrophotometer at 663 and 645 nm for chl a and chl b respectively. Chl contents were calculated using the equations proposed by Arnon (1949).

3B.5.11 Histochemical detection of H_2O_2 and $O_2^{\bullet-}$

In stress condition $O_2^{\bullet-}$ locating in leaf that was detected following Chen *et al.* (2010) with slight modification. 0.1% 3-diaminobenzidine (DAB) and 0.1% nitrobluetetrazolium chloride (NBT) solution were used to stain the leaf for H_2O_2 and $O_2^{\bullet-}$ detection, respectively. Leaves were stained in those solutions for 24 h under a dark condition. Incubated leaves were then blenched by immersing in boiling ethanol. Brown spots were detected as H_2O_2 due to the reaction with DAB and blue spots were $O_2^{\bullet-}$ produced by reacting with NBT (Thordal-Christensen *et al.*, 1997). Photographs were then taken by placing the leaves on glass.

3B.5.12 Extraction and analysis of ascorbate and glutathione

Five percent meta-phosphoric acid was kept in ice and fresh wheat leaves of 0.5 g was measured. Then these leaves were homogenized with 3 mL ice-cold 5% meta-phosphoric acid and 1mM ethylenediaminetetraacetic acid (EDTA) using a mortar and pestle. The homogenate was centrifuged at 11,500 × g for 12 min at 4 °C, and the supernatant was collected to analyze for AsA and GSH. Ascorbate content was measured according to the method of Huang *et al.* (2005) with some modifications. The supernatant was collected and neutralized with 0.5 M K-P buffer (pH 7.0). The oxidized fraction was reduced by 0.1 M dithiothretitol. AsA was assayed spectrophotometrically at 265 nm in 100 mM K-P buffer (pH 7.0) with 0.5 units of ascorbate oxidase (AO). A specific standard curve of AsA was used for quantification.

The GSH pool was assayed according to a previously described method (Yu *et al.*, 2003) with modifications as described by Paradiso *et al.* (2008). Aliquots (0.2 mL) of supernatant were neutralized with 0.3 mL of 0.5 M K-P buffer (pH 7.0) and GSH is oxidized by 5, 5-dithio-bis (2- nitrobenzoic acid) (DTNB) and reduced by nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of GR. GSH content was evaluated by the rate of absorption changes at 412 nm of 2-nitro-5-thiobenzoic acid (NTB) generated from the reduction of DTNB. Oxidized glutathione (GSSG) was determined after removing GSH by 2-vinylpyridine 31 derivatization. Standard curves with known concentrations of GSH and GSSG were used. The content of GSH was calculated by subtracting GSSG from total GSH.

3B.5.13 Determination of protein

Protein concentration of each sample was measured following the method of Bradford (1976) using BSA (Bovin Serum Albumin) as standard.

3B.5.14 Enzyme extraction and assays

Using a pre-cooled mortar and pestle, 0.5 g of wheat leaf tissue was homogenized in 1 ml of 50 mM ice-cold K-P buffer (pH 7.0) containing 100 mM KCl, 1 mM ascorbate, 5 mM β-mercaptoethanol and 10% (w/v) glycerol. The homogenates were centrifuged at 11,500× g for 15 min and the supernatants were used for determination of enzyme activity. All procedures were performed at 0−4^{0}C.

3B.5.15 Ascorbate peroxidase (APX, EC: 1.11.1.11)

According to Nakano and Asada (1981) APX (EC: 1.11.1.11) activity was calculated. 50 mM K-P buffer (pH 7.0), 0.5 mM AsA, 0.1 mM H2O2, 0.1 mM EDTA were used as reaction buffer with enzyme extract and final volume owas 700 µl.

Absorbance was in decreaseing trend and collected at 290 nm for 1 min using an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$.

3B.5.16 Catalase (CAT, EC: 1.11.1.6)

CAT (EC: 1.11.1.6) activity was assayed according to Hasanuzzaman *et al.* (2012b) by observing the decrease in absorbance at 240 nm for 1 min caused by the decomposition of H_2O_2. The reaction mixture contained 50 mM K-P buffer (pH 7.0), 15 mM H_2O_2, and enzyme solution in a final volume of 700 µL. The reaction was initiated with the enzyme extract and activity was calculated using extinction coefficient 39.4 $M^{-1}cm^{-1}$.

3B.5.17 Monodehydroascorbate reductase (EC: 1.6.5.4)

It was measured according to the method of Hossain *et al.* (1984). For determination of MDHAR activity, distilled water, reaction buffer containing 50 mM Tris-HCl buffer (pH 7.5), 0.2 mM NADPH, 2.5 mM AsA, 0.5 units of AO (reaction initiator), and enzyme solution were used and final volume was 700 µL. Absorbance was read at 340 nm for 1 min. MDHAR activity was calculated using an extinction coefficient of 6.2 mM^{-1} cm^{-1} and expressed as nmol min^{-1} mg^{-1} protein.

3B.5.18 Dehydroascorbate reductase (EC: 1.8.5.1)

Its activity was measured by the procedure of Nakano and Asada (1981). 50 mM K-P buffer (pH 7.0), 2.5 mM GSH, 0.1 mM EDTA, and 0.1 mM dehydro ascorbic acide (DHA) was used as reaction buffer. To determine DHAR, distilled water, buffer solution, DHA, and enzyme solution was mixed and change in absorbance was read at 265 nm for 1 min. Activity of DHAR was calculated using an extinction coefficient of 14 mM^{-1} cm^{-1} and expressed as nmol $min^{-1}mg^{-1}$ protein.

3B.5.19 Glutathione Reductase (GR, EC: 1.6.4.2)

GR (EC: 1.6.4.2) assayed was done by the method of Hasanuzzaman *et al.* (2011b). The reaction mixture contained 0.1 M K-P buffer (pH 7.0), 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH, and enzyme solution in a final volume of 1 ml. changes in absorbance at 340 nm was recorded for 1 min. The activity was calculated using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$.

3B.5.20 Glyoxalase I (Gly I, EC: 4.4.1.5)

It was measured according to Hasanuzzaman *et al.* (2011a). the assay mixture contained 100 mM K-P buffer (pH 7.0), 15 mM magnesium sulphate, 1.7 mM GSH and 3.5 mM MG in a final volume of 700 µ l. The reaction was started by the addition of MG and the increase in absorbance was recorded at 240 nm for 1 min. The activity was calculated using the extinction coefficient of 3.37 $mM^{-1}cm^{-1}$.

3B.5.21 Glyoxalase II (Gly II, EC: 3.1.2.6)

Glyoxalase II (EC: 3.1.2.6) activity was determined according to the method of Principato et al. (1987) using the mixture of 100 mM Tris-HCl buffer (pH 7.2), 0.2 mM DTNB, and 1 mM *S*-D-lactoylglutathione (SLG). The change in absorbance was recorded at 412 nm. Glutathione formation was observed at 412 nm for 1 min and Gly II activity was calculated using an extinction coefficient of 13.6 mM^{-1} cm^{-1}. It is expressed as µmol $min^{-1}mg^{-1}$.

3B.5.22 Statistical analysis

The data obtained for different parameters were statistically analyzed following computer based software XLSTAT 2016 (AddinSoft, 2016) and mean separation was done by DMRT at 5% level of significance.

Chapter 4

RESULTS AND DISCUSSION

Experiment: 1

This experiment was conducted to study the growth and yield of wheat affected by GA and drought stress on different growth stage of wheat.

Data was collected on different growth stage and yield of wheat. The analyses of variance (ANOVA) of the data on different growth and yield parameters are presented in Appendix IV-XIII.

Findings of this experiment are represented by table and graphs. All possible interpretations are given below:

4A.1 Crop growth characters

4A.1 Plant height

Plant height is a visible growth parameter that determines either the treatments have any effects on crop growth or not. In my experiment seedlings were allowed to grow up to 20 days without any disturbance. Then data was collected with a definite period of time interval.

4A.1.1 Effect of GA on plant height

Plant height was taken at different days. From the data it was clear that GA has a positive role in increasing plant height. When first data was measured at 20DAS almost all plot shows similar plant height. But after 45 DAS, 70 DAS and at harvest stage data collection it was clear that GA helps to increase plant height. From the data (Fig. 2), it was found that at 45DAS average plant height was 42.22 cm, at 70DAS plant height was 64.23 cm and at harvest stage it was 67.98 cm where GA was not sprayed whereas it was 46.93 cm 67.79 cm and 71.24 cm respectively due to spraying GA. It was found that GA increased 11.21% plant height at harvest stage. GA has a role in stem elongation by increasing cell division and cell enlargement and it is proved by Alabadí *et al.* (2008).

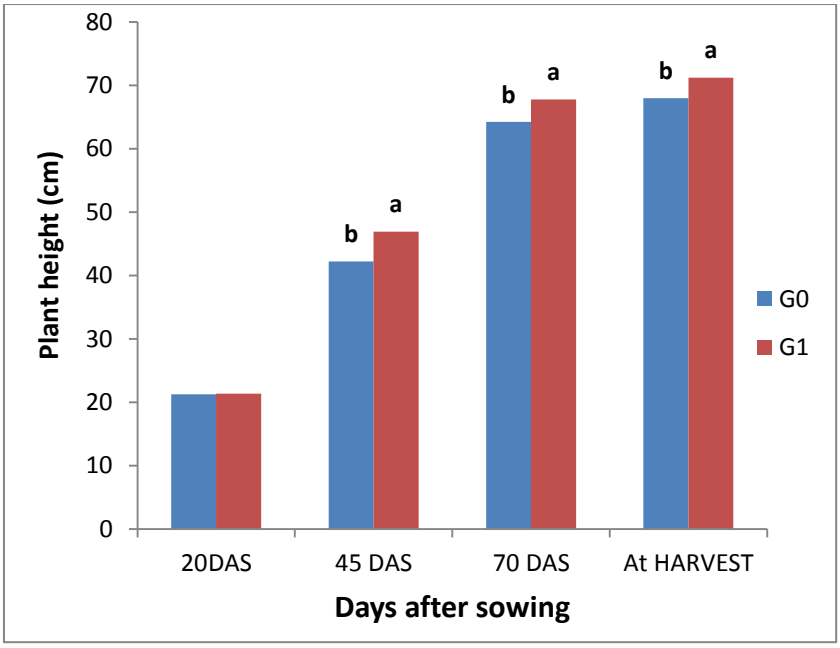


Figure 2. Effect of GA on plant height at different DAS (SE ± $_{0.05}$ = 0.0145, 0.3404, 0.2086 and 0.2321 at 20DAS, 45DAS, 70DAS and harvest respectively).

G0= No Gibberellic acid, G1= Gibberellic acid spraying

4A. 1.2 Effect of drought stress on plant height

Drought stress has a negative effect on plant height and growth. Plant height significantly reduced due to water stress and it was showed that in T_0 treatment where no water was applied, found lowest height (39.345 cm, 60.78 cm, 63.487 cm) at 45DAS, 70DAS and during harvest respectively. The highest plant height was found in T_1 that was control (no stress was imposed). Here plant height was found about 47.23 cm, 72.59 cm and 75.967 cm at 45DAS, 70DAS and during harvest respectively.

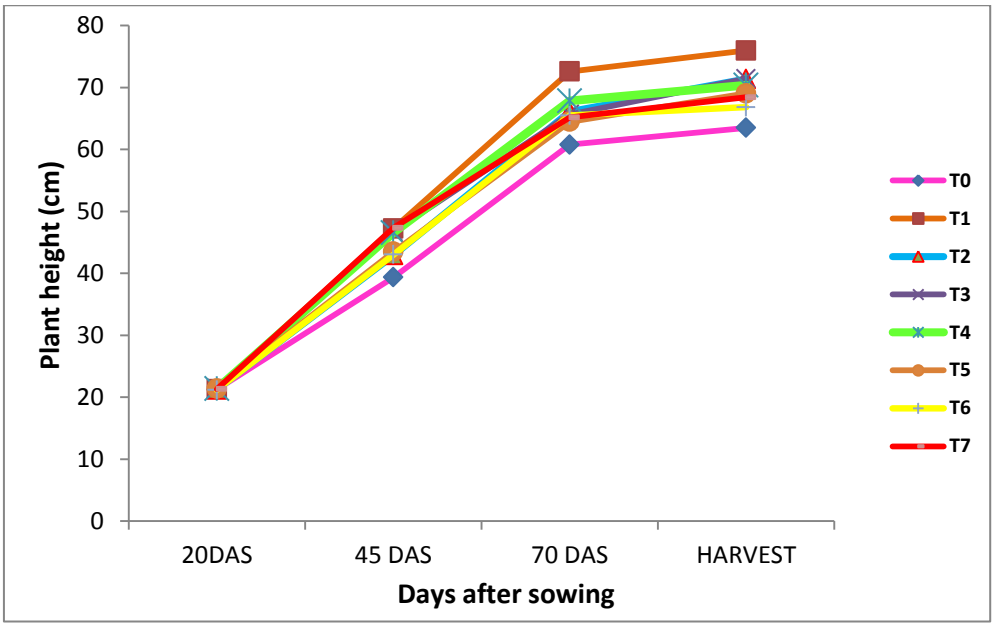
It was observed that due to stress at CRI stage in case of T_2 (stress at CRI stage), T_5 (stress at CRI stage + flowering stage), T_6 (stress at CRI stage + grain development stage), plant height was reduced. Again, due to stress at flowering stage plant height was also reduced in T_3, T_5, and T_7. In this case of T_5 which got stress at both CRI and flowering stage plant height was in more reduced condition though T_5 and T_7 was statistically similar.

Finally data was collected at harvest stage, after giving stress at grain development stage. Here, it was found that, ultimately, lower plant height was in T_6. T_7 and T_5 and

were statistically similar and taller than T_7. T_2 and T_3 was shorter due to stress condition at CRI stage and stress at flowering stage but at grain development stage they got sufficient irrigation and gave positive result to irrigation but T_3 was not so much responsive and shorter than T_2 and T_3 due to stress. In all stages, highest plant height was in T_1 and T_0 was shorter than all others treatments.

If effect of water stress is compared within different growth stage condition except T_0 and T_1, it was found that T_6 (stress at flowering and grain development) is more sensitive.

Zhai *et al.* (2003) supported the findings. He said that plant height reduced at drought stress though Gupta *et al.* (2001) found booting or flowering stage was more critical.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 3. Effect of drought stress on plant height at different growth stage of wheat (SE ± $_{0.05}$ = 0.4514, 1.2081, 0.9618 and 0.1573 at 20 DAS, 45 DAS, 70 DAS and harvest respectively).

4A. 1.3 Interaction effect of GA and drought stress

Gibberellic acid and drought stress both has an effect on crops. At 45 DAS, the tallest plant was in G_1T_1, G_1T_3 and G_1T_7 and shortest plant was G_1T_0. G_1T_2 and G_1T_6 were statistically similar and taller than G_1T_0. G_1T_4 was statistically similar with both G_1T_3 and G_1T_5 but G_1T_5 was also similar with G_1T_6. Whereas without GA, it was seen that G_0T_1, G_0T_3, G_0T_4, G_0T_7 was statistically similar with G_1T_2 and G_1T_6. Full stress condition G_0T_0 had the shortest plant height but G_0T_2 and G_0T_6 was similar with G_0T_0.

At 70 DAS, we found that G_1T_1 was taller (75.18 cm) than others and shortest plant was G_0T_0 (59.25). G_1T_0 was statistically similar with G_0T_0. It was seen that after spraying GA, G_1T_2 was taller than G_0T_2.

AT harvest stage it was found that G_1T_1 was the tallest plant. G_1T_2 and G_0T_1 were statistically similar. Here it may be happened due to GA because when GA was applied at CRI stage, due to the function of GA, G_1T_2 was not affected by stress. Also from Table 1 we may explain that plant has recover capacity that's why after getting irrigation, G_0T_2 performed better than previous growth stage. From Table 1 it was clear that spraying GA without any stress showed tallest plant whereas G_0T_0 (63.16) was the shortest plant that was statistically similar with G_0T_6 and G_1T_0. It was found that without GA, stress imposed both in CRI stage and grain development stage was critical. Stress at CRI and flowering stage was also sensitive but in case of GA, G_1T_6 and G_1T_7 was critical stage. The results showed that GA has a protective role and it was supported by Pavlista *et al.* (2014). He also stated that GA increased the plant height in different environmental situation.

Table 1: Interaction effect of GA and drought stress on plant height at different DAS

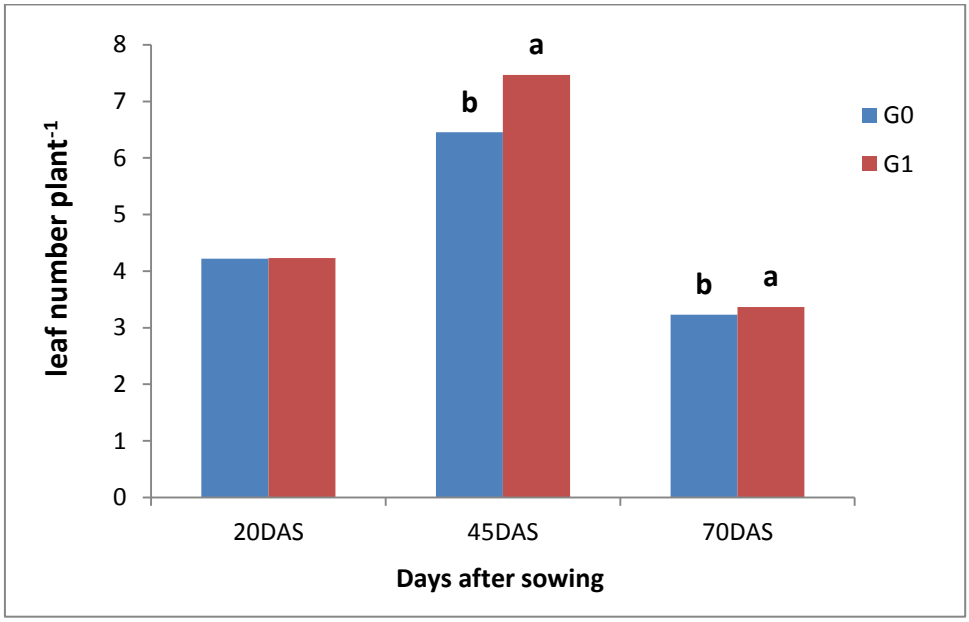
Interactions	Plant height(cm) at			
	20 DAS	**45 DAS**	**70 DAS**	**At harvest**
G_0T_0	21.17	37.99 e	59.25 i	63.16 i
G_0T_1	21.24	44.82 c	70.00 bc	74.65 b
G_0T_2	21.19	39.42 de	60.67 hi	68.46 fg
G_0T_3	21.30	44.69 c	60.67 efg	70.58 de
G_0T_4	21.37	44.63 c	66.88 cdef	69.93 e
G_0T_5	21.26	40.39 d	63.69 fgh	66.10 h
G_0T_6	21.19	39.78 de	63.56 fgh	63.81 i
G_0T_7	21.27	46.04 c	64.72 efg	67.18 gh
G_1T_0	21.32	40.70 d	62.31 ghi	63.81 i
G_1T_1	21.31	49.64 a	75.18 a	77.29 a
G_1T_2	21.26	46.40 c	71.55 b	74.26 b
G_1T_3	21.44	48.73 a	66.22 def	72.28 c
G_1T_4	21.36	48.47 ab	68.77 bcd	70.81 de
G_1T_5	21.46	46.58 bc	65.28 defg	71.98 cd
G_1T_6	21.19	46.25 c	67.42 cde	69.82 ef
G_1T_7	21.35	48.62 a	65.64 defg	69.67 ef
SE $\pm_{0.05}$	NS	0.96	1.70	0.63
CV (%)	1.28	2.64	3.17	1.12

Here, G0= No GA, G1= GA spraying and others are Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7).** NS= Not significant.

4A.2 Leaf number plant^{-1}

4.2.1 Effect of GA on leaf number plant^{-1}

Like plant height GA has a role on leaf number/plant. Number of leaves per plant increased from 20 to 45 DAS and then gradually reduced. It may be attributed to the compensation of the early produced leaves for the newly produced ones mobilizing assimilate upward leaves (Nahar, 2013). From the Fig. 4 it was seen that GA increase plant leaf number after 45 DAS but in case of 20 DAS and 70 DAS it was statistically similar. At 45 DAS leaves plant^{-1} for GA was 7.46 whereas without GA it was 6.45 leaves plant^{-1}.



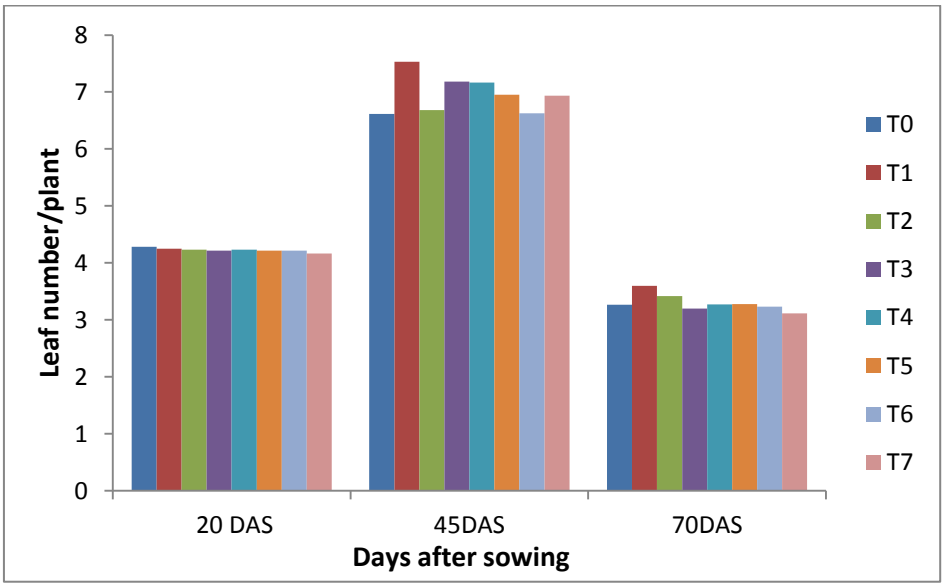
G_0= no GA and **G_1**= GA spraying

Figure 4. Effect of GA on leaf number at 20, 45 and 70 DAS (SE $_{0.05}$ = 0.0375, 0.1026, and 0.0318 at 20DAS, 45DAS and 70DAS respectively).

4A. 2.2 Effect of drought stress on plant Leaf number

Leaf number plant^{-1} of wheat showed statistically significant variation due to the different levels of drought stress. We found that leaf number was maximum when no drought stress was applied and it was 3.6 leaves/plant at 45 DAS and minimum number of leaves was found at stress condition and they were significantly similar with each others.

Again at 70 DAS, maximum number of leaves was also found in no stress condition (7.53) and minimum leaves was found at full stress condition (6.61), stress at CRI stage (6.68) and stress at CRI+ grain development stress (6.62). From stress at CRI+ flowering and stress at flowering + grain development stage we found 6.95 and 6.93 leaves/ plant which were statistically similar. From Figure 5 it is observed that drought stress reduced the leaves number significantly and stress at CRI stage is more critical than others for leaves. Although Schneekloth *et al.* (2009) found tillering stage was more critical.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 5. Effect of drought stress on leaf number at different DAS ($SE\pm_{0.05}$ = 0.0449, 0.1026 and 0.0940 at 20DAS, 45DAS, and 70 DAS respectively)**.**

4A. 2.3 Interaction effect of GA and drought stress

From the Table 2 it was found that in all stage and treatments leaf numbers showed significant variations. Maximum leaf number was found in G_1T_1 and it was 7.83 leaves/plant and 3.60 leaves plant^{-1} at 45 DAS and 70 DAS respectively. G_0T_0 or full stress condition (6.10) , G_0T_2 or stress at CRI stage (6.10), and G_0T_6 or stress at CRI+ grain development stage (6.10) gave minimum leaf number at 45DAS but G_1T_0 (7.13), G_1T_2 (7.27), G_1T_6 (7.15) which were statistically similar with G_0T_1 (7.23) carried higher number of leaves compared to G_0T_0, G_0T_2, and G_0T_6. Stress at CRI

stage or CRI+ other stage gave lower number of leaves without GA. But applying GA the effect of drought stress was reduced and increased the leaf numbers. Similar result was found by Kaya *et al.* (2006) who stated that GA has potentiality to mitigate drought stress.

Table 2 Interaction effect of GA and drought stress on number of leaves plant^{-1} at different DAS

Treatments level	Leaves number plant^{-1} at				
	20 DAS	45 DAS		70 DAS	
G_0T_0	4.33	6.10	g	3.27	bcd
G_0T_1	4.26	7.23	c	3.60	a
G_0T_2	4.26	6.10	g	3.23	bcd
G_0T_3	4.20	6.80	d	3.20	bcd
G_0T_4	4.20	6.53	d	3.30	bcd
G_0T_5	4.16	6.30	ef	3.13	cd
G_0T_6	4.13	6.10	g	3.07	d
G_0T_7	4.20	6.47	e	3.03	d
G_1T_0	4.23	7.13	c	3.27	bcd
G_1T_1	4.23	7.83	a	3.60	a
G_1T_2	4.20	7.27	c	3.60	a
G_1T_3	4.23	7.57	ab	3.20	bcd
G_1T_4	4.26	7.80	a	3.24	bcd
G_1T_5	4.26	7.60	ab	3.42	ab
G_1T_6	4.30	7.15	c	3.40	abc
G_1T_7	4.13	7.40	bc	3.20	bcd
SE± (0.05)	NS	0.13		0.13	
CV (%)	1.84	2.45		4.94	

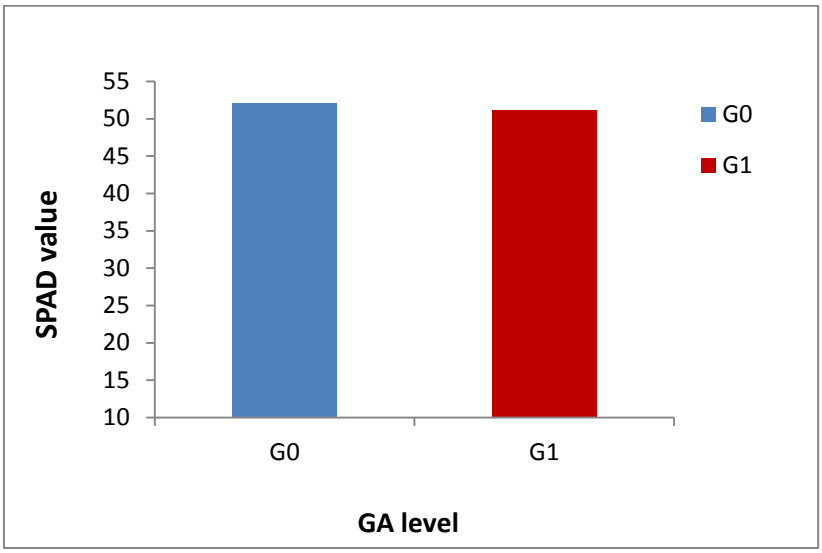
Here, G_0= No GA, G_1= GA spraying and others are Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7).** NS= Not significant.

At 70 DAS, it was seen that maximum leaves number was found in G_0T_0 (3.60), G_1T_1 (3.60), G_2T_2 (3.60) and minimum leaves were in G_0T_6 (3.07) and G_0T_7 (3.03) and they are statistically similar. G_1T_5 (3.42) shows better result than G_0T_5 (3.13) and same things happened between G_0T_2 and G_1T_2, G_0T_6 and G_1T_6. So, it may be assumed that GA has a positive effect to mitigate the drought stress, that's why the difference is significant.

4A.3 SPAD VALUE

4A.3.1 Effect of GA on SPAD value

GA has a significant effect on chlorophyll content. SPAD value was measured from flag leaf. In the experiment GA increased the SPAD value but it was not statistically different from without GA treatment though Akter (2014) found in her experiment that, GA increased the SPAD value**.**



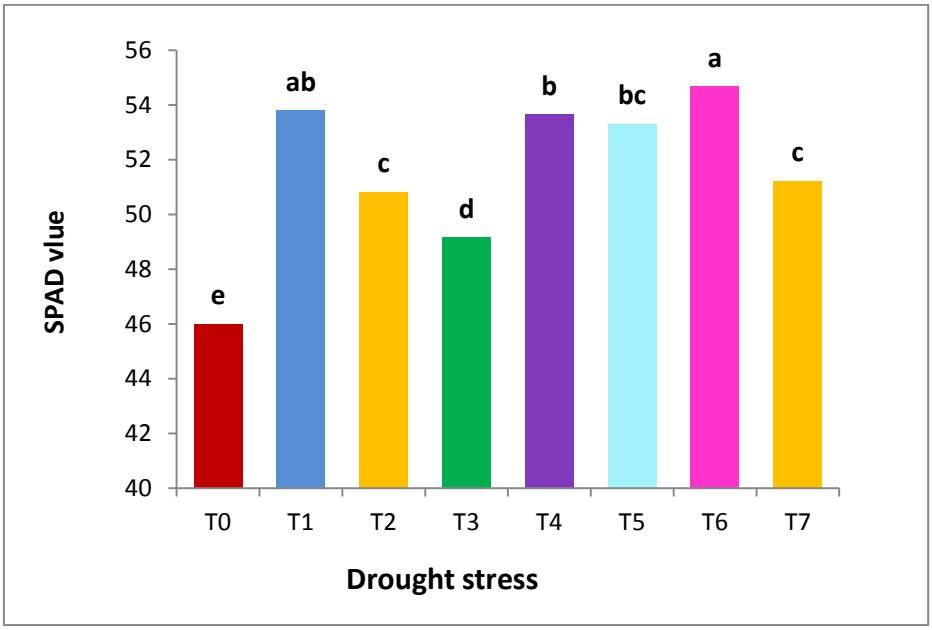
Here **G_0** =No GA, **G_1**= GA spraying

Figure 6. Effect of GA on SPAD value (SE± $_{0.05}$ = 0.2790)

4A.3.2 Effect of drought stress on SPAD value

Drought stress causes significant changes in chlorophyll content. It was found that, due to different level of drought stress, SPAD value has changed. In full stress condition, the lowest SPAD value was found in T_0 and it was 45.993 and T_1 (53.787)

and T_6 (54.677) gave the highest SPAD value. It was clear that full drought stress reduced the chlorophyll content 16.95 % than no stress condition. T_3 also gave lower reading and regarded as sensitive stage and Zhang *et al.* (2006) found the same result. He described that booting stage was more critical.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7).**

Figure 7. Effect of drought stress on SPAD value ($SE\pm_{0.05}$ = 0.4743)

4A. 3.3 Interaction effect of GA and drought stress on SPAD value

From the Table 3 it was found that GA and drought has combined interaction on leaf chlorophyll index (SPAD values). Minimum reading was found in G_0T_0 (43.53) but G_1T_0 (48.46) performed better than it. Most interesting thing is in case of drought when GA was not applied, SPAD value was higher but when in full irrigated condition with GA, SPAD value was not significantly increased. The result showed that in most of the treatments, at different drought stress level SPAD value reduced and it was significant but due to GA spraying, SPAD value increased very negligible amount which was not statistically different. Al-Shaheen *et al.* (2014) stated that drought stress reduced the chlorophyll content but GA helped to increase it.

Table 3 Interaction effect of GA and drought stress on SPAD value

Interactions	SPAD VALUE
G_0T_0	43.53 i
G_0T_1	54.29 bc
G_0T_2	51.64 ef
G_0T_3	49.13 gh
G_0T_4	53.47 bcd
G_0T_5	54.59 b
G_0T_6	56.63 a
G_0T_7	52.93 cde
G_1T_0	48.46 h
G_1T_1	53.29 bcde
G_1T_2	50.01 fg
G_1T_3	49.19 gh
G_1T_4	53.84 bc
G_1T_5	52.03 de
G_1T_6	52.73 cde
G_1T_7	49.51 gh
$SE\pm_{(0.05)}$	0.67
CV (%)	1.59

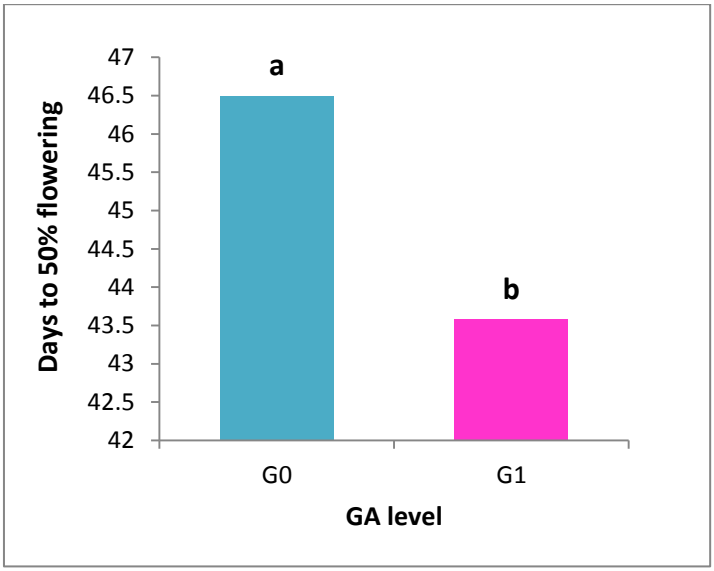
Here, G_0= No GA, G_1= GA spraying and others are Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**.

4A.4 DAYS TO 50% FLOWERING

4A.4.1 Effect of GA on 50% flowering days

GA has an effect on flowering and it increases the flower number as well as it induces flower signaling. In the present experiment in case of GA spraying it took less time to induce flowering (43.583 days) but when GA was not applied, flowering started after

46.5 days. Gupta and Chakrabarty (2013) stated that GA has a role in floral expression.

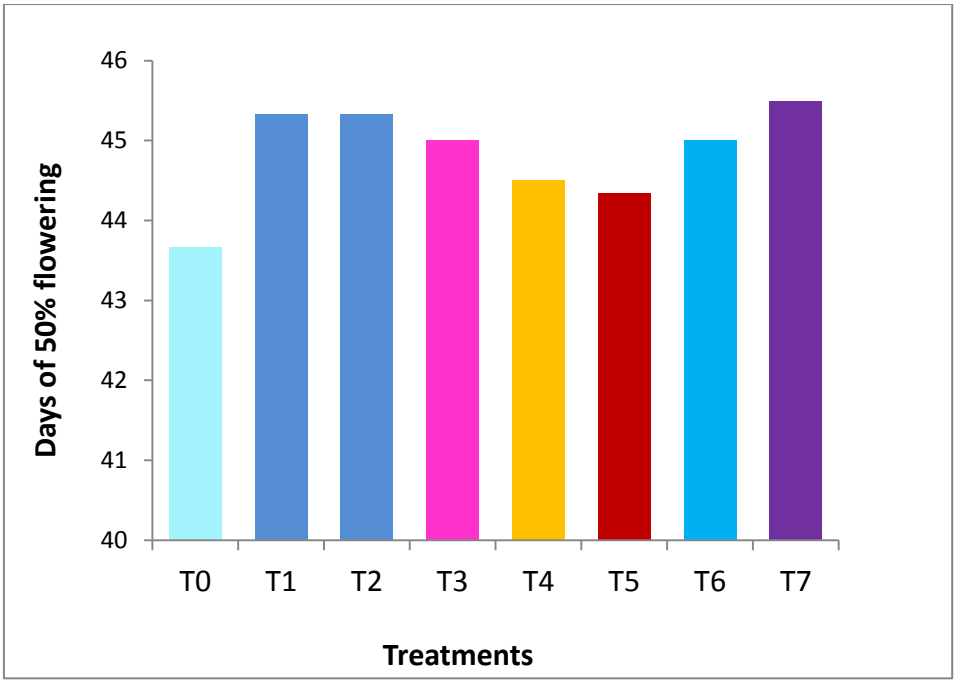


Here $\mathbf{G_0}$ =No GA, $\mathbf{G_1}$= GA spraying

Figure 8. Effect of GA on 50% flowering (SE± $_{0.05}$ = 0.2917).

4A. 4.2 Effect of drought stress on 50% flowering

In full stress condition, flowering initiation was very quickly and it took only 43.66 days. Stress in CRI stage + flowering stage took less days to initiate flowering (44.43 days) also stress condition at grain development stage, flowering initiation was fast (44.50). From Figure 9 it was noticed that full irrigated condition got moderate time for flowering (45.3). It was because in full stress conditions plants want to avoid stress but when it got moisture (like after CRI stage flowering stage was not under stress) it again try to move from flowering stage to vegetative stage as a result delay in flowering. Lopes and Reynolds (2010) supported this and described that under drought stress wheat produced quick flower to avoiding the effect of drought but in irrigation condition it delayed.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 9. Effect of drought stress on 50% flowering ($SE\pm_{0.05}$ = 0.3054).

4A. 4.3 Interaction effect of GA and drought stress on 50% flowering

From the Table 4 it was found that minimum days required for 50% flowering were in G_1T_1, G_1T_2, G_1T_4, and G_1T_6. All need same time and it was 43.33 days. G_1T_0, G_1T_3 and G_1T_7 were statistically similar and they need 43.667 days for flowering. These treatments required less time than G_0T_3 (46.333) and G_0T_7 (47.333) except G_0T_0 (43.667). It was because GA induced early flowering and that is why GA spraying at these treatments gave early flowering but G_0T_0 induced early flowering because when plant is in adverse situation, it wants to complete its life cycle very quickly (Lopes and Reynolds, 2010) and that's why G_0T_0 initiated flowering quickly. G_0T_1 (47.333) and G_0T_2 (47.333) took more time for flowering and they were statistically similar. It may be due to the availability of moisture. When wheat plants are in full irrigated condition or after adapting with stress condition when they got sufficient soil

moisture i.e. G_0T_2, they tried to complete their optimum vegetative growth and then moved towards reproductive stage.

Table 4 Interaction effect of GA and drought stress on days of 50% flowering

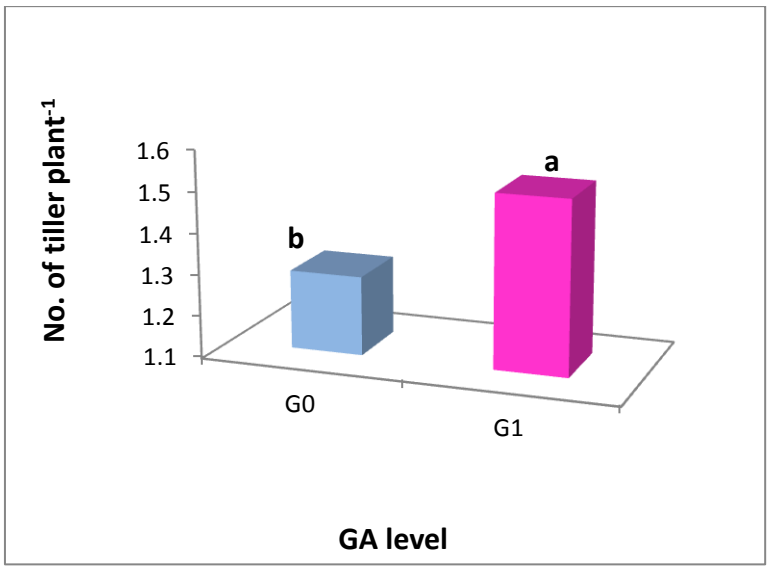
Interactions	Days of 50% flowering
G_0T_0	43.66 fg
G_0T_1	47.33 ab
G_0T_2	47.33 ab
G_0T_3	46.33 cd
G_0T_4	45.66 de
G_0T_5	43.76 fg
G_0T_6	46.66 bc
G_0T_7	47.33 ab
G_1T_0	43.66 fg
G_1T_1	43.33 g
G_1T_2	43.33 g
G_1T_3	43.66 fg
G_1T_4	43.33 g
G_1T_5	44.33 ef
G_1T_6	43.33 g
G_1T_7	43.66 fg
$SE\pm_{(0.05)}$	0.431
CV (%)	1.17

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7).**

4A.5 Number of tillers plant^{-1}

4A.5.1 Effect of GA on tiller number plant^{-1}

GA always plays a role in plant growth and development. It helps to increase tiller number per plant. In Figure 10 it was seen that GA increased the tiller number about 15.13%. From the experiment it was found that without GA tiller number/plant was 1.29 whereas after spraying GA the tiller number was 1.52 per plant. Islam (2013) founded that GA increased the tiller number.

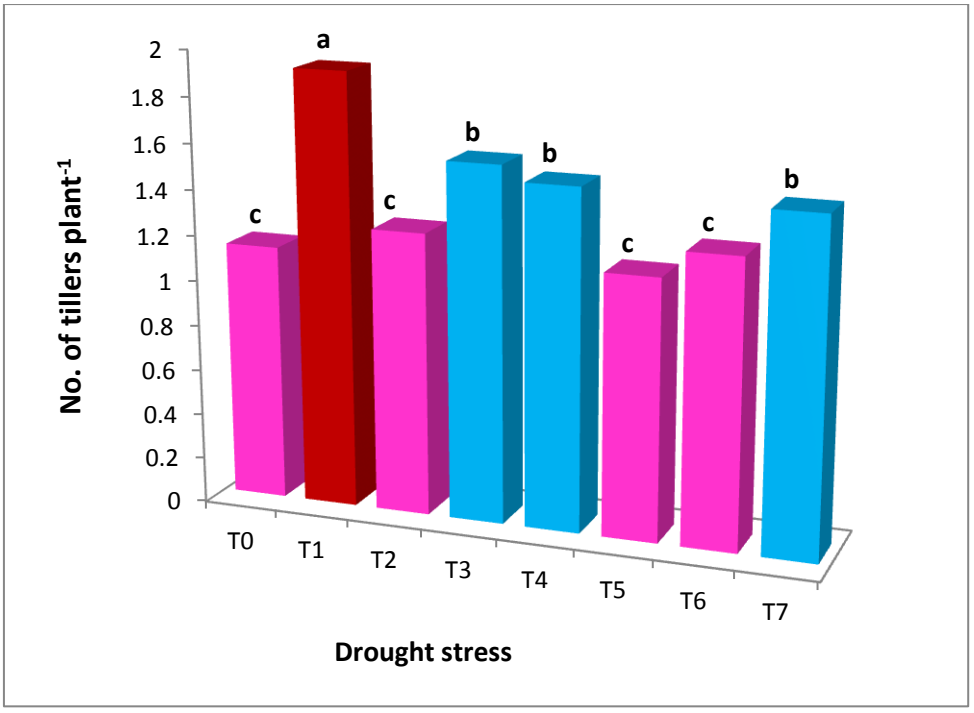


Here **G_0** =No GA, **G_1**= GA spraying

Figure 10. Effect of GA on tiller number plant^{-1} (SE± $_{0.05}$ = 0.0182).

4A. 5.2 Effect of drought stress on tiller number plant^{-1}

In case of no stress condition (T_1) wheat gave higher tiller number (1.92) per plant and full stress condition ((1.13), stress at CRI stage (1.25), stress at CRI stage + flowering stage (1.15), stress at CRI stage + grain development stage (1.26) gave lowest number of tiller per plant. From the experiment it was found that minimum number of tiller was found in stress at CRI stage or CRI + other stage. So it is clear that drought stress reduced the tiller number plant^{-1} and for wheat about 22.06% and drought stress is critical for CRI stage for tiller production. Nahar (2013) and Sarkar (2015) found the same result that drought stress reduced the tiller number.



Treatments are: Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**.

Figure 11. Effect of drought stress on tiller number/plant (SE± $_{0.05}$ = 0.0833).

4A. 5.3 Combined effect of GA and drought stress on tiller number plant^{-1}

Highest number of tiller was found in no stress condition with GA and second highest was no stress condition without GA. So, here we found that without drought stress or full irrigation condition gave higher number of tiller but GA was also responsible for increasing the tiller number because it was found higher tiller number was in G_1T_1 (2.10) than G_0T_1 (1.73). From Table 5 it was observed that the lowest number of tiller was found in G_0T_0 (0.93), G_0T_2 (1.00), G_0T_5 (0.93). G_0T_6 (1.13) also produced lower number of tillers and it was statistically similar with them but higher number of tiller was produced from G_1T_0 (1.33), G_1T_2 (1.50), G_1T_5 (1.37) and G_1T_6 (1.40) than those. Here the result showed that when drought stress imposed on CRI stage it reduced the tiller number, but in drought stress if GA was applied, it reduced the adverse effect of drought stress and increased the tiller number per plant. Iqbal and Ashraf (2013) also found the same result. They stated that GA increased the tiller number in stress

condition although, Gupta *et al.* (2001) found drought at booting stage reduced the tiller number.

Table 5 Effect of GA and drought stress interaction on tiller number plant^{-1}

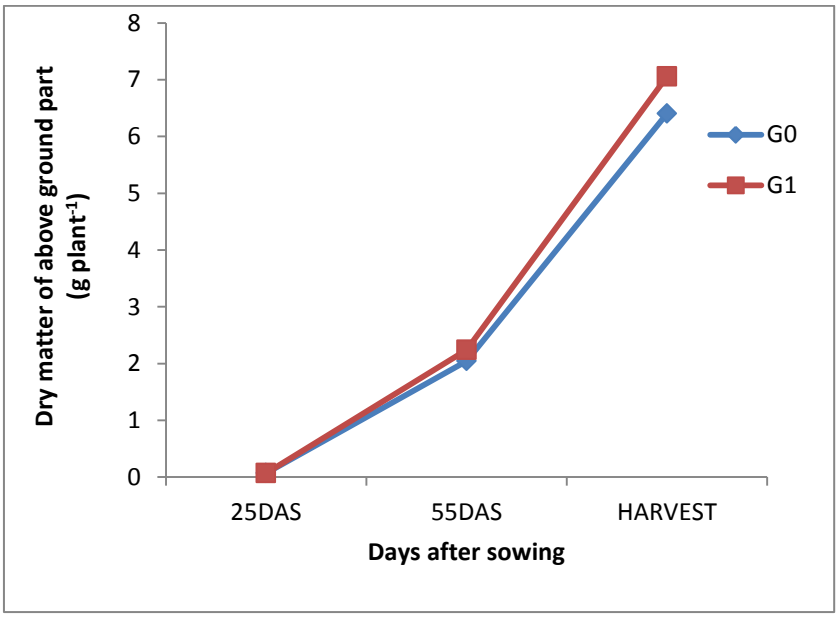
Interactions	Tiller number plant^{-1}	
G_0T_0	0.93	f
G_0T_1	1.73	b
G_0T_2	1.00	f
G_0T_3	1.67	bc
G_0T_4	1.47	cd
G_0T_5	0.93	f
G_0T_6	1.13	ef
G_0T_7	1.47	cd
G_1T_0	1.33	de
G_1T_1	2.10	a
G_1T_2	1.50	bcd
G_1T_3	1.47	cd
G_1T_4	1.53	bcd
G_1T_5	1.37	de
G_1T_6	1.40	d
G_1T_7	1.47	cd
SE±$_{(0.05)}$	0.1179	
CV (%)	10.26	

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

4A.6 Above ground dry matter plant^{-1}

4A.6.1 Effect of GA on dry matter of above ground part

From the Fig. 12 it was visible that GA increases the dry matter in plant. Islam (2013) found the same findings. As, GA increased the plant height and leaf number so dry matter also increased. At 25 DAS, dry matter is statistically similar in GA and without GA but at 55 DAS and at harvest stage dry matter increased in G_1 2.24 g and 7.05 g respectively than G_0.



Here **G_0** =No GA, **G_1**= GA spraying

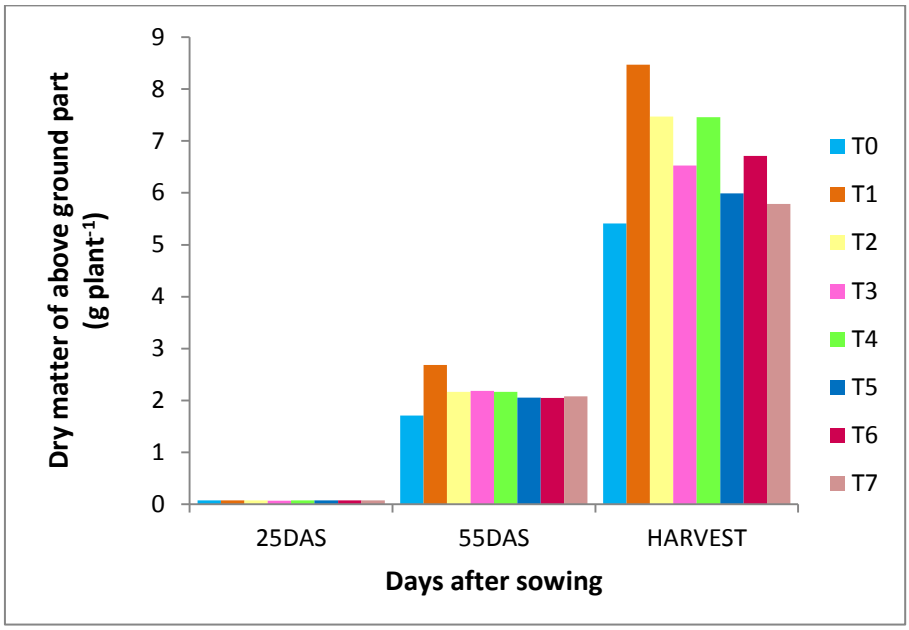
Figure 12. Effect of GA on dry matter of above ground part at different DAS (SE± 0.05 = 2.063E-03, 0.0236 and 0.0505 at 25DAS, 55DAS, and at harvest respectively).

4A. 6.2 Effect of drought stress on dry matter of above ground part

At 25 DAS dry matter for all treatments almost same and statistically similar. At 55 DAS it was seen that dry matter of above ground part was highest at no stress condition (2.6811) whereas lowest dry matter was found in full stress condition (1.7085) and others treatments were statistically similar.

At harvest stage, it was found that, like 55 DAS highest dry matter was in T_1 (8.4710) and lowest dry matter was in T_0 (5.4126). When stress was imposed on two growth stage like CRI+ flowering or flowering + grain development it produce less amount of

dry matter than drought stress only CRI stage or flowering stage. Dalirie *et al.* (2010) explained drought stress reduced the dry matter production and its effect was severe when plants face terminal drought stress.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 13. Effect of drought stress on dry matter of above ground part at different DAS (SE± $_{0.05}$ = 2.802E-03, 0.0979 and 0.0617 at 25DAS, 55DAS, and at harvest respectively).

4A. 6.3 Effect of GA and drought stress on dry matter of above ground part at different DAS

From Table 6 it was observed that at 25 DAS dry matter production is statistically similar with each other but at 55 DAS and at harvest changes in dry matter was very significant and treatments to treatments difference was clear. At 55 DAS it was found that highest dry matter was found in G_1T_1 (2.8795) and second highest was G_0T_1 (2.4827). The lowest dry matter was found in G_0T_0 (1.6011). G_1T_0 also gave lower dry matter but it was statistically similar with both G_0T_0 and others like stress at CRI stage or stress at flowering stage or both. From the Table and data, we saw that

irrigation increased the dry matter than drought stress but when GA was applied with irrigation, it produced more dry matter and it was found almost all the treatments where GA was sprayed. It was calculated that G_1T_1 produced about 16% more dry matter than G_0T_1 and in case of full stress condition, 13.42 % more dry matter production in G_1T_0 than G_0T_0. Islam, (2013) and Pan *et al.* (2003) found the same results.

Table 6 Interaction effect of GA and drought stress on dry matter of above ground part at different DAS

Interactions	dry matter (g) of above ground part at different DAS		
	25 DAS	55 DAS	HARVEST
G_0T_0	0.070	1.60 h	5.11 l
G_0T_1	0.072	2.48 b	8.06 c
G_0T_2	0.070	1.93 fg	6.63 g
G_0T_3	0.067	2.08 defg	6.38 h
G_0T_4	0.074	2.21 bcde	7.24 e
G_0T_5	0.070	2.02 defg	5.69 k
G_0T_6	0.072	1.97 efg	6.44 h
G_0T_7	0.074	2.03 defg	5.63 k
G_1T_0	0.072	1.81 gh	5.71 j
G_1T_1	0.074	2.87 a	8.87 a
G_1T_2	0.074	2.39 bc	8.32 b
G_1T_3	0.069	2.28 bcd	6.66 g
G_1T_4	0.073	2.23 bcde	7.67 d
G_1T_5	0.076	2.07 defg	6.28 h
G_1T_6	0.070	2.11 cdef	6.98 f
G_1T_7	0.072	2.12 cdef	5.94 i
SE± 0.05	NS	0.0873	0.1384
CV (%)	6.72	7.91	1.59

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7). NS= Not significant.

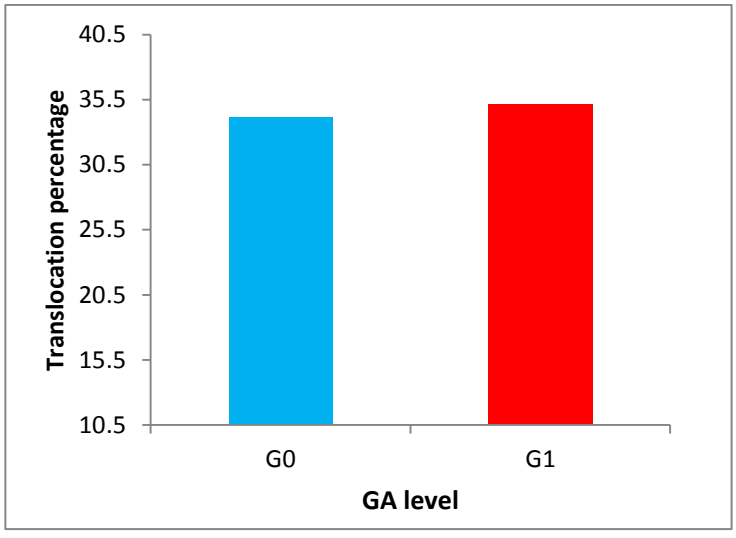
Again, at harvest stage it was visible that G_1T_1 (8.87) produce more dry matter and second and third highest dry matter production was in G_1T_2 (8.31) and G_0T_1 (8.06) respectively. From this data it was clear to say due to effect of GA, G_1T_1 produced higher dry matter than G_0T_1 also in case of stress at CRI stage, GA had worked and that is why G_1T_2 produced higher dry matter than G_0T_2 (6.63) even it was higher than G_0T_1. It was proved that GA has effect to mitigate drought stress and in every treatment GA played to increase dry matter production by protecting wheat plant from drought stress.

The minimum dry matter was found from G_0T_0. If single stage is considered then flowering stage was considered more sensitive to drought stress for dry matter production but the most critical stage for dry matter production was CRI+ flowering and flowering + grain development stage.

4A.7 Translocation percentage

4A.7.1 Effect of GA on translocation percentage

Translocation means plants to distribute water and nutrients to other parts of the plant for proper growth and development. Translocation allows plant to move photosyntheats from vegetative part to reproductive parts. If translocation is higher than yield will be higher.



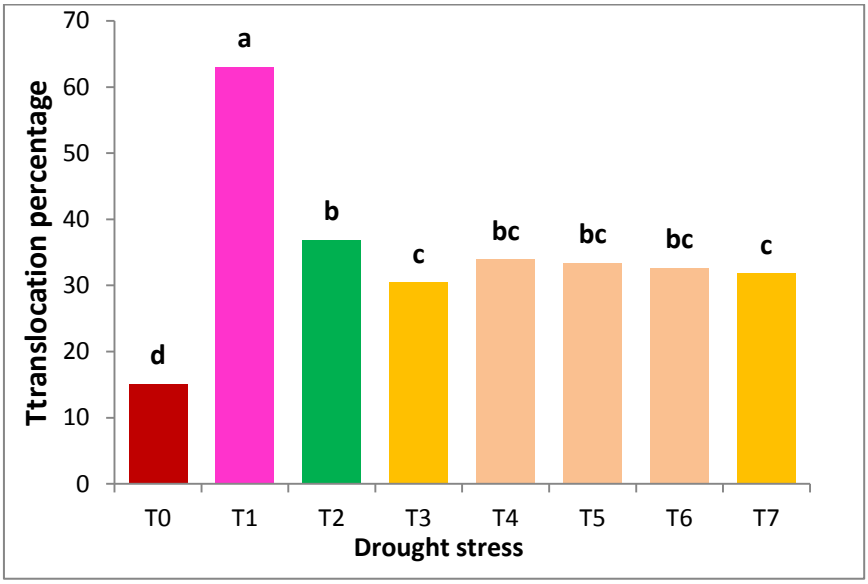
Here **G_0** =No GA, **G_1**= GA spraying

Figure 14. Effect of GA on translocation percentage (SE± $_{0.05}$ = 0.7094)

From the Fig. 14 it was observed that GA increased the translocation percentage at very negligible amount and both G_1 and G_0 were statistically similar. Patrick and Mulligan (1989) said GA increased the assimilates production and translocation which was controversy with my findings but Yim *et al.* (1997) said GA has little role in starch accumulation.

4A. 7.2 Effect of drought stress on translocation percentage

From the Fig. 15 it was observed that drought stress reduced the translocation percentage. The highest translocation (62.98%) was found in T_1 (no stress condition) whereas T_0 (full stress condition) showed the lowest percentage of translocation (15.116 %). About one-fourth reduction of translocation happened due to drought stress. When stress was in T_3 (flowering stage) and T_7 (flowering + grain development stage) translocation percentage reduced drastically and it was 30.402 and 31.796 respectively. It was because due to the shortage of water plants couldn't transfer the food materials to the grain. Zhang *et al*. (1998) and Yang *et al*. (2000) described that transfer of stored material from vegetative part to reproductive part is very much essential to increase the yield.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 15. Effect of drought stress on translocation percentage (SE± $_{0.05}$ = 2.2301).

4A. 7.3 Interaction effect of GA and drought stress on translocation percentage

From the Table 7 it was clear that G_1T_1 (64.145) and G_0T_1 (61.816) is responsible for more translocation percentage. Though G_1T_1 was a little bit higher than G_0T_0 but it was not statistically different. Lowest translocation was found in G_0T_0 (15.852) and G_1T_0 (14.379) and they were statistically similar.

Table 7 Interaction effect of GA and drought stress on translocation percentage

Interactions	Translocation percentage
G_0T_0	15.85 d
G_0T_1	61.81 a
G_0T_2	35.57 bc
G_0T_3	30.17 c
G_0T_4	32.40 bc
G_0T_5	33.33 bc
G_0T_6	32.25 bc
G_0T_7	31.74 bc
G_1T_0	14.37 d
G_1T_1	64.14 a
G_1T_2	38.20 b
G_1T_3	30.63 c
G_1T_4	35.46 bc
G_1T_5	33.37 bc
G_1T_6	32.93 bc
G_1T_7	31.85 bc
$SE_{(0.05)}$	3.153
CV (%)	11.15

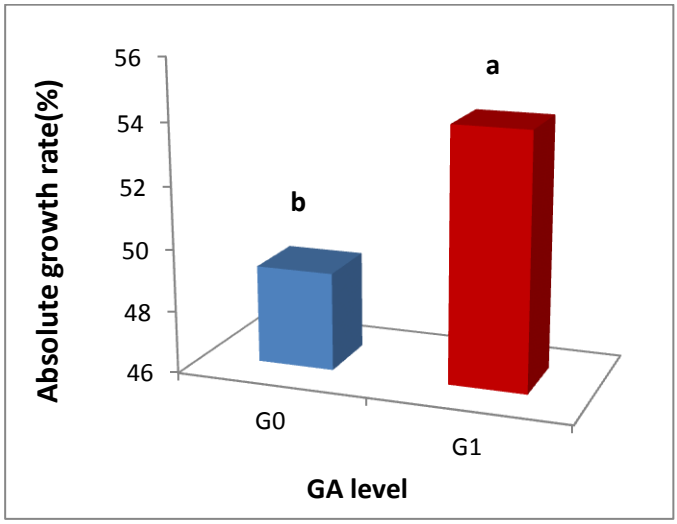
Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

So it was clear that GA has a negligible role in increasing translocation even under drought stress GA couldn't show its efficiency but water has a significant role in case of drought stress either with GA or without GA. In both treatment with G_0T_3 (30.17) and G_1T_3 (30.63) showed lowest translocation percentage. It was because when stress was in flowering stage wheat plants could not transfer the food materials from leaf to reproductive organ or it may be happened due to the drastic effect of drought stress on floral development. Others treatments were statistically similar with each other. Gebbing and Schnyder, (1999) said pre-anthesis assimilates reserved in the stem and leaf sheath is responsible for increasing 25-30% yield in wheat but Yang et al. (2000) stated that dought at grain filling stage is more sensitive to assimilate transfer.

4A.8 Absolute growth rate

4A.8.1 Effect of GA on absolute growth rate

From the Fig. 16 it is clear that, GA has an effect on absolute growth rate of crop. GA increased the plant absolute growth rate. As it was previously discussed that GA is responsible for the plant growth and development, so it was common that GA also increased the plant absolute growth rate. In case of G_1 it was 54.16 whereas in G_0 it was 49.18.

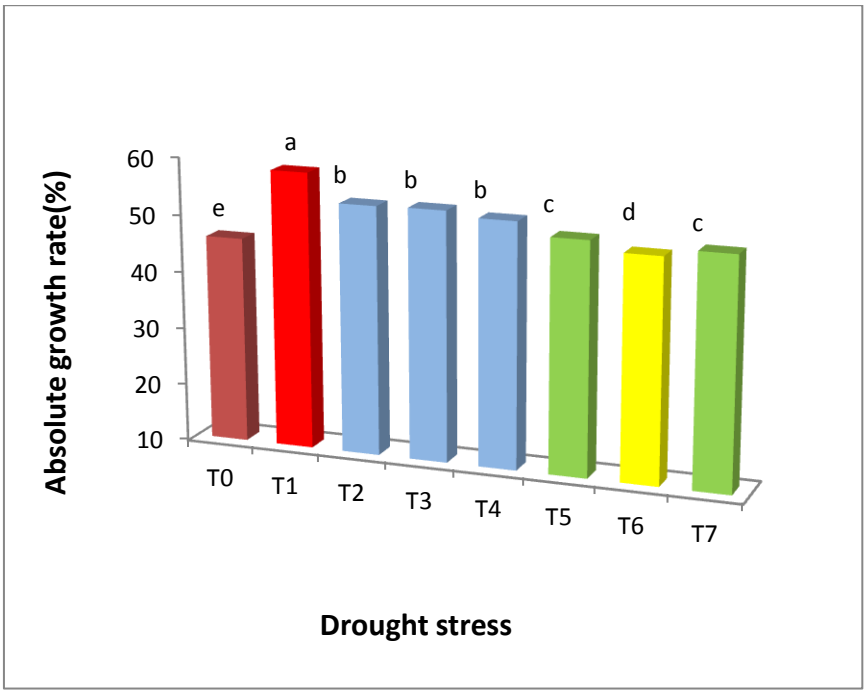


Here **G_0** =No GA, **G_1**= GA spraying

Figure 16. Effect of GA on absolute growth rate (SE $_{0.05}$ = 0.3045).

4A. 8.2 Effect of drought stress on absolute growth rate

Absolute growth rate was higher in no stress condition (58.240) and the lowest growth rate was found in full stress condition (45.917). It was proved that water is very much essential for plant growth and development. Plant height, leaf number dry weight all were increased due to irrigation and in full stress condition those are in lower value as previously explained. Like the other value, here absolute growth rate was also reduced due to the stress condition. Again from the data it was found that stress at any single stage like CRI stage or stress at flowering or grain development stage is more resistant to increase growth rate than the T_5, T_6 or T_7. It was observed that CRI + grain development stage is more susceptible for drought stress.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 17.Effect of drought stress on absolute growth rate (SE $_{0.05}$ =0.4361).

4A. 8.3 Interaction effect of GA and drought stress on absolute growth rate

It was observed from the Table 8 that absolute growth rate was higher in G_1T_1 (60.85) and the lowest was in G_0T_0 (45.65). G_1T_2 (57.61) performed better than G_0T_1 (55.63) and G_1T_1 also better than G_0T_1 (55.63). It was because, in G_1T_1 irrigation helps to increase growth rate as well as when GA was applied it showed better performance than G_0T_1.

Table 8 Interaction effect of GA and drought stress on absolute growth rate

Interactions	Absolute growth rate(%)	
G_0T_0	45.64	ij
G_0T_1	55.63	c
G_0T_2	49.23	g
G_0T_3	51.87	f
G_0T_4	51.66	f
G_0T_5	46.70	hi
G_0T_6	44.85	j
G_0T_7	47.82	h
G_1T_0	46.18	hij
G_1T_1	60.85	a
G_1T_2	57.61	b
G_1T_3	55.26	c
G_1T_4	53.76	de
G_1T_5	54.31	cd
G_1T_6	52.85	ef
G_1T_7	52.52	ef
$SE_{(0.05)}$	0.6167	
CV (%)	1.46	

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

Moreover at G_1T_2, due to GA, stress could not damage the wheat plants drastically because GA protected the plants from stress. So, the ultimate result was drought reduced the plant growth rate but GA helped to increase it either in stress condition or without drought stress condition.

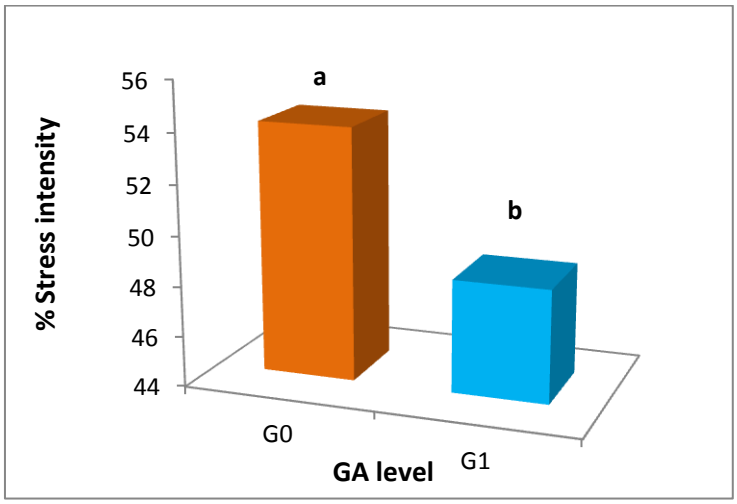
If we considered finding out the most sensitive stage, it was G_0T_6 (44.856) or stress at CRI+ grain development stage. G_1T_6 also showed the same findings but due to GA, it was less damaged.

4A.9 Stress intensity (SI)

Stress intensity refers to extend of damage caused by the different type of biotic and abiotic stress. It depends on stress duration, plants type and so on. The more the stress intensity increase, the more the damage will be occurred.

4A.9.1 Effect of GA on stress intensity

From the Figure. 18 it is clear that stress intensity percentage was higher in G_0 (54.09) that mean more damage was occurred in G_0 but in G_1 (48.53) damage was lower than G_0. So, it may be assumed that GA worked to protect plants from drought stress condition.

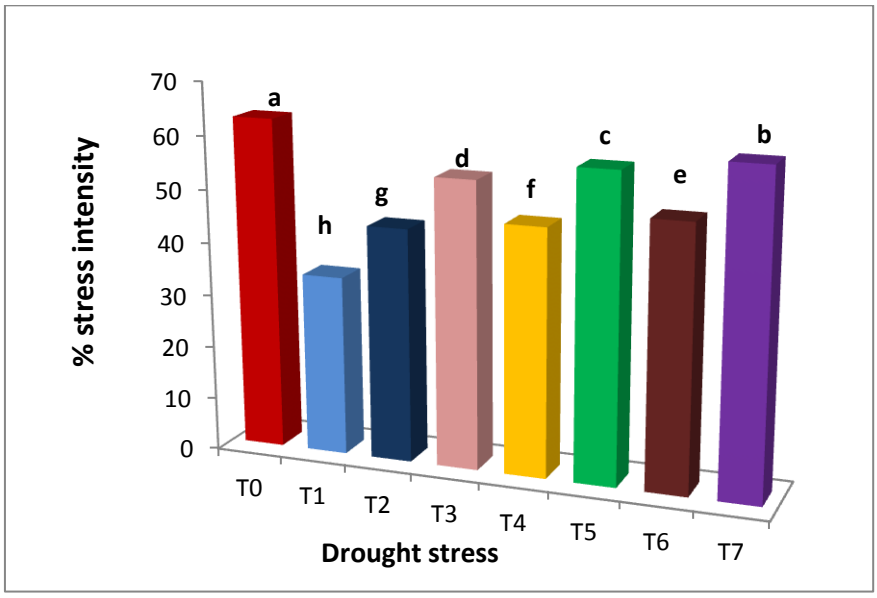


Here **G_0** =No GA, **G_1**= GA spraying

Figure 18. Effect of GA on stress intensity (SE± $_{0.05}$ = 0.4460).

4A. 9.2 Effect of drought stress on stress intensity

As it is well known that drought stress has a negative effect on plants and depending on its severity damage may be severe. It was observed from the experiment that due to drought stress, higher amount of damage occurred. The highest value of stress intensity was found in full drought stress condition (62.91) and the lowest intensity was found from the no stress condition (34.07). In field condition, plants naturally face many types of problems and that's why it is tough to get the maximum yield but by maintaining proper cultural operations like irrigation supply, yield can be increased. Here we found irrigation may increase up to 45.83 potentiality of wheat. It was noticed that stress intensity was higher in CRI stage (44.33) and then grain development stage (46.77) it means in CRI stage, more damage was occurred. Samarah (2005) said under drought stress, stress intensity was increased. If it is lower than plant is resistant to increase yield but in susceptible plant SI is higher and reduced the yield although Gao *et al*. (2009) said for yield stability water required higher in jointing and anthesis stage.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 19. Effect of drought on stress intensity (SE± $_{0.05}$ = 0.4533).

4A. 10.3 Interaction effect of GA and drought stress on stress intensity

It had already discussed that both GA and irrigation has a positive effect to reduce the stress intensity. From the combined effect we found that the lowest stress intensity was found in G_1T_1 (31.782) and then G_1T_2 (36.828) and G_0T_1 (36.371).

Table 9 Interaction effect of GA and drought stress on stress intensity

Interactions	Stress intensity %	
G_0T_0	65.451	a
G_0T_1	36.371	j
G_0T_2	51.834	f
G_0T_3	56.271	de
G_0T_4	48.697	h
G_0T_5	60.736	c
G_0T_6	50.384	fg
G_0T_7	63.016	b
G_1T_0	60.364	c
G_1T_1	31.782	k
G_1T_2	36.828	j
G_1T_3	52.28	f
G_1T_4	44.851	i
G_1T_5	54.956	e
G_1T_6	49.196	gh
G_1T_7	58.011	d
$SE\pm_{(0.05)}$	0.6410	
CV (%)	1.53	

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

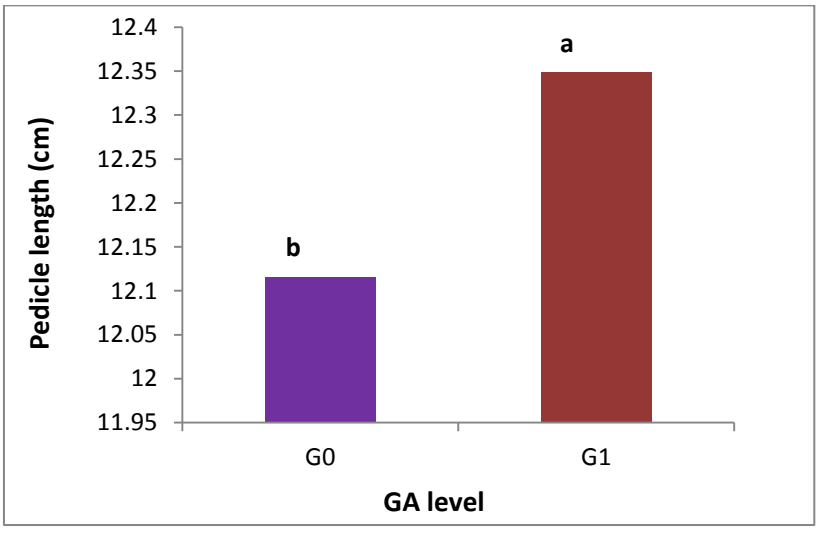
The maximum stress intensity was found in G_0T_0 (65.45) but G_1T_0 showed 58.01 stress intensity. Again, In case of full irrigated condition or no stress condition, value of G_1T_1 was lower than G_0T_1 again G_0T_1 and G_1T_2 was statistically similar as well as almost all GA treatments showed positive result. So it may say GA has a role in reducing stress intensity in case of both drought stress or irrigated condition. Colebrook *et al.* (2014) told about the role of GA in stress mitigation and from his discussion it was clear that GA plays role to reduce stress intensity.

From the Table 9 it was also visible that when stress was imposed on flowering+ grain development stage it caused more damage and increased the stress intensity. Stress only at grain development stage was more resistant to drought stress than other stage. Here, G_0T_7 showed 63.01 % and G_1T_7 showed 58.01 % stress intensity.

4A.10 Pedicle length

4A.10.1 Effect of GA on pedicle length

From the data we found that GA increased the pedicle length of wheat spike. In G_1 pedicle length was 12.35 cm where as in G_0 it was 12.12 cm. Gupta and Chakrabarty (2013) stated about the role of GA in floral development. There they mentioned that GA played a role in increasing pedicle length.

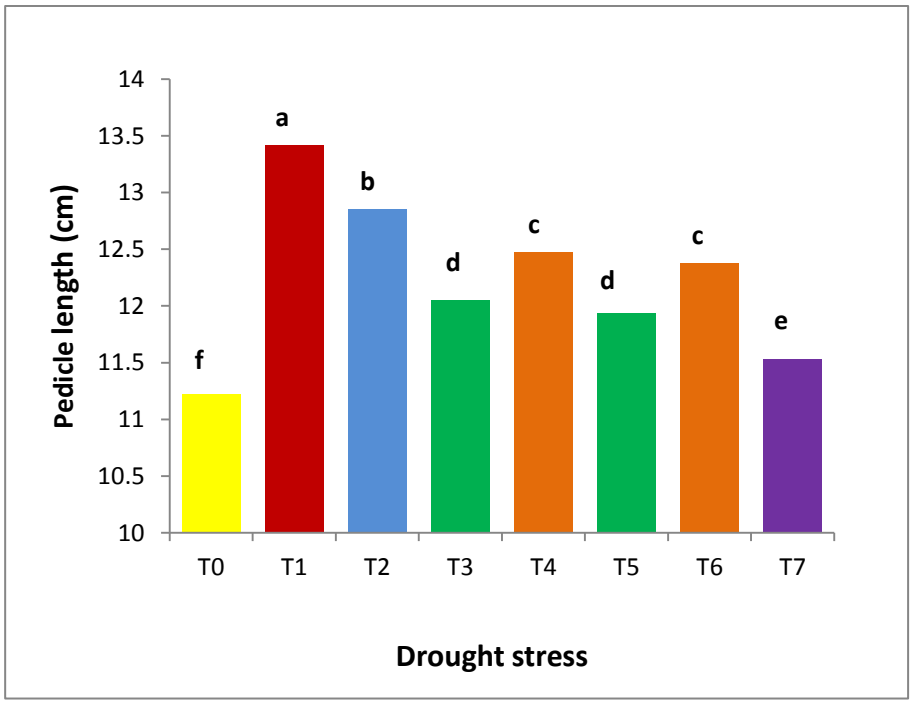


Here **G_0** =No GA, **G_1**= GA spraying

Figure 20. Effect of GA on pedicle length (SE± $_{0.05}$ = 0.0227).

4A. 10.2 Effect of drought stress on pedicle length

Drought stress reduces the wheat pedicle length. It was observed that maximum pedicle length was found in T_1 or no stress condition (13.42 cm) and the lowest pedicle length was in full stress condition (11.21 cm). About 20% pedicle length decreased due to the drought stress (Fig. 21). Again among the different growth stage of wheat it was noticed that when stress was only at CRI stage (T_2) pedicle length reduction was negligible but the most sensitive stage for drought stress was flowering + grain development (T_7). Pedicle length was 11.53 cm at this stage though T_3 (12.05) and T_5 (11.93) stage also showed susceptibility towards drought stress.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 21. Effect of drought stress on pedicle length ($SE\pm_{0.05}$ = 0.1380).

4A. 10.3 Interaction effect of GA and drought stress on pedicle length

From the Table 10 it was observed that maximum pedicle length was found in G_0T_1 (13.373), G_1T_1 (13.467) and G_1T_2 (13.127) and the lowest pedicle length was found in G_0T_0 (11.11). Though G_1T_0 (11.327) and G_0T_7 (11.497) was responsible for higher pedicle length but they were statistically similar with G_0T_0 and also G_1T_7 (11.57). It may be said that both GA and water helped to increase the pedicle length even in stress at CRI stage when GA was applied it gave similar result to no stress condition because GA had worked on it.

Table 10 Interaction effect of GA and drought stress on pedicle length

Interactions	Pedicle length (cm)	
G_0T_0	11.11	g
G_0T_1	13.37	a
G_0T_2	12.57	b
G_0T_3	11.98	de
G_0T_4	12.41	bc
G_0T_5	11.65	ef
G_0T_6	12.32	bcd
G_0T_7	11.49	fg
G_1T_0	11.33	fg
G_1T_1	13.46	a
G_1T_2	13.13	a
G_1T_3	12.12	cd
G_1T_4	12.54	b
G_1T_5	12.22	bcd
G_1T_6	12.43	bc
G_1T_7	11.57	f
$SE\pm_{(0.05)}$	0.195	
CV (%)	1.95	

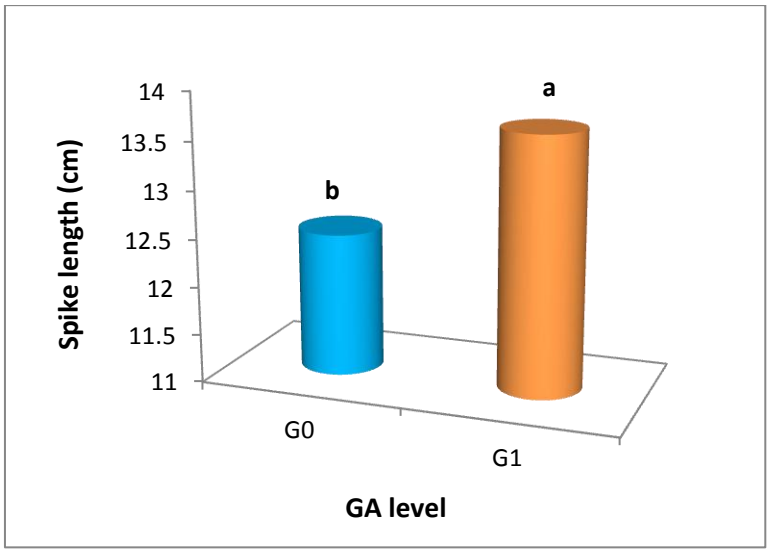
Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

Here the resistant stage for drought stress was CRI stage. Grain development stage was showed same result but it was also statistically similar with other stages. The most susceptible stage was G_0T_7 (11.497) even instead of spraying GA, flowering + grain development stage showed that when stress was applied from starting of flowering stage to end of grain development it caused damage severely.

4A.11 Spike length

4A.12.1 Effect of GA on spike length

In case of wheat, spike length is a yield determining parameters. GA increased the length of spike of wheat. Without GA application, spike length was found 12.491 cm whereas GA treated wheat plants produced 13.66 cm long spike.



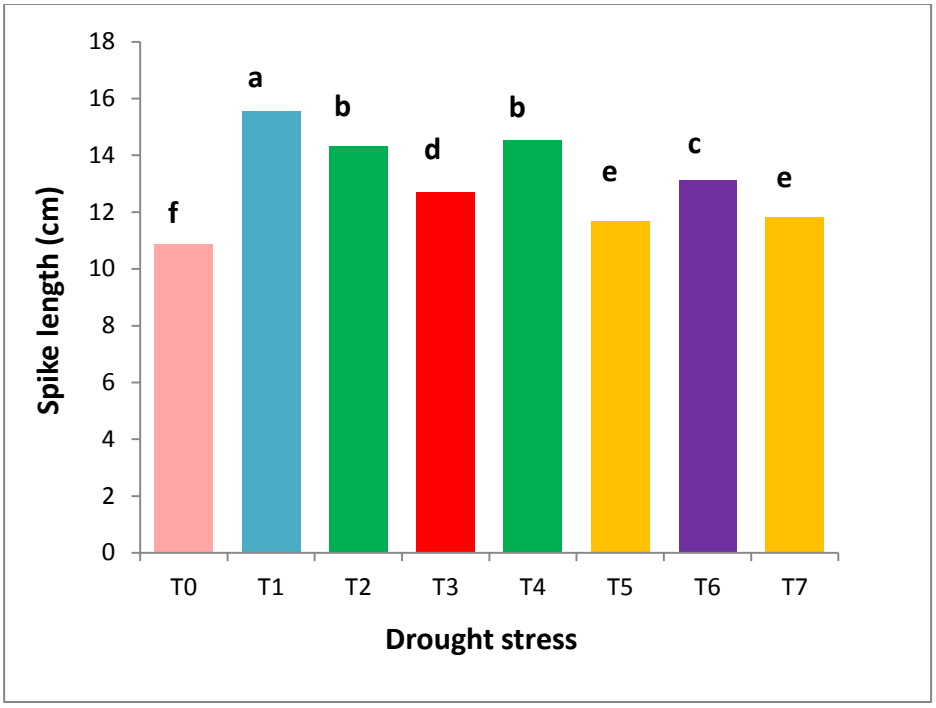
Here **G_0** =No GA, **G_1**= GA spraying

Figure 22. Effect of GA on spike length (SE± $_{0.05}$ = 0.0681).

4A. 12.2 Effect of drought on spike length

Drought stress reduced the spike length in wheat, from the data it was found that spike length was highest in T_1 (15.56 cm) and the lowest value was found in T_0 (10.87 cm). Drought stress reduced the spike length 43.14% than the no stress condition. T_5 (11.69 cm) and T_7 (11.81cm) was found as sensitive stage for spike development than other treatments though T_3 (12.70 cm) was critical than T_2 and T_4. T_2 (14.32cm) and T_4

(14.51 cm) showed statistically similar result. Sangtarash (2010) found the same effect on drought stress. He noticed drought stress reduce the spike length.



Treatments are: Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 23. Effect of drought stress on spike length ($SE\pm_{0.05}$ = 0.1821).

4A. 12.3 Interaction effect of GA and drought stress on spike length

GA and drought stress creates an opposite interaction on wheat. Drought stress reduced the spike length whereas GA increased it. From the experiment it was found that the maximum spike length was found in G_1T_1 (15.94 cm) and it was higher than G_0T_1 (15.19). Again the lowest length was 10.014 cm that found in G_0T_0. Full stress condition with GA produced 11.734 cm spike length which was statistically similar with G_0T_6 (11.684 cm) and G_0T_7 (11.33 cm). The stage which was suffered from drought stress mostly was CRI + flowering stage or G_0T_5 (10.93) and it was statistically similar with G_0T_7 (11.33 cm) though G_1T_5 and G_1T_7 gave better result than them. Mukhopadhyay and Bankar (1983) stated that GA increased the spike

length. And it was also supported by Islam (2013) who found higher spike length at drought stress by applying GA.

The result concluded that in no stress condition spike length was increased but with GA application it increased more also in case of full stress condition GA increased the spike length than full stress condition without GA. Besides this it was also observed that G_0T_5 and G_0T_7 were considered as the sensitive stage for spike length increased under drought stress.

Table 11 Interaction effect of GA and drought stress on spike length

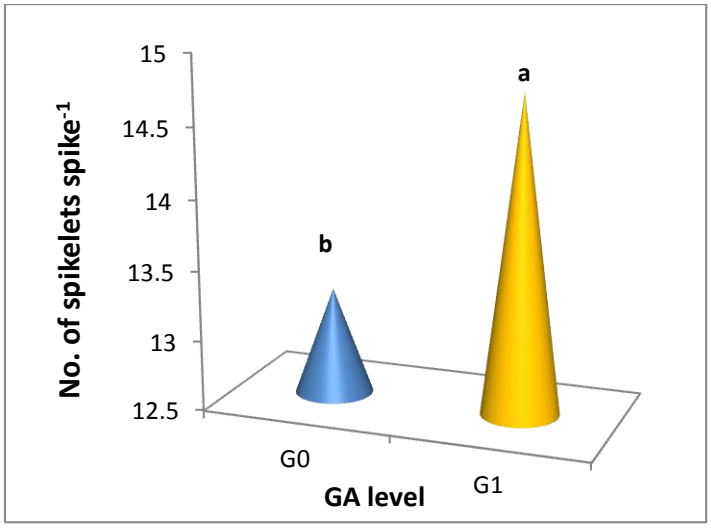
Interactions	Spike length (cm)
G_0T_0	10.01 j
G_0T_1	15.19 b
G_0T_2	13.61 e
G_0T_3	12.56 Fg
G_0T_4	14.59 cd
G_0T_5	10.93 i
G_0T_6	11.68 h
G_0T_7	11.33 hi
G_1T_0	11.73 h
G_1T_1	15.94 a
G_1T_2	15.03 bc
G_1T_3	12.84 f
G_1T_4	14.43 d
G_1T_5	12.46 fg
G_1T_6	14.56 cd
G_1T_7	12.31 g
$SE\pm_{(0.05)}$	0.257
CV (%)	2.41

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

4A.12 Number of spikelets spike^{-1}

4A.12.1 Effect of GA on spikelets spike^{-1}

From the data it was showed that GA increased the spikelets spike^{-1}. Here G_0 produced 13.26 spikelets spike^{-1} whereas it was 14.76 at G_1.



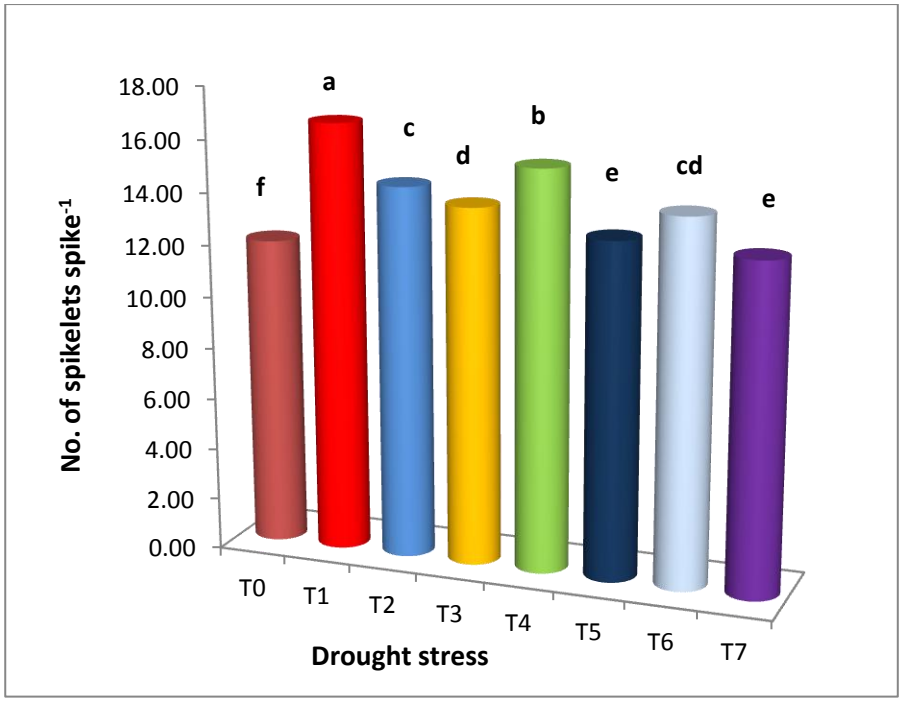
Here **G_0** =No GA, **G_1**= GA spraying

Figure 24. Effect of GA on no. of spikelets spike^{-1} (SE±$_{0.05}$ = 0.0924).

4A. 12.2 Effect of drought stress on spikelets spike^{-1}

Drought stress reduces the spikelets spike^{-1}. From the data we found that, maximum no. of spikelets spike^{-1} was found from T_1 (16.635) and the lowest number was from T_0 (11.972). About 41% no. of spikelets reduced due to the drought stress. Besides it was also noticed that stress at CRI + flowering stage (T_5) and stress at flowering + grain development stage (T_7) was more critical. They were statistically similar and produced 13.00 and 12.712 spikelets per spike respectively. If we want to consider any single stage like only CRI or only flowering or grain development stage for drought susceptibility then flowering stage will be more critical.

Drought stress reduced the spikelets spike^{-1} and this result was supported by Sarkar (2015); Nahar (2013) and Zhang *et al.* (2006).



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 25. Effect of drought stress on spikelets/spike ($SE\pm_{0.05}$ =0.2171).

4A. 13.3 Interaction effect of GA and drought stress on spikelets/spike

Highest number of spikelets/spike was found from G_1T_1 (17.12) and lowest number was from G_0T_0 (11.5). G_1T_0 produced 12.443 spikelets and G_0T_1 produced 16.15 spikelets/ spike as well as in every treatment when GA was applied it produced more spikelets/spike both in stress condition and irrigated condition. It was also seen that like spike length, spikelets per spike was minimum in G_0T_5 (11.53) and G_0T_7 (12.09). G_1T_7 (13.33) also produce lower number of spikelets than other treatments with GA. So the sensitive stage was CRI + flowering stage and flowering and grain development stage.The findings from the Table also represents that GA actually works to increase the number of spikelets per spike as well as it helped heat plant to alleviate the effect of drought stress. Sangtarash (2010), Nahar (2013) and Khan et al. (2009) found the lower amount of spikelets/spike under drought stress.

Table 12 Interaction effect of GA and drought stress on spikelets spike^{-1}

Interactions	Spikelets spike^{-1}	
G_0T_0	11.5	h
G_0T_1	16.15	b
G_0T_2	13.44	e
G_0T_3	13.15	e
G_0T_4	15.39	c
G_0T_5	11.53	h
G_0T_6	12.86	ef
G_0T_7	12.09	gh
G_1T_0	12.44	fg
G_1T_1	17.11	a
G_1T_2	15.4	c
G_1T_3	14.51	d
G_1T_4	15.51	bc
G_1T_5	14.46	d
G_1T_6	15.29	c
G_1T_7	13.33	e
$SE\pm_{(0.05)}$	0.3070	
CV%	2.68	

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stasge (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

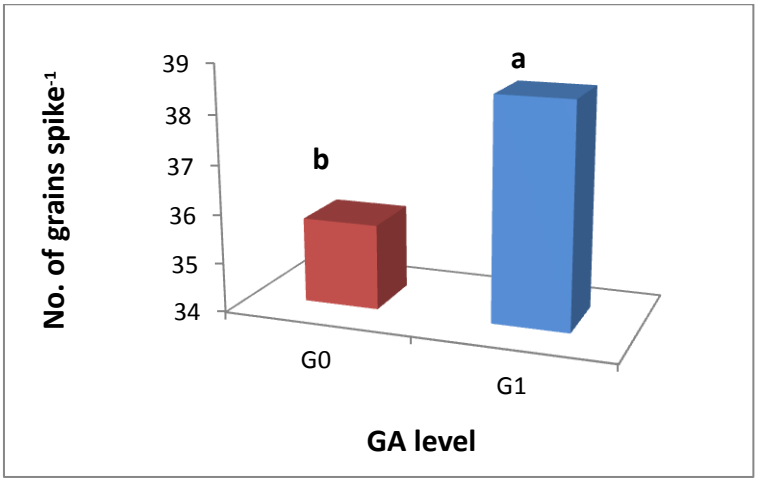
Khan et al. (2009) found highest filled grain spike^{-1} (31.90) when irrigation was given at CRI stage but Sangtarash (2010) stated that early stem elongation period was most sensitive. Rahman and Wilson (1977) stated GA increased the spikelets spike^{-1}.

Islam (2013) also stated that even under drought stress GA increased the spikelets number**.**

4A.13 Number of grains spike^{-1}

4A.13.1 Effect of GA of grains spike^{-1}

GA increased the grains number spike^{-1}. It was found that GA increased about 8% grains spike^{-1}. It was measured that GA produced 38.53 grains where as G_0 produced 35.74 grains spike^{-1}. This result was expected as it was previously discussed that GA increased the spike length and number of spikelets. Rebetzke and Richards (2000) also stated the effect of GA on increasing the grain number.

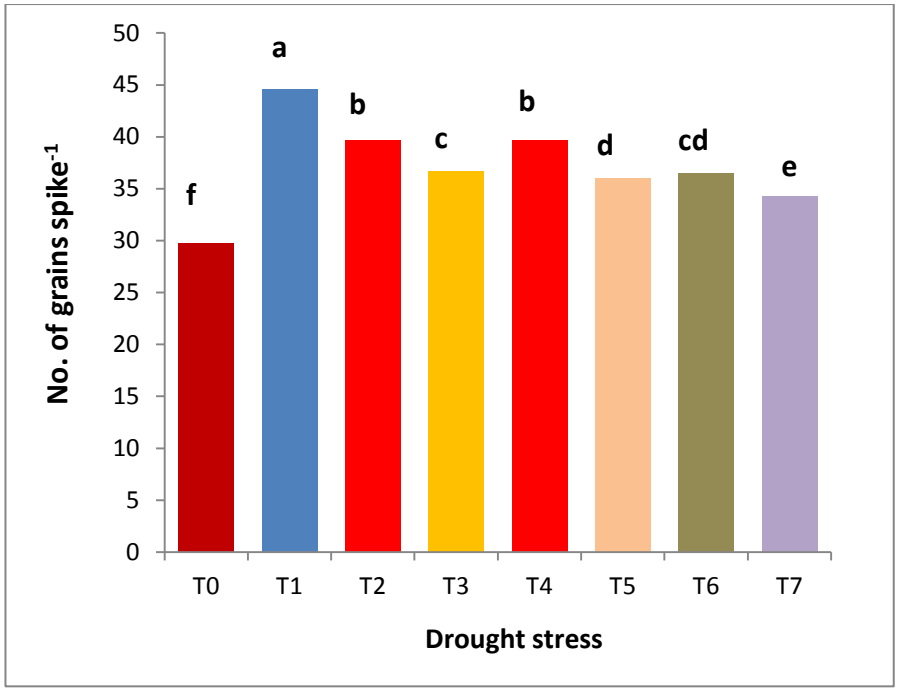


Here **G_0** =No GA, **G_1**= GA spraying

Figure 26. Effect of GA on no. of grains spike^{-1} (SE± $_{0.05}$ =0.0910).

4A. 13.2 Effect of drought stress on grains spike^{-1}

Drought stress has a negative effect on wheat, from the data it was found that grain number was maximum in T_1 (44.53) and the lowest value was found in T_0 (29.74). About 33.25% grain number spike^{-1} were reduced due to drought stress than the no stress condition. The T_7 (34.24) was found as the sensitive stage because when drought stress occurred both in flowering and grain development stage, it reduced the number of grains/spike. Mushtaq et al. (2011) stated that skipping irrigation at grain filling stage and tillering stage reduced the grains. Same result was found by Nahar (2013).



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 27. Effect of drought stress on no. of grains spike^{-1} (SE± $_{0.05}$ = 0.2753).

4A. 13.3 Interaction effect of GA and drought stress on grains spike^{-1}

GA and drought stress both has a controversy effect on wheat. Drought stress reduced the no. of grains spike^{-1} whereas GA increased it. From the experiment it was found that the maximum no. of grains spike^{-1} was found in G_1T_1 (45.86) and it was higher than G_0T_1 (43.2). Again the lowest no. of grains spike^{-1} was 28.96 that found in G_0T_0. Full stress condition with GA produced 30.52 grains spike^{-1}. The stage which was suffered from drought stress mostly was flowering + grain development stage or G_0T_7 (32.83) though G_1T_7 gave better result than others.

The result concluded that in no stress condition no. of grains spike^{-1} was increased but with GA application it increased more also in case of full stress condition GA helped to increased the no. of grains spike^{-1} than full stress condition without GA. Besides this it was also observed that G_0T_7 was considered as the sensitive stage for no. of grains spike^{-1} under drought stress. GA increased the grain production in drought stress

(Islam, 2013). Irrigation given at both CRI stage and pre flowering stage with 200 ppm GA gave better performance.

Table 13 Interaction effect of GA and drought stress no. of grains spike^{-1}

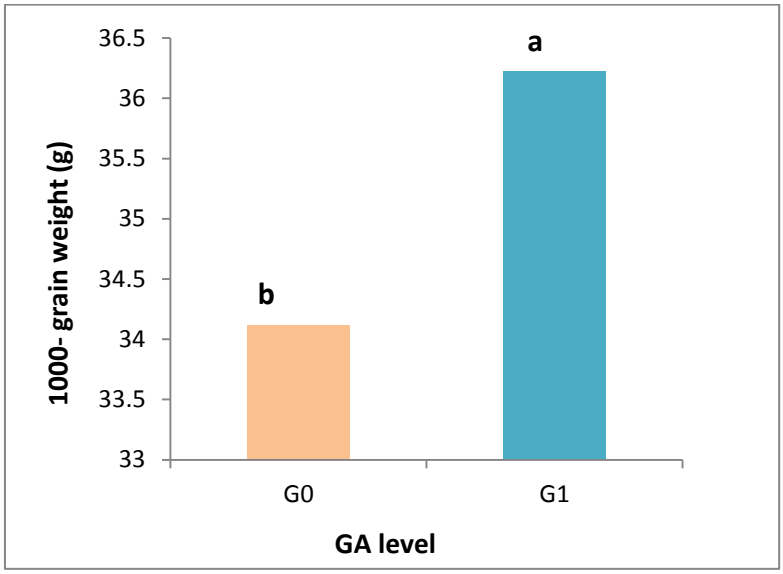
Interactions	No. of grains spike^{-1}
G_0T_0	28.96 k
G_0T_1	43.2 b
G_0T_2	36.21 g
G_0T_3	36.11 g
G_0T_4	39.12 d
G_0T_5	34.66 h
G_0T_6	34.83 h
G_0T_7	32.83 i
G_1T_0	30.52 j
G_1T_1	45.86 a
G_1T_2	43.16 b
G_1T_3	37.26 f
G_1T_4	40.26 c
G_1T_5	37.32 f
G_1T_6	38.16 e
G_1T_7	35.65 g
SE± $_{(0.05)}$	0.389
CV (%)	1.28

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

4A.14 1000-grain weight

4A.14.1 Effect of GA on 1000-grain weight

It was found that GA increased the 1000-grain weight. 1000-seed weight of GA treated plants was 36.22 g where as it was 34.12 g for G_0. It was previously discussed that GA increased the plant growth and development so it may play role to increase the grain weight. Islam *et al.* (2014) showed that GA increased the 1000-grain weight.



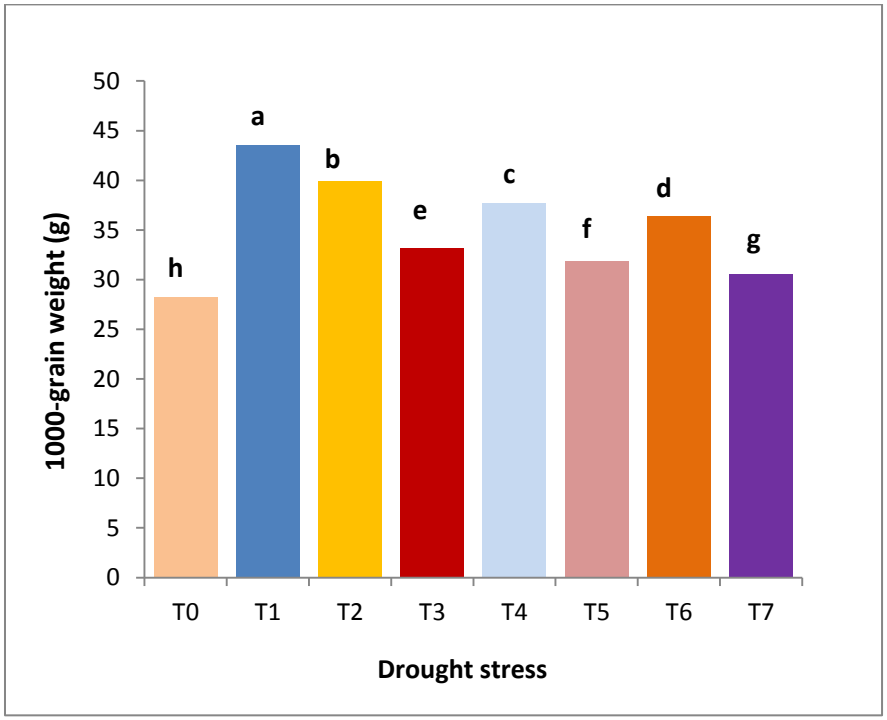
Here **G_0** =No GA, **G_1**= GA spraying

Figure 28. Effect of GA on 1000-grain weight (SE± $_{0.05}$ = 0.2417).

4A. 14.2 Effect of drought stress on 1000-grain weight

Drought stress reduces the 1000-grain weight. From Figure 29 we can see that, maximum weight of 1000-grain was found from T_1 (43.5 g) and the lowest weight was from T_0 (28.2). About 54% weight of 1000 seeds was reduced due to the drought stress. Besides it was also noticed that stress at flowering + grain development stage (T_7) and then stress at CRI + flowering stage (T_5) was more sensitive to drought stress. It was 30.5 g and 31.8 g of 1000-seed weight of T_7 and T_5 respectively. If we want to consider any single stage like only CRI or only flowering or grain development stage for drought susceptibility then flowering stage will be more critical. Drought stress always reduced the grain weight and it was supported by Ali *et*

al. (2013) as well as Ghodsi *et al.* (1998) mentioned that reproductive stage was more sensitive to drought stress than vegetative stage.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 29. Effect of drought stress on 1000-grain weight (SE± $_{0.05}$ = 0.2755).

4A. 14.3 Interaction effect of GA and drought stress on 1000-grain weight

The highest 1000 grain weight was found from G_1T_1 (44.55 g) and lowest number was from G_0T_0 (27.49 g). G_1T_0 produced 29.02 g weight of 1000 grains and G_0T_1 produced 42.57 g as well as in every treatment when GA was applied it increased the 1000 gain weight both in stress condition and irrigated condition. It was also seen that like spike length, spikelets per spike and others, 1000 grain weight was minimum in G_0T_5 (30.61 g) and G_0T_7 (30.12) and they were statistically similar. G_1T_7 (30.97 g) also produced lower number grains weight than other treatments with GA as well as it was statistically similar with G_0T_5 and G_0T_7. So the sensitive stage was CRI + flowering stage and flowering and grain development stage. The findings from the

Table 14 are that GA actually works to increase the weight of 1000 grains as well as it helped the plant to alleviate the effect of drought stress. Dong *et al.* (2009) said that different growth hormones like GA, ABA increased the 1000-grain weight. Abdel and AL-Rawi (2012) also used 200% GA on lentil and he found grain weight was increased in drought condition.

Table 14 Interaction effect of GA and drought stress on 1000-grain weight

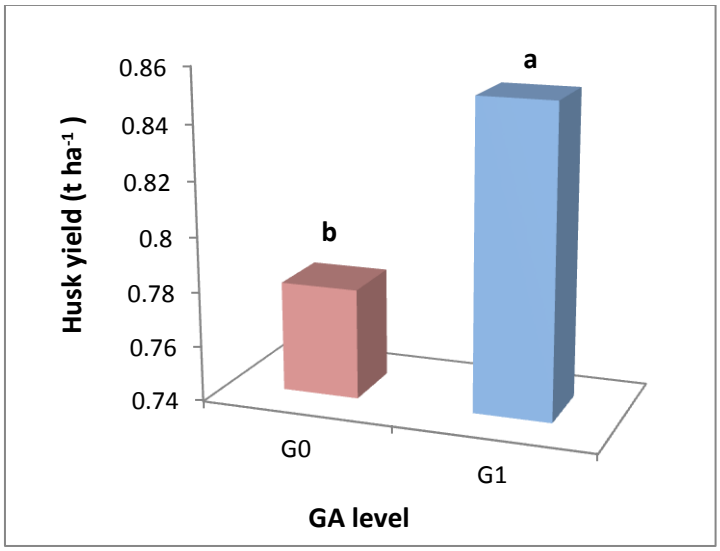
Interactions	1000-grain weight (g)	
G_0T_0	27.499	k
G_0T_1	42.579	b
G_0T_2	38.766	d
G_0T_3	31.75	h
G_0T_4	36.396	e
G_0T_5	30.611	i
G_0T_6	35.24	f
G_0T_7	30.106	ij
G_1T_0	29.022	j
G_1T_1	44.552	a
G_1T_2	41.085	c
G_1T_3	34.516	f
G_1T_4	38.984	d
G_1T_5	33.173	g
G_1T_6	37.474	e
G_1T_7	30.977	hi
$SE\pm_{(0.05)}$	0.3897	
CV (%)	1.60	

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

4A.15 Husk yield

4A.15.1 Effect of GA on husk weight

Gibberellic acid increased the husk weight. From the previous data we found that GA increased spike length, spikelets spike^{-1}, 1000 grain weight so ultimate yield of husk was increased due to the spray of GA. Data showed that husk yield was found about 0.85 t ha^{-1} from G_1 and 0.78 t ha^{-1} was found from G_0. About 10% husk yield increased due to the spray of GA. The similar result was found by Islam *et al.* (2014) and Islam (2013).

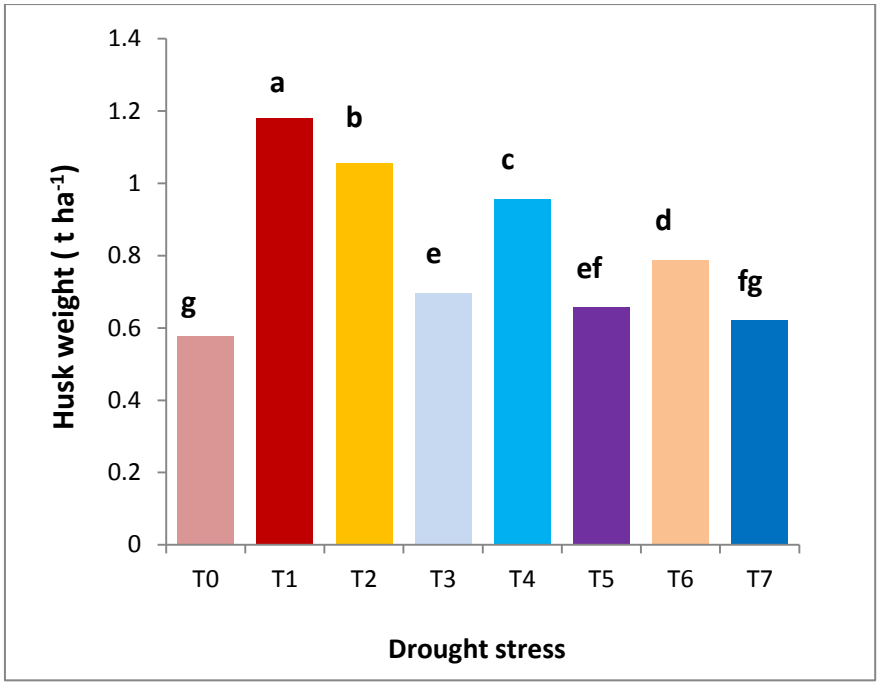


Here **G_0** =No GA, **G_1**= GA spraying

Figure 30. Effect of GA on husk weight (SE± $_{0.05}$ = 0.0116).

4A. 15.2 Effect of drought stress on husk weight

Drought stress reduced the husk weight. The lowest husk weight was found from T_0 (0.57 t ha^{-1}) and highest weight was from T_1 (1.17 t ha^{-1}). About double amount of husk weight was reduced due to the drought stress. Again the sensitive stage for reduction of husk weight was T_7 (0.62 t ha^{-1}) though T_5 (0.65 t ha^{-1}) was statistically similar with T_7 and T3 (0.69). So we may say that reduction of husk weight will be reduced if wheat plants face continuous drought stress from flowering to grain development stage.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 31. Effect of drought stress on husk weight (SE± $_{0.05}$ = 0.0214).

4A. 15.3 Interaction effect of GA and drought stress on husk weight

From the table it was found that maximum husk yield was found from G_1T_1 (1.33 t ha^{-1}) and it was statistically similar with G_1T_2 (1.18 t/ha). The lowest amount of husk was found from G_0T_0 (0.4744 t/ha). G_0T_1 and G_1T_0 produced 1.0269 and 0.68 t ha^{-1} respectively also G_0T_2 produced 0.93 t ha^{-1} husk. The result implied that GA increased the husk weight both in full stress condition and no stress condition than without GA as well as the role of GA may be understood from comparing the G_0T_2 and G_1T_2. It was found that GA protected the wheat plants from stress at CRI stage that's why husk weight increased.

G_1T_7 produced 0.5919 t ha^{-1} husk and it was statistically similar with G_0T_7 (0.64) and G_1T_3 (0.65 t ha^{-1}). Again, G_0T_5 (0.69 t ha^{-1}) and G_1T_5 (0.62 t ha^{-1}) was statistically similar with G_0T_7 (0.64) and G_1T_3 (0.65 t ha^{-1}). So we may assumed that either GA

applied or not flowering + grain development stage was more critical as well as flowering stage and CRI + flowering stage was also sensitive to drought stress.

Table 15 Interaction effect of GA and drought stress on husk weight (t ha^{-1})

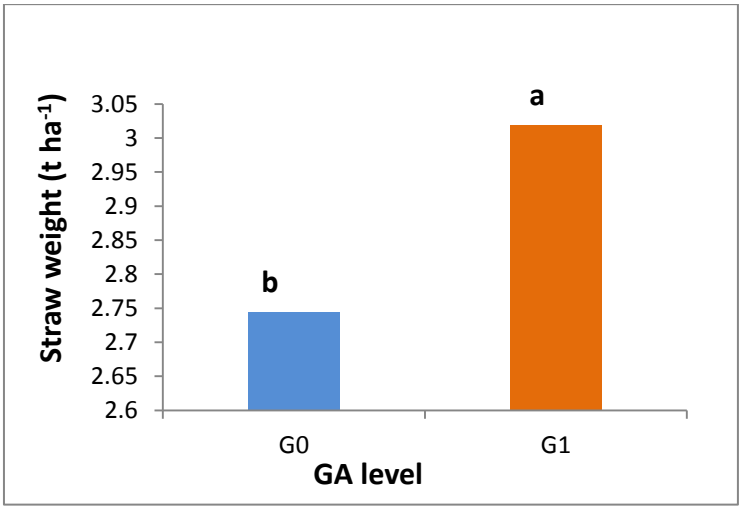
Interactions	Husk weight (t ha^{-1})
G_0T_0	0.47 i
G_0T_1	1.02 b
G_0T_2	0.93 c
G_0T_3	0.74 e
G_0T_4	0.87 c
G_0T_5	0.69 ef
G_0T_6	0.85 d
G_0T_7	0.64 fgh
G_1T_0	0.68 efg
G_1T_1	1.33 a
G_1T_2	1.17 a
G_1T_3	0.65 fgh
G_1T_4	1.03 b
G_1T_5	0.62 gh
G_1T_6	0.71 e
G_1T_7	0.59 h
$SE\pm_{(0.05)}$	0.0302
CV (%)	4.54

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

4A.16 Straw yield

4A.16.1 Effect of GA on straw weight

Gibberellic acid increased the straw weight. From the previous data it was found that GA increased the vegetative growth, dry matter and here straw weight was increased due to the spray of GA. Data showed that straw weight was found 3.02 tha^{-1} from G_1 and 2.74 tha^{-1} was found from G_0. About 10% straw weight was increased due to the spray of GA.



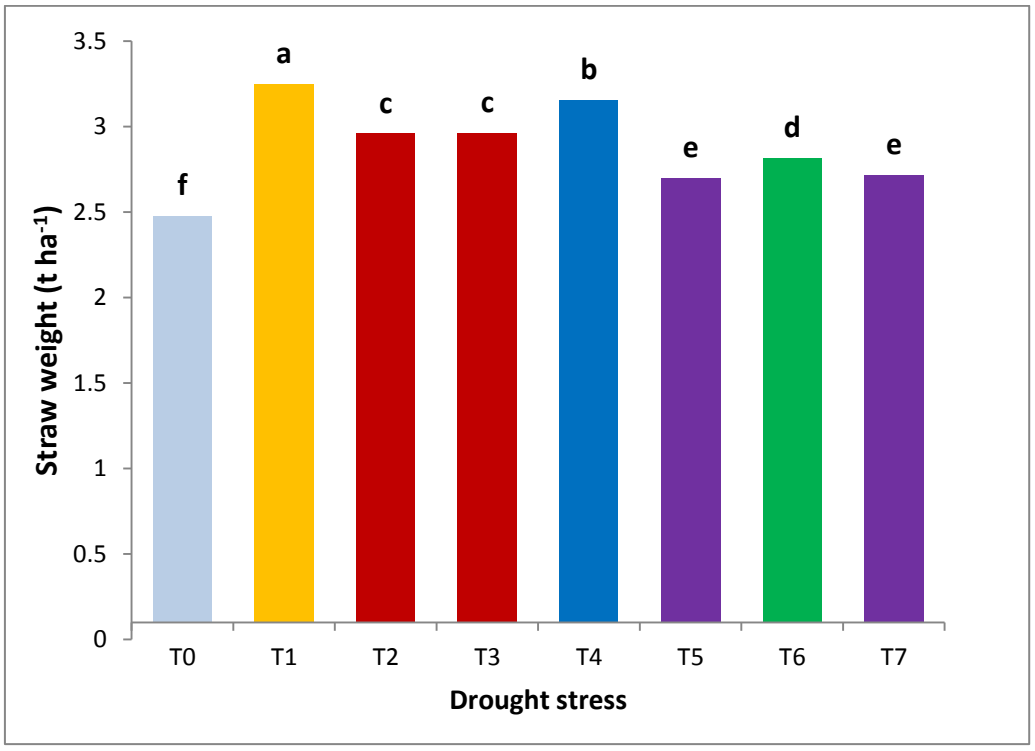
Here **G_0** =No GA, **G_1**= GA spraying

Figure 32. Effect of GA on straw weight (SE± $_{0.05}$ = 0.0211).

4A. 16.2 Effect of drought stress on straw weight

If plant gets optimum moisture condition in its growing period, growth and development also be optimum. In the experiment it was found that the maximum straw weight was in T_1 (3.24 t ha^{-1}) and the lowest amount of straw was in T_0 (2.48 t ha^{-1}). It was because due to lack of water, plant could not grow vigorously and for this reason, plants straw weight was decreased. T_5 (2.7 t ha^{-1}) and T_7 (2.71 t ha^{-1}) was regarded as most sensitive stage for drought stress to produce maximum weight of straw. Here it was found that stress at grain development stage was less affected by drought stress for straw yield.

Plants already completed its maximum vegetative growth before reached to reproductive stage, so it may be happened that grain development stage was less sensitive for straw production under drought stress. Saleem (2003) found the reduced straw yield from the drought stress. Johari-Pireivatlou (2010) found the flowering stage was critical for straw production.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag (**T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 33. Effect of drought stress on straw weight (SE± $_{0.05}$ = 0.0349).

4A. 16.3 Interaction effect of GA and drought stress on straw weight

Combined effect of GA and drought stress on straw weight showed that maximum straw was produced from G_1T_1 (3.36 t ha^{-1}) though G_1T_2 (3.27 t ha^{-1}) was statistically similar with it. G_0T_1 produced 3.13 t ha^{-1} straw. Like other parameters straw yield was increased in G_1T_2 due to the positive role of GA. The minimum straw weight was found from G_0T_0 (2.43 t ha^{-1}) though G_1T_0 produced 2.53 t ha^{-1} straw.

So it was clear here that GA had worked in drought stress condition to protect the plants from serious damage.

Table 16 Interaction effect of GA and drought stress on straw weight

Interactions	Straw weight (t ha^{-1})
G_0T_0	2.4295 i
G_0T_1	3.1364 cd
G_0T_2	2.6489 fg
G_0T_3	2.8583 E
G_0T_4	3.1189 cd
G_0T_5	2.5366 h
G_0T_6	2.5596 fgh
G_0T_7	2.6614 f
G_1T_0	2.5309 h
G_1T_1	3.3605 a
G_1T_2	3.2725 ab
G_1T_3	3.0722 d
G_1T_4	3.1982 bc
G_1T_5	2.8629 e
G_1T_6	3.0728 d
G_1T_7	2.7752 e
$SE\pm_{(0.05)}$	0.0493
CV (%)	2.10

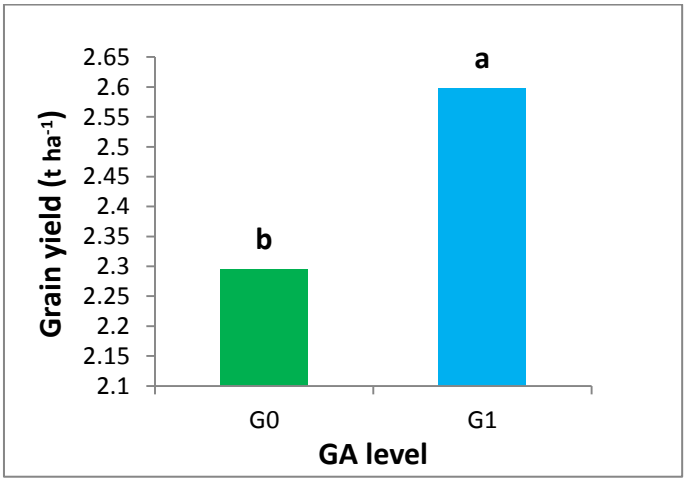
Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

The most sensitive growing stage for drought stress was CRI + flowering stage (2.5366 t ha^{-1}) though G_0T_6 (2.5596 t ha^{-1}) was statistically similar with G_0T_5. In case of GA application, G_1T_7 (2.7752 t ha^{-1}) and G_1T_5 (2.8629 t ha^{-1}) was the critical stage for straw production under drought stress. It may be occurred because most of vegetative growth happened during CRI and flowering stage. So when plants face stress at this time straw production was decreased. Islam *et al.* (2014) reported that GA increased straw yield up to 4.6 t ha^{-1} and according to Islam (2013) in drought stress with GA increased straw production.

4A.17 Grain yield

4A.17.1 Effect of GA on grain yield

GA increased the total grain yield. Grain yield from G_1 was 2.59 t ha^{-1} where as it was 2.29 t ha^{-1} for G_0. Previously it was found that GA increased the plant growth, spikelets number, 1000-grain weight, husk weight so it may play role to increase the grain yield.



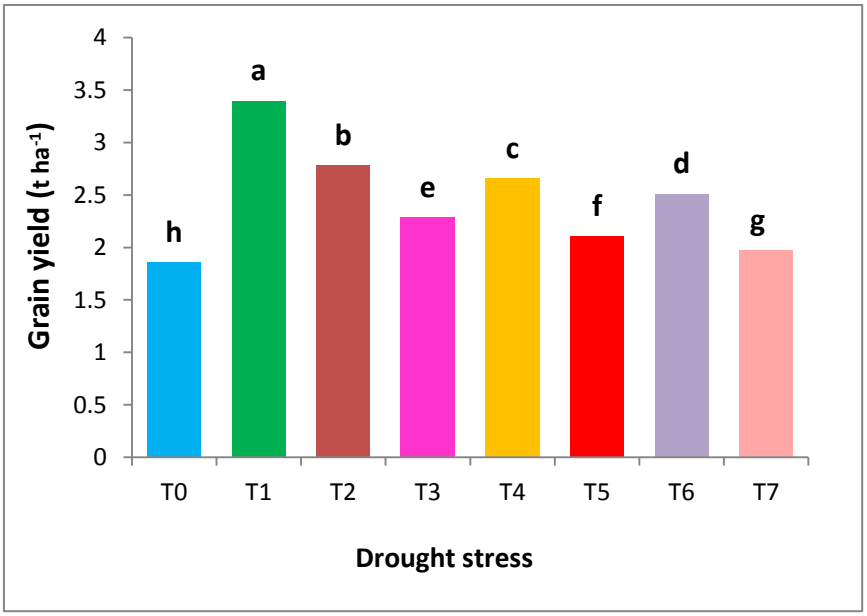
Here **G_0** =No GA, **G_1**= GA spraying

Figure 34. Effect of GA on grain yield (SE± $_{0.05}$ = 0.0223).

4A. 17.2 Effect of drought stress on grain yield

Drought stress reduces the grain yield. From the data we found that, maximum grain yield was found from T_1 (3.39 t ha^{-1}) and the lowest number was from T_0 (1.854 t ha^{-1}). About 45% grain yield was reduced due to the drought stress. Besides it was also noticed that stress at flowering + grain development stage (T_7) was more critical. It

produced 1.97 t ha^{-1} yields. Stress at CRI + flowering stage (T_5) also sensitive and it produced 2.11 t ha^{-1} yield which was little bit higher than T_7. If it was considered to any single stage like only CRI or only flowering or grain development stage for drought susceptibility then flowering stage was more critical. It produced 2.286 t ha^{-1} grains. Schneekloth *et al.* (2009) reported that drought stress decreased the yield in wheat but if it is in tillering stage yield reduction may be up to 46% but Zhang *et al.* (2006) said that drought stress should be avoided at the booting and heading of wheat. Another finding from Khan *et al.* (2009) was that CRI stage was more critical for drought stress.



Treatments are: Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**.

Figure 35. Effect of drought stress on grain yield (SE± $_{0.05}$ = 0.0227).

4A. 17.3 Interaction effect of GA and drought stress on grain yield

Highest grain yield was found from G_1T_1 (3.6109 t ha^{-1}) and lowest yield was from G_0T_0 (1.7274 t ha^{-1}) though G_1T_0 produced 1.9818 t ha^{-1} yield. G_0T_1 produced 3.1815 t ha^{-1} which was statistically similar with G_1T_2 (3.1586 t ha^{-1}). Where GA was applied it increased the grain yield both in stress condition and irrigated condition. It was also seen that like spike length, spikelets per spike and others, grain yield was also increased by GA. G_0T_5 (1.96 t ha^{-1}) and G_0T_7 (1.85 t ha^{-1}) were statistically

similar and produced lower yield. G_1T_5 and G_1T_7 also produced lower yield and it was 2.25 and 2.09 t ha^{-1} respectively. So the sensitive stage was CRI + flowering stage and flowering and grain development stage. The findings from the Table 17 are GA actually works to increase the grains yield as well as it helped the plant to alleviate the effect of drought stress. Rebetzke and Richards (2000) noticed GA inreased grain yield as well as Kaya *et al.* (2006) also stated that GA increased the capability to alleviate the drought stress and increased the yield.

Table 17 Interaction effect of GA and drought stress on grain yield

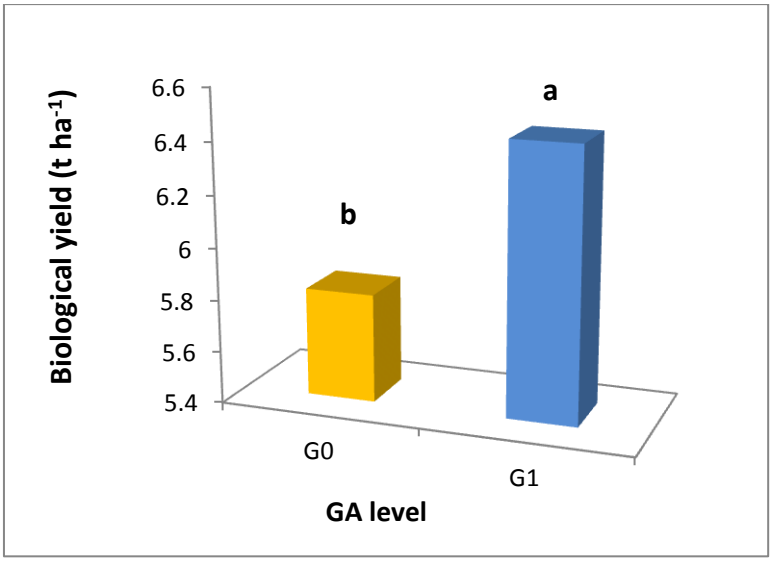
Interactions	**Grain yield (t ha^{-1})**	
G_0T_0	1.7274	j
G_0T_1	3.1815	b
G_0T_2	2.4083	e
G_0T_3	2.1865	fgh
G_0T_4	2.5652	d
G_0T_5	1.9632	hi
G_0T_6	2.4808	de
G_0T_7	1.8492	ij
G_1T_0	1.9818	hi
G_1T_1	3.6109	a
G_1T_2	3.1586	b
G_1T_3	2.386	ef
G_1T_4	2.7574	c
G_1T_5	2.2522	fg
G_1T_6	2.5402	d
G_1T_7	2.0995	gh
$SE\pm_{(0.05)}$	0.0321	
CV (%)	1.60	

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

4A.18 Biological yield

4A.18.1 Effect of GA on biological yield

Biological yield consists of both grain weight and straw weight. As both were increased by GA, ultimately biological yield also increased in G_1. G_1 (6.44 t ha^{-1}) produce about 19% higher biological yield than G_0 (5.81 t ha^{-1}).



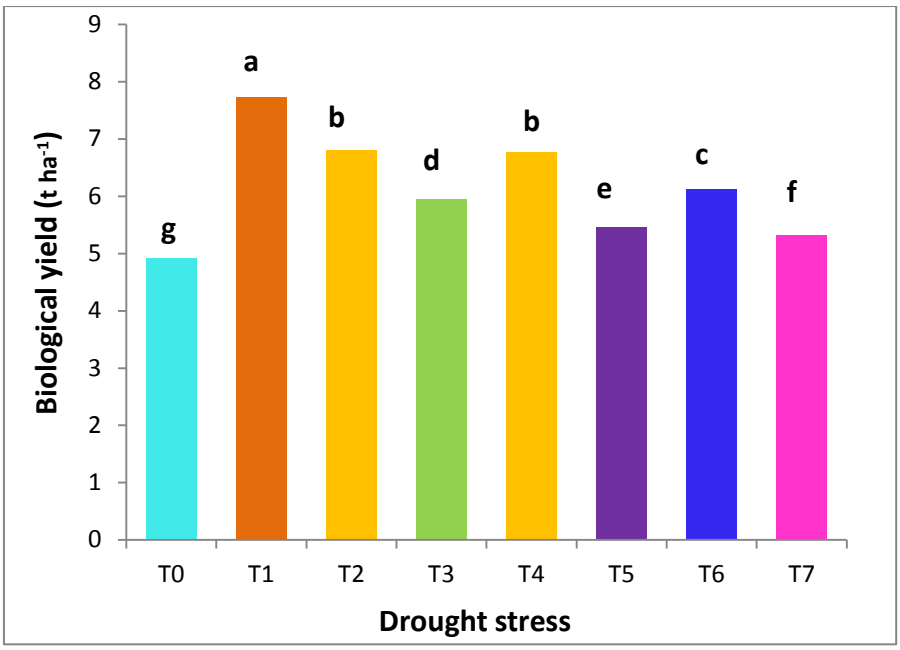
Here **G_0** =No GA, **G_1**= GA spraying

Figure 36. Effect of GA on biological yield (SE± $_{0.05}$ = 0.0523).

4A. 18.2 Effect of drought stress on biological yield

In the experiment it was found that the maximum biological yield was in T_1 (7.72 t ha^{-1}) and the lowest amount of biological yield was in T_0 (4.91 t ha^{-1}). It was because due to lack of water, plant could not grow vigorously and yield was also poor. For this reason, plants biological yield was decreased. T_7 (5.31 t ha^{-1}) and then T_5 (5.46 t ha^{-1}) was regarded as most sensitive stage for drought stress to produce maximum biological yield. Here we saw stress at grain development stage was less affected by drought stress for biological yield because in this stage straw weight was higher. So it may be happened that grain development stage is less sensitive for reduction of biological yield under drought stress. Khan *et al.* (2009) stated that if irrigation was

given at CRI stage for single irrigation, biological yield will be higher. He found 8.06 t ha^{-1} biological yields at optimum watering condition.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 37. Effect of drought stress on biological yield ($SE\pm_{0.05}$ = 0.0517).

4A. 18.3 Interaction effect of GA and drought stress on biological yield

Combined effect of GA and drought stress on biological yield showed that maximum biological yield was produced from G_1T_1 (8.10 t ha^{-1}) and G_1T_2 produced second higher biological yield (7.61 t ha^{-1}). G_0T_1 produced 7.34 t ha^{-1} biological yield. Like other parameters biological yield was also increased in G_1T_2 due to the positive role of GA. The minimum biological yield was found from G_0T_0 (4.63 t ha^{-1}) though G_1T_0 produced 5.19 t ha^{-1} straw. So here it was clear that GA had worked in drought stress condition to protect the plants from serious damage.

The most sensitive growing stage for biological yield under drought stress was G_0T_5 (5.19 t ha^{-1}) and G_0T_7 (5.15 t ha^{-1}) and they were statistically similar. It may be occurred because most of vegetative growth happened during CRI and flowering

stage. So when plants face stress at this time biological yield production was decreased. In case of GA application, G_1T_7 (5.4665 t ha^{-1}) and G_1T_5 (5.7361 t ha^{-1}) was also found as the critical stage under drought stress but they produced higher biological yield than G_0T_5 and G_0T_7. Atikulla (2013) showed when irrigation was given at CRI stage maximum biological yield was obtained and it was supported by Khan *et al.* (2009). On the other hand Islam (2013) found that irrigation at CRI or preanthesis stage with GA gave maximum biological yield and it was 9.93 t ha^{-1}.

Table 18 Interaction effect of GA and drought stress on biological yield

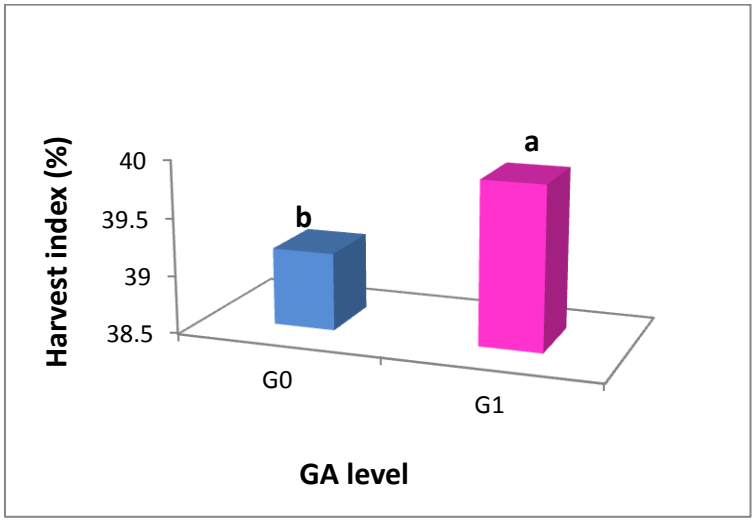
Interactions	Biological yield (t ha^{-1})	
G_0T_0	4.6313	j
G_0T_1	7.3448	c
G_0T_2	5.9881	f
G_0T_3	5.7856	g
G_0T_4	6.5565	e
G_0T_5	5.1917	i
G_0T_6	5.8977	fg
G_0T_7	5.1585	i
G_1T_0	5.1928	i
G_1T_1	8.1028	a
G_1T_2	7.6107	b
G_1T_3	6.1117	f
G_1T_4	6.9947	d
G_1T_5	5.7361	G
G_1T_6	6.3325	e
G_1T_7	5.4665	h
$SE\pm_{(0.05)}$	0.0731	
CV (%)	1.46	

Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

4A.19 Harvest index (HI)

4A.19.1 Effect of GA on Harvest index

GA increased the harvest index of wheat. Without GA application, harvest index was found 39.173 whereas GA treated wheat plants produced 39.909. G_1 increased about 22% harvest index than G_0. Austin *et al.* (1980) stated that GA increased the HI.

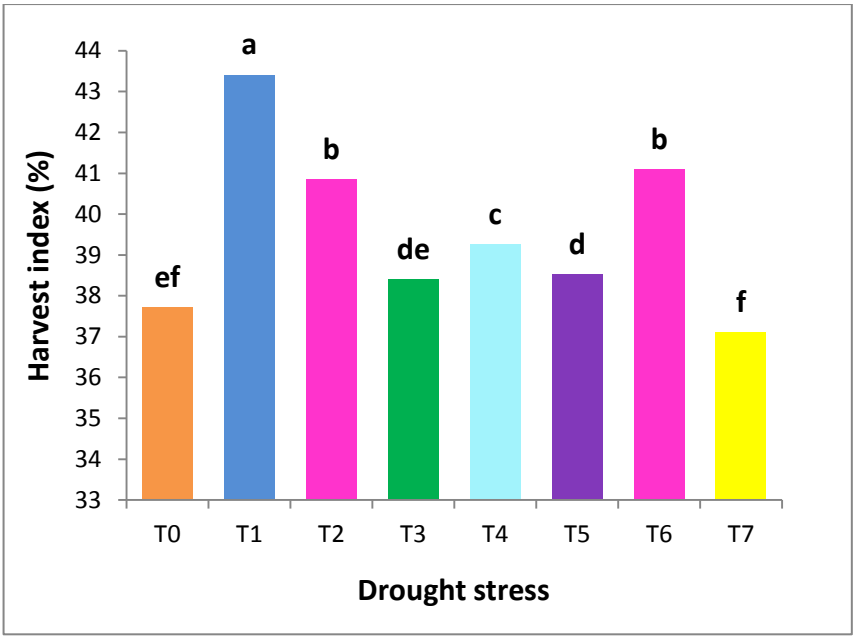


Here, **G_0**=No GA and **G_1** = GA spraying

Figure 38. Effect of GA on Harvest index (SE± $_{0.05}$ = 0.0454).

4A. 19.2 Effect of drought stress on Harvest index

From the experiment it was found that maximum reduction of harvest index was in full drought stress condition (37.70) but it was statistically similar with T_7 or stress at Flowering + grain development stage (37.10). T_3 also reduced the harvest index and it was 38.39. In T_5 (38.52) harvest index was also reduced**.** The highest harvest index was found from T_1 (43.40). Except T_1 minimum reduction of harvest index was in T_2 (40.85) and T_6 (41.08). Atikullah (2013) found 40.16% HI from full irrigated condition where as in stress condition he found only 32.94%. He found CRI stage was the most critical stage though Gupta *et al.* (2001) found anthesis stage as critical.



Here, Full stress condition **(T_0)**, No stress **(T_1),** Stress at CRI stage **(T_2),** Stress at flowering stage **(T_3),** Stress at grain development stag **(T_4),** Stress at CRI and flowering stage **(T_5),** Stress at CRI and grain development stage **(T_6),** Stress at flowering and grain development stage **(T_7)**

Figure 39. Effect of drought stress on Harvest index ($SE\pm_{0.05}$ = 0.3457).

4A. 19.3 Interaction effect of GA and drought stress on Harvest index

The maximum harvest index was found from G_1T_1 (43.489) and G_0T_1 (43.319). They were statistically similar. G_0T_6 (42.061) and G_1T_2 (41.499) produced second highest HI. The lowest harvest index was G_0T_7 (35.839). G0T0 produced 37.269% HI and it was similar with G_0T_3 (37.768) and G_1T_0 (38.143) but G1T0 was also statistically similar with G_1T_3 (39.018) and G_1T_7 (38.366). It was seen that GA has a role to increase the harvest index but it was not so much significant for irrigated condition but when it was in drought stress condition, as GA plays role to mitigate drought stress, it increased the harvest index.

Again the most critical stage for reduction the HI was flowering + grain development stage. Stress at flowering stage and stress at CRI + flowering stage was also found as sensitive stage to drought stress. Abdel and AL-Rawi (2012) noticed that GA was responsible for increasing harvest index about 14.94%. Islam (2013) also found

45.91% HI when irrigation was given at CRI and pre-flowering stage and 200ppm GA was sprayed. Again, Islam *et al.* (2014) also found (46.1%) HI after spraying GA.

Table 19 Interaction effect of GA and drought stress on Harvest index

Interactions	Harvest index (%)
G_0T_0	37.269 i
G_0T_1	43.319 a
G_0T_2	40.208 c
G_0T_3	37.768 hi
G_0T_4	39.116 ef
G_0T_5	37.8 hi
G_0T_6	42.061 b
G_0T_7	35.839 j
G_1T_0	38.143 ghi
G_1T_1	43.489 a
G_1T_2	41.499 b
G_1T_3	39.018 efg
G_1T_4	39.411 cde
G_1T_5	39.243 def
G_1T_6	40.104 cd
G_1T_7	38.366 fgh
SE±$_{(0.05)}$	0.4889
CV (%)	1.51

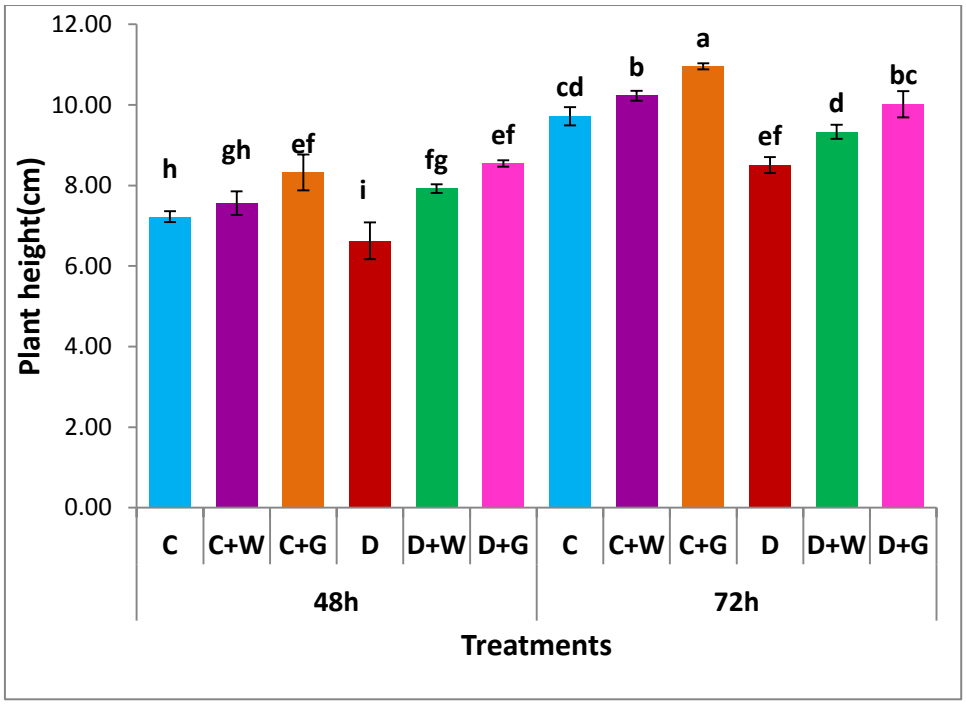
Here, G_0= No GA, G_1= GA spraying and others are Full stress condition (T_0), No stress (T_1), Stress at CRI stage (T_2), Stress at flowering stage (T_3), Stress at grain development stag (T_4), Stress at CRI and flowering stage (T_5), Stress at CRI and grain development stage (T_6), Stress at flowering and grain development stage (T_7).

Experiment: 2

4B. 1 Growth parameters

4B. 1.1 Plant height

As it was previously discussed in experiment 1 that drought stress disturbed the plant normal growth but GA helped to increase it. In this experiment it was also found that the shortest plant height was found in drought stress whereas the maximum plant height was found in control with GA spraying (Plate 1 and Plate 2). It was happened in case of both 48 h and 72 h treatments. GA played a role to increase plant height than control. For 48 h 15.17 % and for 72 h 11.29% plant height was increased.



Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 40. Effect of drought stress and GA on plant height of wheat

This experiment also showed that drought stress reduced the plant height about 8.3% and 12.48% for 48h and 72h respectively and GA helps to increase plant height from drought stress about 20.37% and 12.07% for 48h and 72h respectively. Here water also played a role for increasing plant height in case of drought stress. About 16.36% and 8.85% plant height increased due to water spraying for 48h and 72 h (Figure 40). Lonbani and Arzani (2011) reported that under drought stress plant height reduced significantly. Mehri (2015) also found the same result. Clua *et al*. (2009) stated that under drought stress plant height may decreased due to the damage by ROS, cell damage, photosynthesis reduction and so on however Pavlista *et al.*, (2014) found that GA increased the plant height.

Plate 1. Effect of GA on plant growth after 48 h of drought stress condition of wheat seedlings

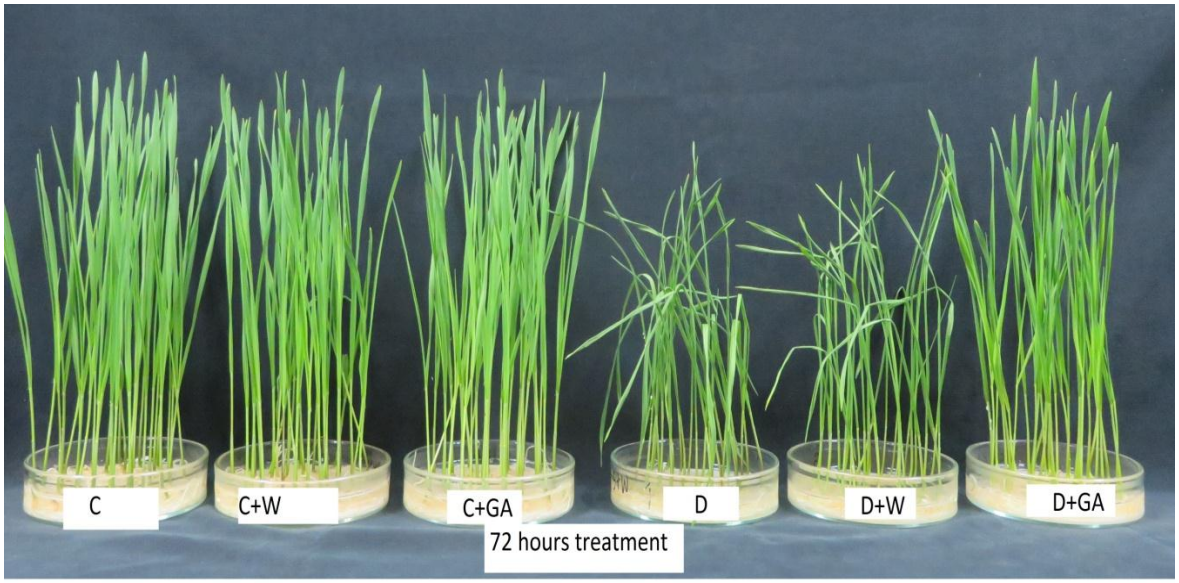
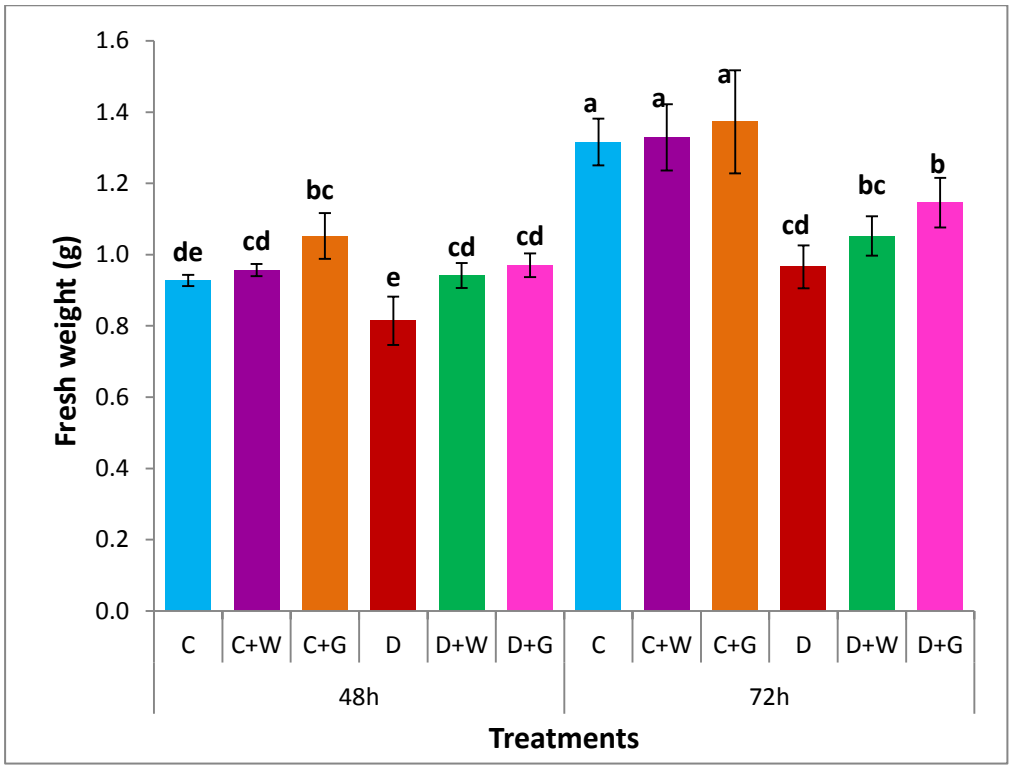


Plate 2. Effect of GA on plant growth after 72 h drought stress condition of wheat seedlings

4B.1. 2 Shoot fresh weight (g)

From the experiment it was found that in drought stress fresh weight was reduced and due to GA spraying it was increased. About 12.18% and 26.62% fresh weight was reduced in drought stress condition after 48h and 72 h respectively but in GA treated plant 16.15% FW increase after 48h and 18.63% fresh weight increased after 72 h in drought stress condition (Figure 41). Here after 48 h water and GA played statistically similar result for increasing FW but after 72 h water played a negligible role. Drought stress reduced the plant growth and plant biomass. GA helped to increase the plant fresh weight as it was also responsible for plant growth (Sakhabutdinova *et al.*, 2003).



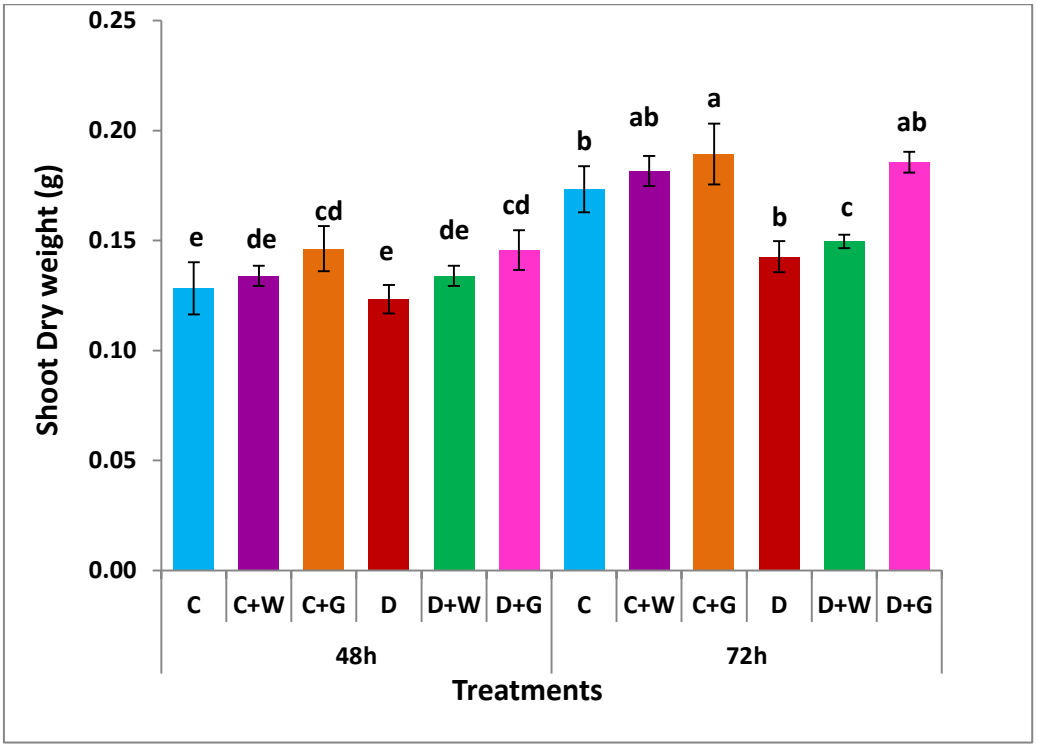
Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 41. Effect of drought stress and GA on FW of wheat

4B.1. 3 Shoot dry weight (g)

It was found that in drought stress dry weight was reduced and due to GA spraying it was increased. About 3.89% and 17.69% dry weight was reduced in drought stress condition after 48h and 72 h respectively but in GA treated plant 18.108% DW increased after 48h and 30.14% DW increased after 72 h in drought stress condition. Here after 48 h water and GA played statistically similar result for increasing DW but after 72 h water played a negligible role.

Schwechheimer (2008) supported the present findings and explained GA helped to increase the plant biomass under environmental stress condition.



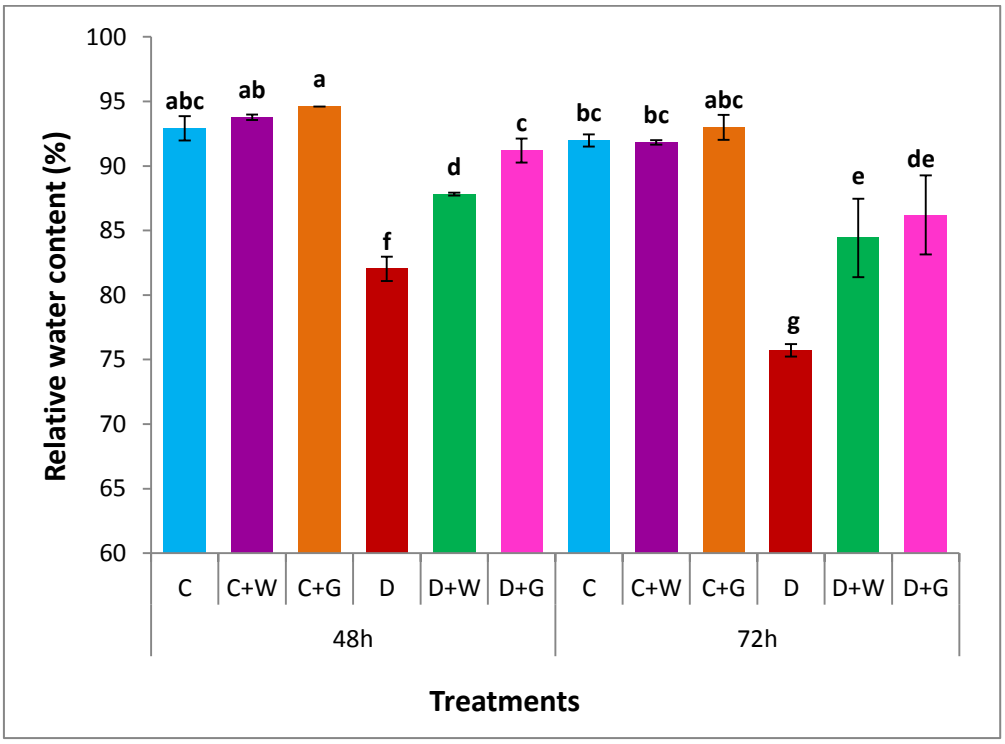
Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 42. Effect of drought stress and GA on DW of wheat

4B.1.4 Relative water content (RWC)

Relative water content decreased in drought stress condition. In this experiment the result showed us that under drought stress RWC decreased 11.71% and 17.68% after 48 h and 72 h respectively than control but in GA treated seedlings after 48h 11.17% and after 72h 13.86% RWC increased than drought (Figure 43).

It was seen that higher relative water content was found in C, C+W and C+G. water played a role in increasing RWC as respectively after 48h and 72h about 7% and 12 % RWC increased due to water spraying than drought. Ahmadizadeh *et al.* (2011b) supported the findings also Kaya *et al.* (2006) reported that in drought stress RWC decreased but GA increased the RWC under drought stress condition.



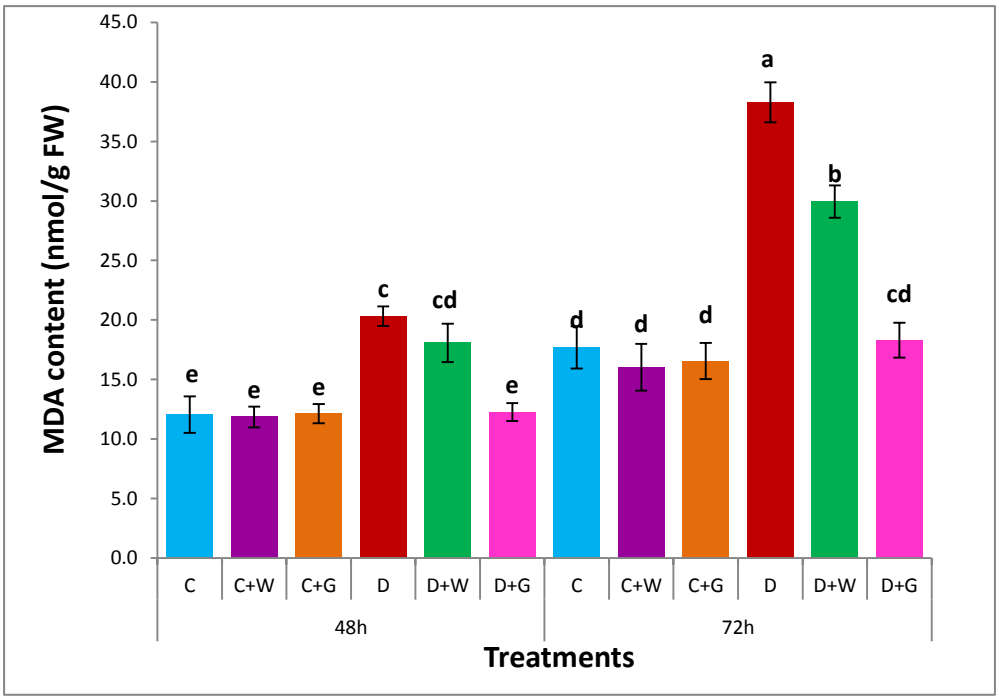
Here C means control, W means water, D means drought by 12% PEG and G means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 43. Effect of drought stress and GA on RWC of wheat

4B.2 Oxidative damage

4B.2.1 Lipid peroxidation (MDA content)

In the present study it was found that in drought stress MDA content was increased whereas GA plays a significant role to decrease the lipid peroxidation in cell. About 68% MDA content increased in drought stress condition and GA decreased the MDA content about 39.64% after 48 h of treatments. Water didn't play any significant role to decrease MDA. Again after 72 h it was seen that MDA content increased about 116.33% but GA reduced about 52.20% MDA content. Here water was responsible for 21.77% reduction of MDA (Figure 44).



Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

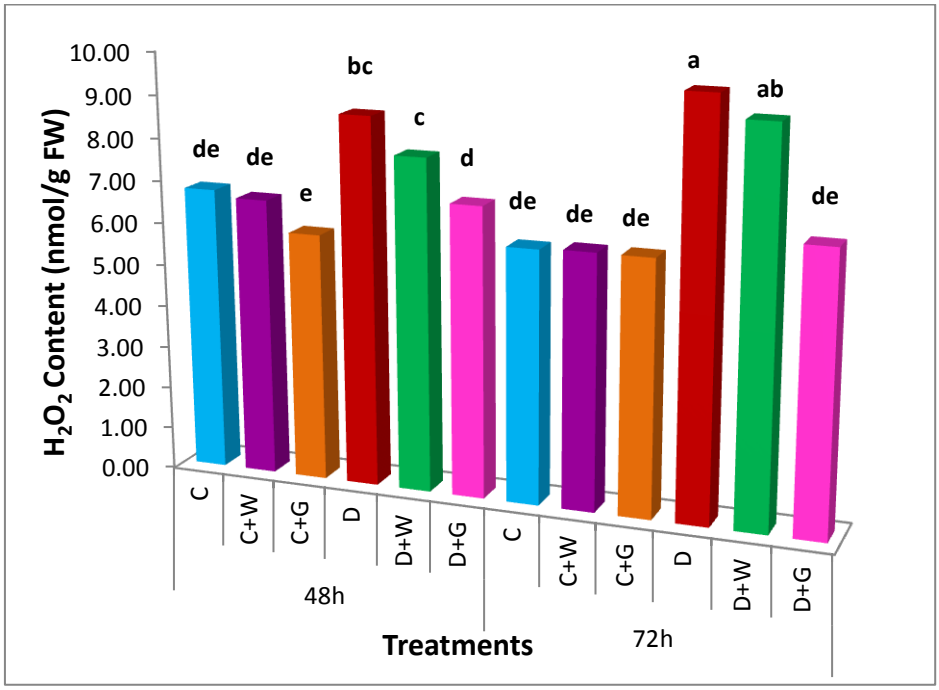
Figure 44. Effect of drought stress and GA on MDA content of wheat.

Cell membrane is one of the most common starting points of damage by drought Yamazaki *et al*. (2003). Under drought stress ROS production is increased and it damages the cell membrane by lipid per oxidation as well as protein degradation (Clua *et al*., 2009). Candan and Tarhan (2003) also stated that MDA content increased

in drought stress and cause cell damage. Sairam and Srivastava (2001) suggested that various kinds of protectants including plant growth regulators help to increase the drought resistance. Simova-Stoilova *et al*. (2008) also supported this.

4B.2.2 H_2O_2 Content

In any kind of abiotic stress, H_2O_2 production is a common phenomenon. Under drought stress its production is increased significantly. It was seen in my experiment that after 48h of drought stress, about 29.28% and after 72 h 61.76% H_2O_2 production was increased and in case of GA spraying, 21.26% and 31.94% H_2O_2 production decreased after 48 h and 72 h respectively (Figure 45). Here water didn't have any significant role to decrease H_2O_2 production.



Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 45. Effect of drought stress and GA on H_2O_2 content of wheat.

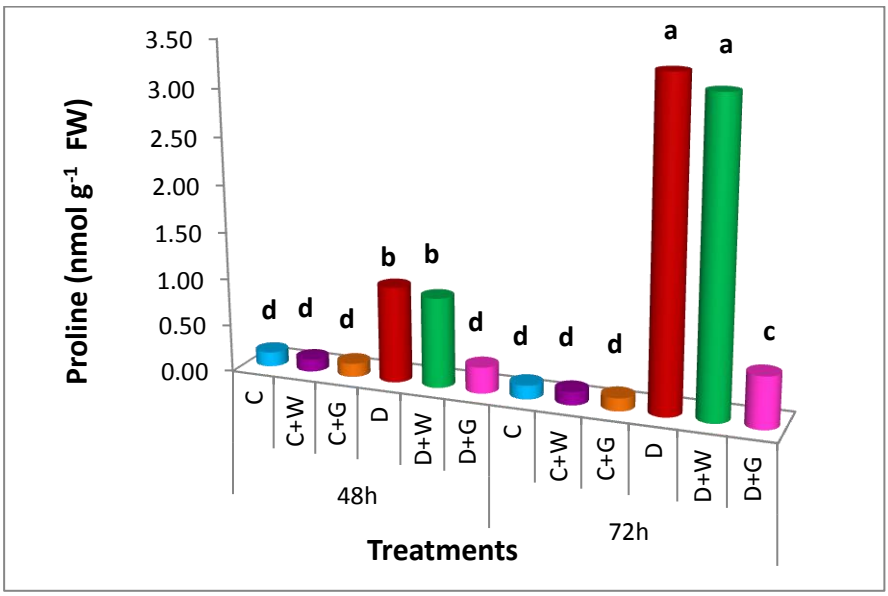
Under drought stress H_2O_2 production is increasing and cause oxidative stress (Qiusheng, *et al*., 2005). Mittler, (2002) and AL-Ghamdi, (2009) also stated that plants under drought stress produced H_2O_2 and also cause lipid per oxidation. Zhu *et*

al. (2004) and Kachout *et al.* (2009) reported that though H_2O_2 is toxic to plants, it can be detoxified by CAT and SOD activity. Fath *et al.* (2001) stated that GA increased the plant cell death by increasing H_2O_2 production but Schopfer *et al.* (2001) found the opposite result. He found that during germination time, GA decreased the oxidative damage including H_2O_2 that supported this experiment finding.

4B.2.3 Proline Content

Proline content increased in wheat seedlings under drought stress. 84.98% proline content increased after 48 h of drought stress but GA reduced 84.011% proline content. Again after 72 h it was increased about 95.72% where as GA reduced about 72.67% proline content. Like H_2O_2, water didn't have any role here.

In the study it was found that GA reduced the proline content but Li et al. (2010) found that free proline increased about 62.9% in case of rapeseed after applying GA in drought stress. Findings of Ahmad (2010) supported the present study as he found that GA application decreased the proline content 12.8% and 21.4% at different level of salt stress.



Here, **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 46. Effect of drought stress and GA on Proline content of wheat

4B.2.4 Chlorophyll

Under drought stress chlorophyll content decreased in wheat seedlings. It was reported that drought stress reduced the chlorophyll a, chlorophyll b and total chlorophyll about 11.76%, 14.98% and 12.46% respectively after 48 h. after 72 h it was 9.67%, 21.55%, 12.12% respectively. GA treated seedlings showed positive result to increasing chlorophyll content. GA increased 4.53, 12.88 and 6.29% chlorophyll a, chlorophyll b and total chlorophyll respectively for 48 h and 6.03, 16.84, 8.07 **%** for 72 h. Here water played statistically similar result like GA. Water also helped to maintain chlorophyll content during drought stress.

Table 20 Effect of GA on chlorophyll content of wheat under drought stress condition

Treatments	chl a (nmol/g DW)	chl b (nmol/g DW)	Chl (a+b) (nmol/g DW)
C 48h	12.49 ± 0.66	3.47 ± 0.20	15.96 ± 0.84
C+W 48h	12.18 ± 0.64	3.19 ± 0.26	15.37 ± 0.83
C+G 48h	12.33 ± 0.65	3.28 ± 0.17	15.61 ± 0.82
D 48h	11.02 ± 0.58	2.95 ± 0.31	13.97 ± 0.78
D+W 48h	11.69 ± 0.62	3.17 ± 0.32	14.86 ± 0.83
D+G 48h	11.52 ± 0.61	3.33 ± 0.20	14.85 ± 0.79
C 72h	13.02 ± 0.69	3.48 ± 0.21	16.49 ± 0.87
C+W 72h	12.80 ± 0.68	3.30 ± 0.22	16.10 ± 0.85
C+G 72h	12.47 ± 0.67	3.43 ± 0.20	15.90 ± 0.84
D 72h	11.76 ± 0.62	2.73 ± 0.34	14.49 ± 0.81
D+W 72h	12.55 ± 0.69	3.23 ± 0.31	15.78 ± 0.83
D+G 72h	12.47 ± 0.66	3.19 ± 0.19	15.66 ± 0.83

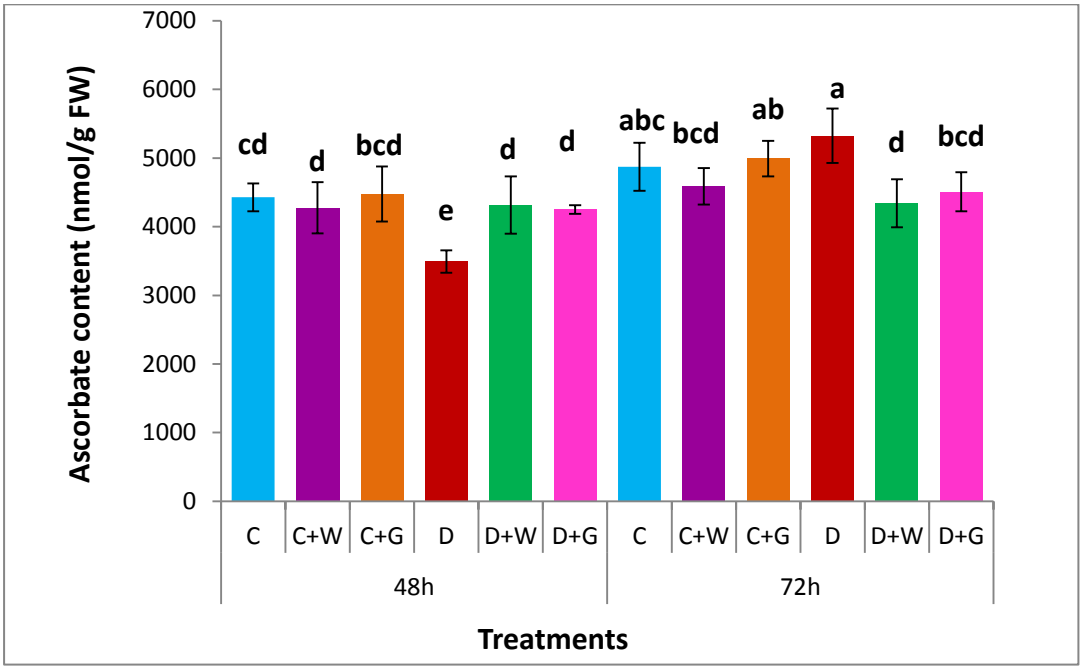
Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Shah (2007) reported that GA helped to restore the normal chlorophyll content. Keyvan (2010) had similar observation in case of Chl a and Chl b. Turkyilmaz, (2012) also found the same result that supported the present findings.

4B.3 Antioxidant enzyme activity

4B.3.1 Ascorbate content

In the experiment it was found that after 48 h of treatment, in drought stress condition ascorbate was reduced, and both water and GA showed statistically similar result; they increased the ascorbate content than drought. After 72 h it was found that ascorbate content increased in drought condition. Though its amount was higher than control but it was statistically similar with control. Both water and GA decreased the ascorbate content after 72 h but water reduced the ascorbate content more than control. Hasanuzzaman and Fujita (2011) stated that in case of mild stress AsA content increased but Tabata *et al.* (2002) found that under drought stress AsA content decreased.



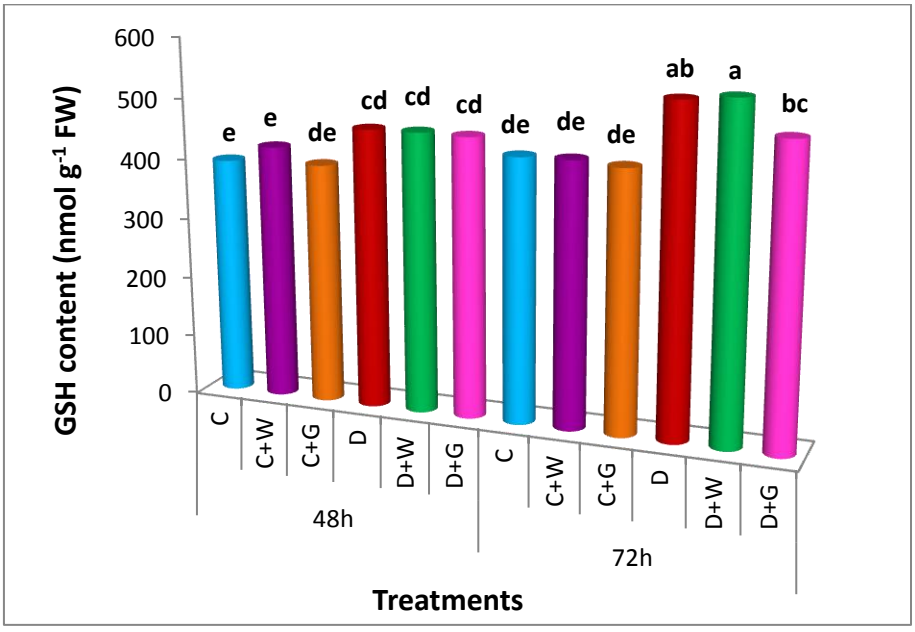
Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 47. Effect of GA on AsA content of wheat under drought stress condition

4B.3.2 GSH content

In the present study we found that under drought stress condition GSH content increased both in 48h and 72 h and it was about 17.41 and 23.96% respectively. In case of 48h like water GA didnt have any role but in case of 72 h GA decreased the GSH content about 8.6% (Figure 48).

Müller *et al.* (2006) and Xu *et al*. (2008) reported that in water deficient condition, reduced glutathione (GSH) increased that supports the present findings. Abedi and Pakniyat (2010) also find the same result where as Ahmadizadeh et al. (2011b) said GA played a role in decreasing GSH content under drought stress.



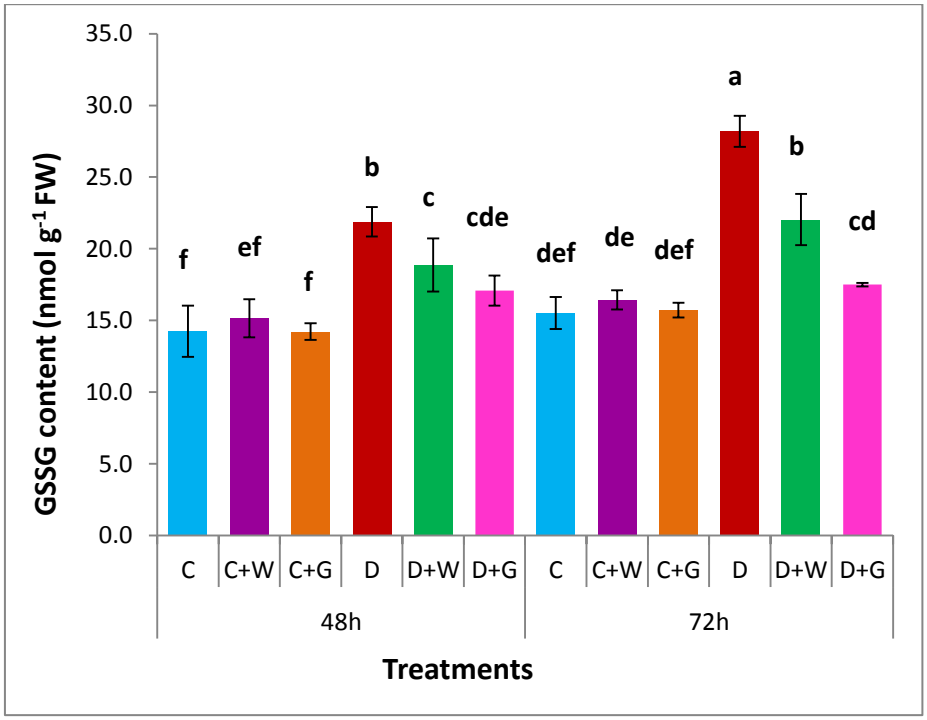
Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 48. Effect of GA on GSH content of wheat under drought stress condition

4B.3.3 GSSG content

In the study it was noticed that GSSG content increased under drought stress. About 53.47 and 46.35% GSSG increased after 48h and 72h drought stress respectively. In case of 48 h water played a little a role but GA decreased the GSSG content about

21.92%. After 72 h GA helped to decrease GSSG content about 37.902% whereas water played role to decrease about 21.84% GSSG content (Figure 49).

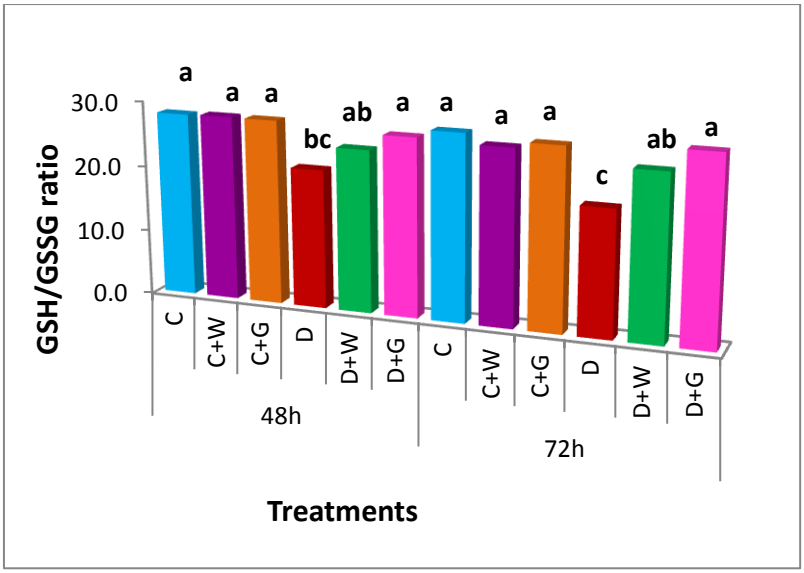


Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 49. Effect of GA on GSSG content of wheat under drought stress condition.

4B.3.4 GSH/GSSG ratio

In the experiment it was observed that GSH/GSSG ratio decreased due to the drought stress and it was about 32% in case of both 48 h and 72 h. Here GA played a significant role in increasing GSH/GSSG ratio. GA increased about 21.966% and 47.025% GSH/GSSG ratio after 48 h and 72 h respectively (Fig. 50). Here water didn't have any significant role.

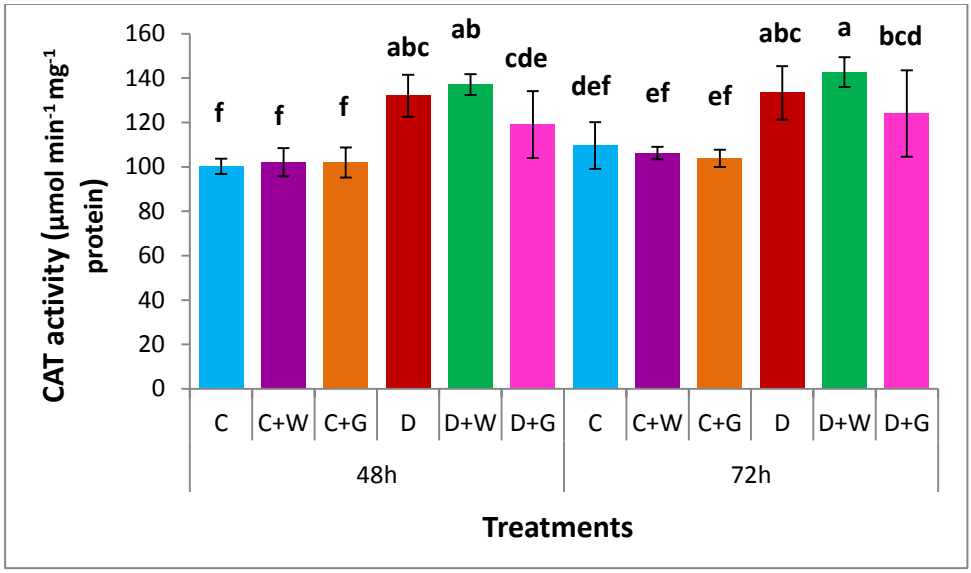


Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 50. Effect of GA on GSH/GSSG ratio of wheat under drought stress condition

4B.3.5 CAT activity

After 48 h CAT increased in drought stress about 17.33% and after 72 h it was 21.703 %. GA increased 9.8 % and 7.0 % CAT activity than drought stress but water was not signeficantlly changed (Figure 51).



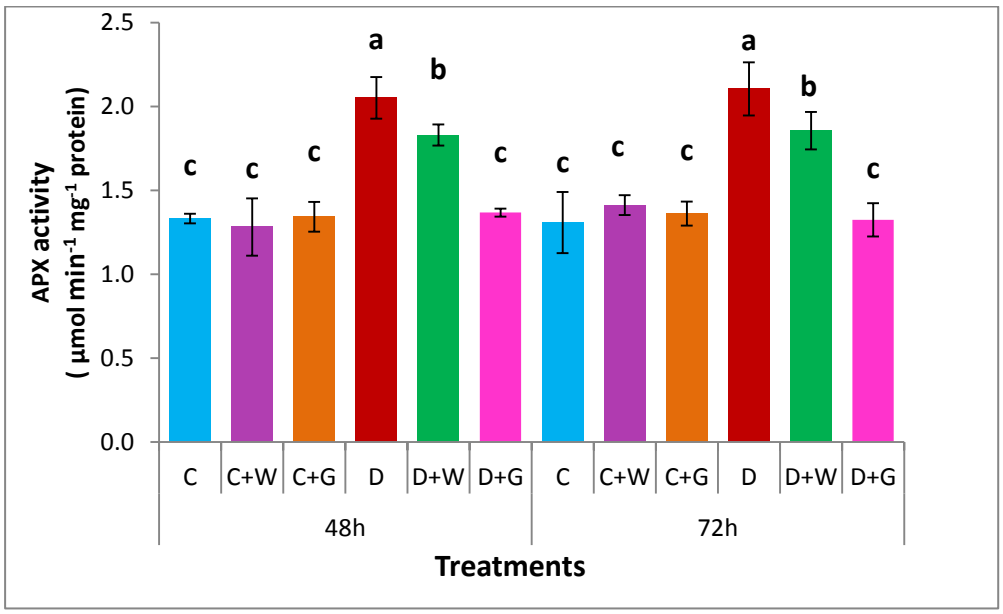
Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 51. Effect of GA on CAT of wheat under drought stress condition

Kachout *et al*. (2009) also found that under drought stress CAT activity increased as an anti oxidative enzyme. With the help of SOD, APX and other enzyme CAT helps to detoxify the ROS (Joanny, 2005). Bakalova *et al*. (2004) also reported that not only in drought but also at any environmental stress CAT activity is increased and played a critical role against the oxidative damage. Li *et al*. (2010) found that under drought stress GA increased the catalase activity.

4B.3.6 APX activity

Under drought stress, APX activity was increased about 53.907 and 60.968% respectively after 48 h and 72 h. in both cases, GA played a role to decrease the catalase activity but water increased it though it was statistically similar with drought stress condition. About 33.304% CAT activity was decreased after 48 h whereas after 72 h its reduction was 37.142% (Figure 52). Sharma and Dubey (2005) found that APX content increased with the increased level of drought. Ahmad (2010) supported these findings as he found GA decreased the APX activity under stress condition.

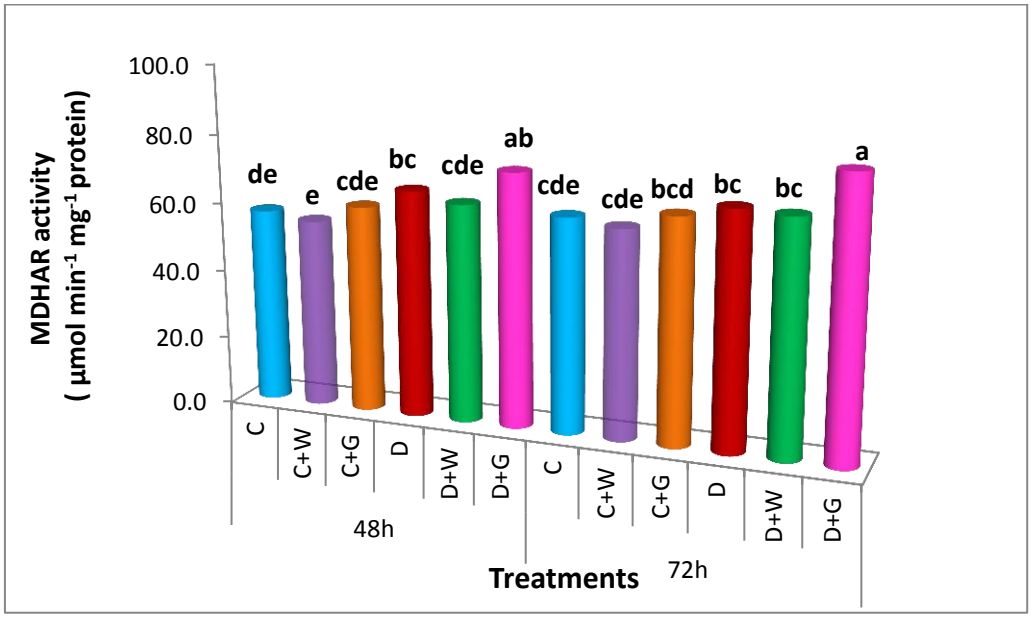


Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 52. Effect of GA on CAT of wheat under drought stress condition

4B.3.7 MDHAR activity

About 16.386% and 9.753% MDHAR activity increased under drought stress compared to control after 48 h and 72 h respectively but 11.47% and 17.68 % MDHAR activity increased due to GA compared to drought at 48 h and 72 h drought stress respectively (Fig. 53). Here water had no role to change the MDHAR activity significantly. Sharma and Dubey (2005) stated that Monodehydroascorbate reductase (MDHAR) was increased due to the drought stress.

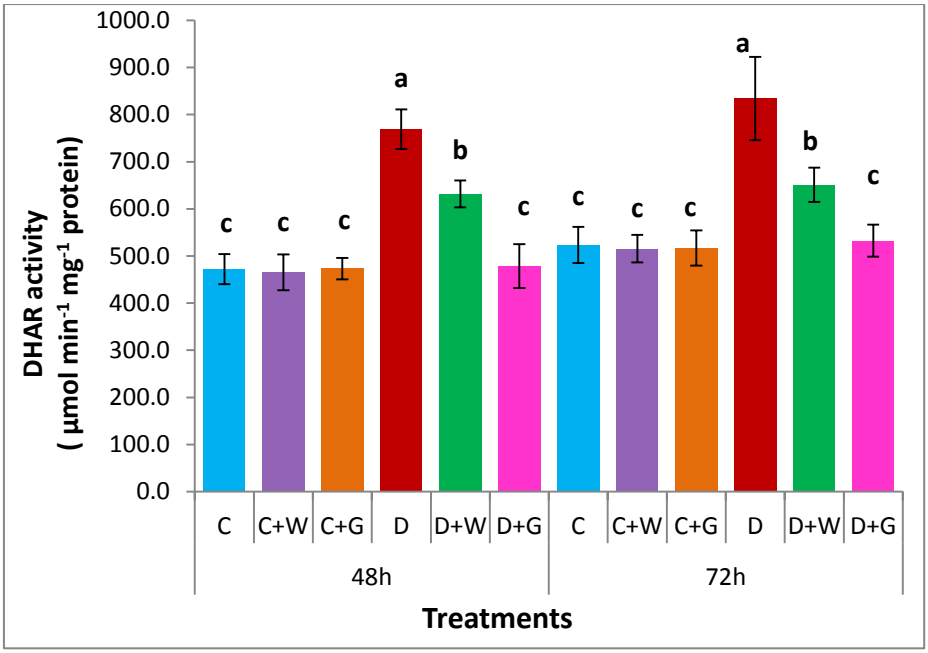


Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 53. Effect of GA on MDHAR of wheat under drought stress condition

4B.3.8 DHAR activity

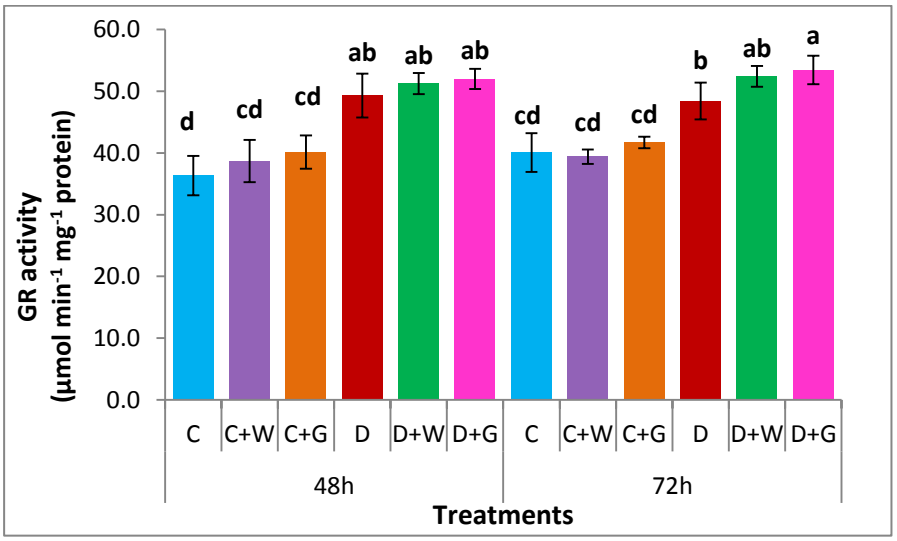
In this present study it was found that about 62.71% and 59.27% DHAR activity was increased in drought stress compare to control after 48 h and 72 h respectively where as at GA treated wheat seedlings about 37.764 and 36.144% DHAR activity was decreased after 48 h and 72 h compare to drought stress. Here ater played a little role. It reduced 17.83% and 21.93% DHAR activity. Sharma and Dubey (2005) found increased level of DHAR content under drought stress.



Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 54. Effect of GA on DHAR of wheat under drought stress condition

4B.3.9 GR activity



Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

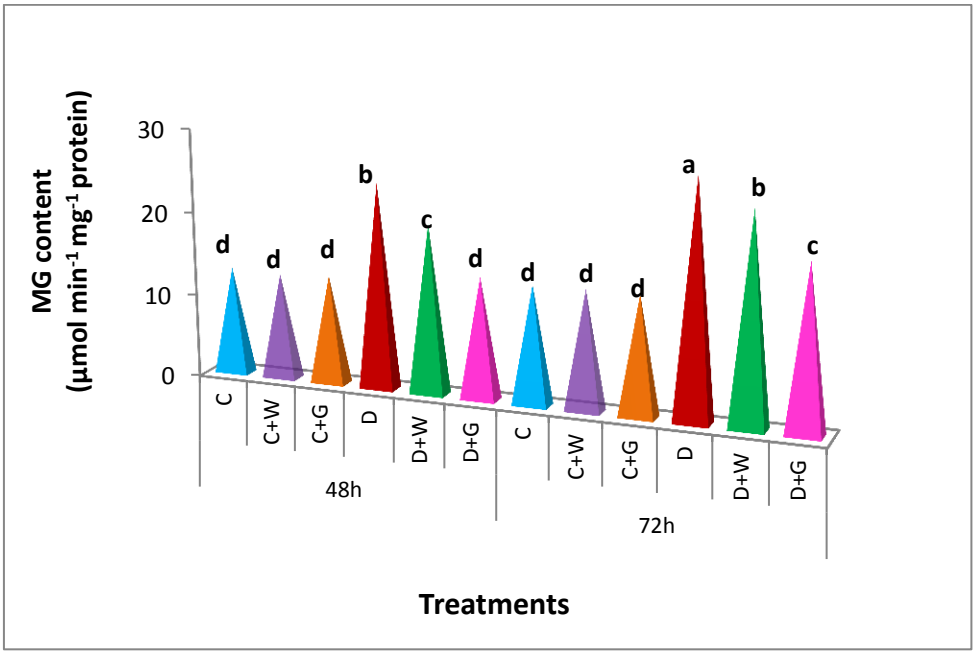
Figure 55. Effect of GA on GR of wheat under drought stress condition

From the Figure 55 we found that, in drought stress GR activity was increased. About 26.29% and 20.85% GR activity was increased after 48 h and 72 h of drought stress compared to control but GA didn't play any significant role to change the GR activity.

4B.4 Glyoxalase system and methylglyoxal detoxification

4B.4.1 Methylglyoxal (MG) content

MG content increased in drought stress compare to control and it was 90.78% and 99.35% after 48 h and 72 h respectively. GA had played a great role to reduce the MG content about 40.75% and 30.45% respectively for 48 h and 72 h stress compare to drought. Water played a little role as it reduced 17.69 and 11.71% MG content respectively. Under any kind of environmental hazards MG content increased and it was found in plant systems (Yadav *et al.* 2005). Singla-Pareek *et al.* (2006) found the same result.

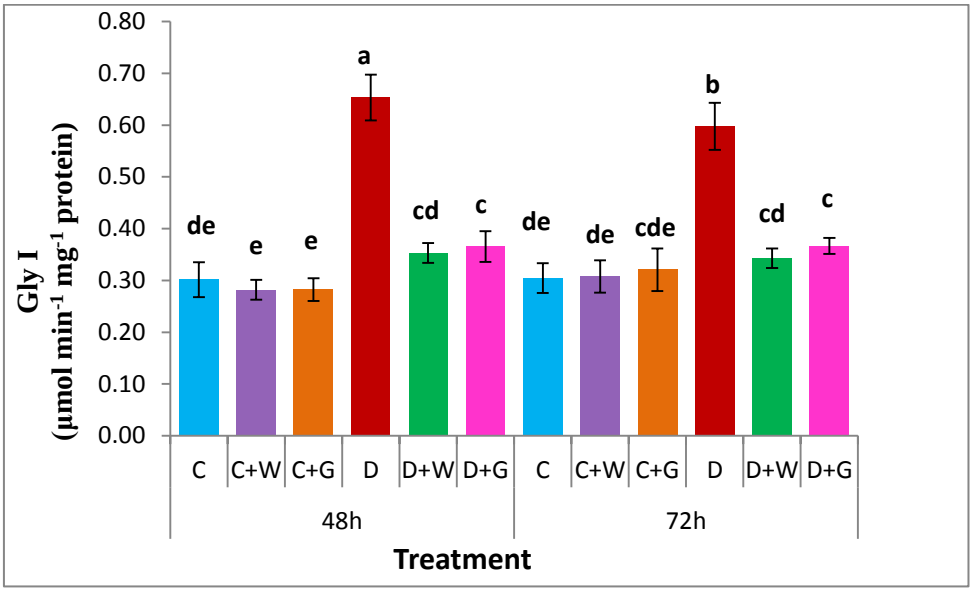


Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 56. Effect of GA on MG of wheat under drought stress condition

4B.4.2 Gly I

It was found that in wheat Gly I activity increased due to drought stress. about 116.655 % and 96.056% Gly I activity was increased in drought compare to control. Here GA 44.061% and water 45.933% reduced the Gly I activity after 48 h compare to drought wher as after 72 h it was 38.576% and 42.61961 % for GA and water respectively (Figure 57).

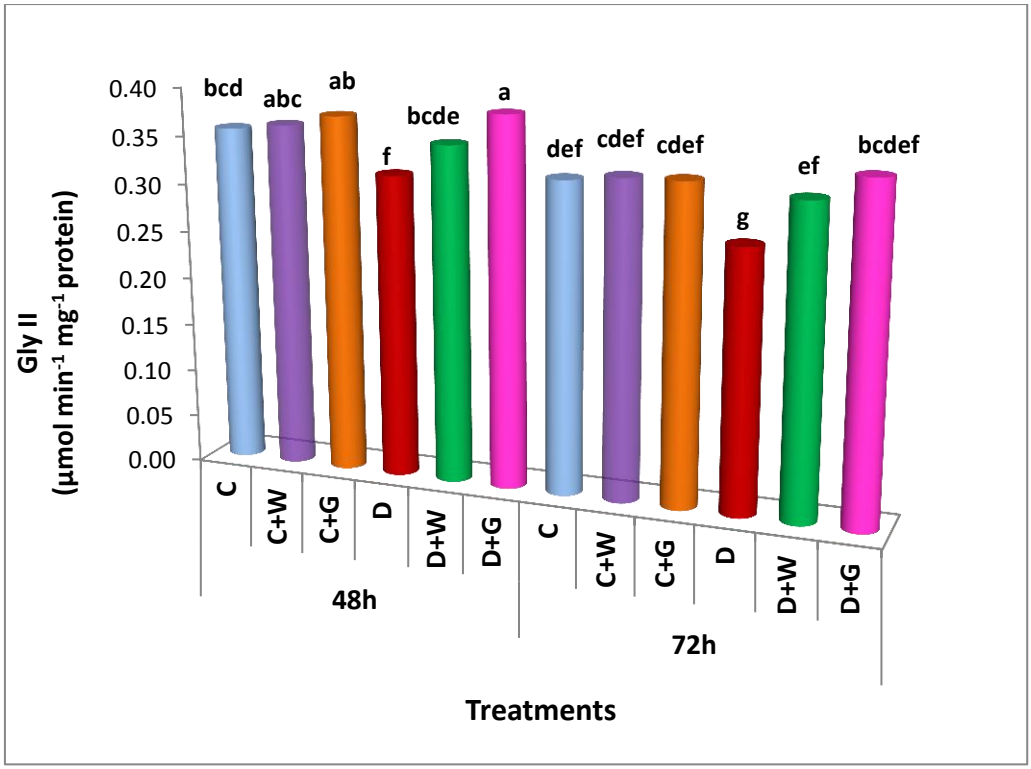


Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 57. Effect of GA on Gly I of wheat under drought stress condition.

4B.4.3 Gly II

In the study it was found that Gly II decreased 10.57% and 15.95% in drought stress after 48h and 72 h respectively compare to control. GA increased 21.60 and 27.21% Gly II after 48 h and 72 h to protect the plant from damage. Water also increased the Gly II activity about 10.93 and 17.97 % respectively after 48 h and 72 h.



Here **C** means control, **W** means water, **D** means drought by 12% PEG and **G** means GA. Mean (±SD) was calculated from three replicates for each treatment. Bars in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Figure 58. Effect of GA on Gly II of wheat under drought stress condition.

In the present experiment it was found that, MG was increased and it was responsible for plant damage at drought stress but in this MG-cycle the GLY I and GLY II content was changed to protect the plant. It was supported by (Yadav *et al.* 2005). He explained that when plant is in drought stress, most of the cases they protected themselves from the MG with the help of glyoxalase syste i.e. changing the GLY I and GLY II content (Yadav *et al*., 2005; Singla-Pareek *et al*., 2006). Hossain *et al.* (2009) also found the same result.

4B.5 Histochemical detection of H_2O_2 and $O_2^{\bullet-}$ generation

Leaves from each treatment were collected and stained by DAB and NBT to observe the ROS production. Brown spot due to the production of H_2O_2 was formed due to the

DAB staining and dark blue spots were formed due to the NBT staining as a result of $O_2^{\bullet-}$ generation compared to control. In case of drought stress, the spots were very prominent and it was in higher amount but GA treated plants leaves showed lower spots that mean lower production of ROS than drought **(**Plate 3).

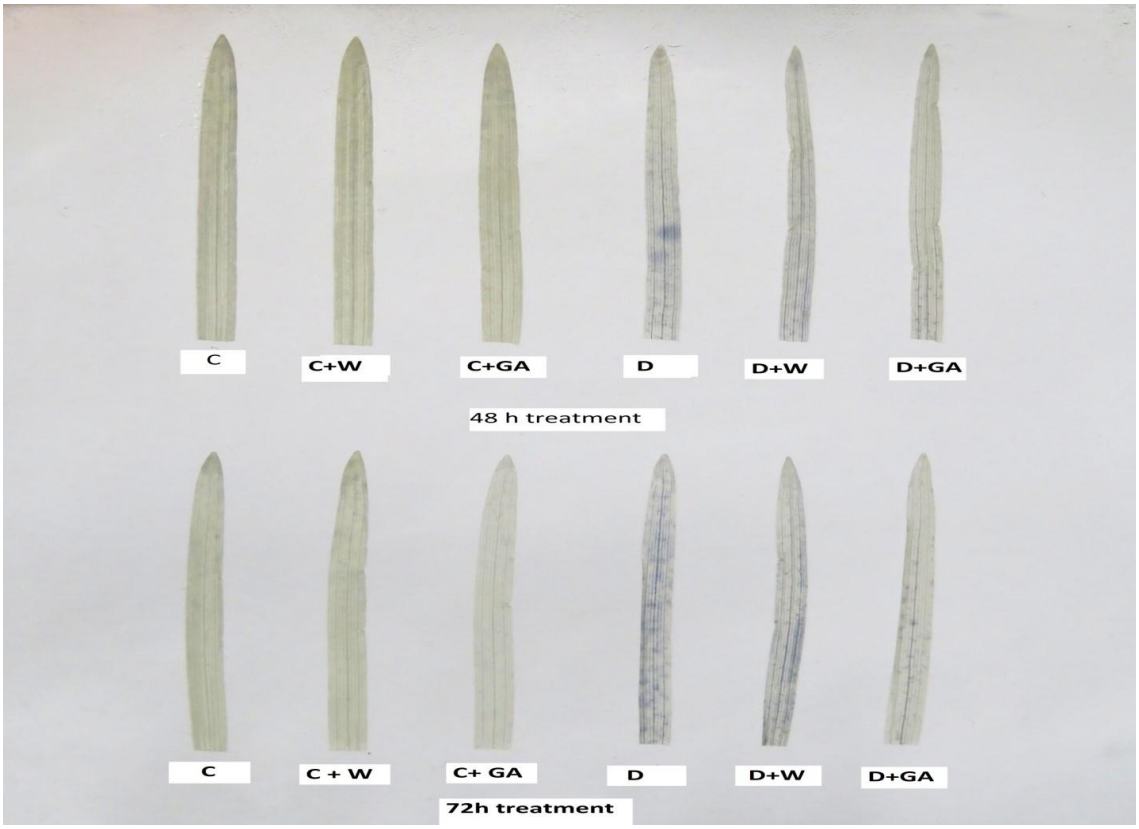


Plate 3. Histochemical detection of superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) in the leaves of wheat seedlings under drought stress and GA.

Chapter 05

SUMMARY AND CONCLUSION

Two experiments were conducted to find out the effect of drought stress on morph-physiological and biochemical changes of wheat as well as mitigation by using GA. The combined effect of drought stress and GA on growth and yield was also estimated.

The first experiment was conducted at Agronomy field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period of November, 2016 to February, 2017. Here effect of drought stress and GA at different growth stage on growth parameters and yield attributes of wheat were observed. The treatments were stress at CRI stage, flowering stage, grain development stage and their combination with GA and without GA.

In this experiment seeds were sown in 15 November, 2016 and harvested at 23 February, 2017.

Growth parameters were collected after a definite period of time and the yield data was collected after harvest.

It was observed that drought stress reduced the plant height. About 16.428% plant height reduced due to full stress condition compare to no stress condition and GA increased 4.5% plant height. Stress at CRI stage and flowering stage was critical for plant height without GA but GA treated plant showed stress at T_6 and T_7 are more sensitive.

At 20 DAS and 70 DAS leaf number was not statistically different because after certain period wheat leaves reduced in number but at 45 DAS it was found that full irrigated condition gave higher number of leaves/plant and CRI and CRI + other stage were the sensitive stage.

SPAD value reduced due to drought stress and GA didn't play any role to change the SPAD value under drought stress. The lowest SPAD value was found in stress at flowering stage.

It was seen that both GA and drought stress induced early flowering. GA has an effect on flower initiation where as in drought stress plants want to complete its life cycle within a short period to avoid the damage.

About 15.06% tiller number was increased due to GA and 22.60% tiller number was decreased due to drought stress compared to control. In CRI stage when drought stress was imposed it reduced the tiller number.

GA increased the dry matter both in irrigated condition and drought stress. It was found that the highest dry matter plant^{-1} was in G_1T_1 (2.87) and second highest was G_0T_1 (2.48). The lowest dry matter was found in G_0T_0 (1.60). Dry matter was reduced drastically at both CRI stage and flowering stage in case of drought stress.

Translocation percentage was higher in full stress condition. Assimilates translocation was higher in G_1T_1 (64.1) and G_0T_1 (61.82) and the lowest translocation was found in G_0T_0 (15.85) and G_1T_0 (14.37).

The lowest stress intensity was found in G_1T_1 (31.78) and then G_1T_2 (36.83) and G_0T_1 (36.37) that means GA worked on CRI stage to reduce the stress intensity. Stress only at grain development stage was more resistant to drought stress than other stage. Here, G_0T_7 showed 63.02% and G_1T_7 showed 58.01 % stress intensity.

Pedicle length, Spike length, no. of grains spike^{-1}, spikelets spike^{-1} all are reduced due to drought stress and in case of CRI + flowering and flowering + grain development stage drought stress caused more damage.

The highest number of spikelets spike^{-1} was found from G_1T_1 (17.12) and lowest number was from G_0T_0 (11.5). G_1T_0 produced 12.443 spikelets and G_0T_1 produced 16.153 spikelets spike^{-1} that means GA worked to increase spikelets per spike. It was found that minimum spikelets per spike were in G_0T_5 (11.53) and G_0T_7 (12.09).

Maximum no. of grains spike^{-1} was found in G_1T_1 (45.86) and it was higher than G_0T_1 (43.2). Again the lowest no. of grains spike^{-1} was 28.96 that found in G_0T_0. Here G_0T_5 and G_0T_7 were more sensitive.

Like others yield contributing parameters, 1000 grain weight, husk weight, straw weight was reduced in drought stress but GA increased the yield. G_1T_1 and G_0T_1 produced highest grain weight, husk weight and straw weight.

About 11.66% yield increased due to the application of GA whereas 45.40% yield reduced due to the drought stress. The highest grain yield was found from G_1T_1 (3.62 t ha^{-1}) and the lowest yield was from G_0T_0 (1.73 t ha^{-1}) though G_1T_0 produced 1.98 t ha^{-1} yield. The G_0T_1 produced the grain yield of 3.18 t ha^{-1} which was statistically similar with G_1T_2 (3.15 t ha^{-1}) and G_0T_5 (1.96 t ha^{-1}) and G_0T_7 (1.85 t ha^{-1}) produced statistically similar and lower yield.

The second experiment was conducted at Laboratory of Plant stress responses, Kagawa University; Kagawa, Japan during the period from March, 2017 to August, 2017. It was conducted in a CRD design. Here effect of GA to mitigate the drought stress on wheat was observed by calculating different biochemical responses.

It was observed that plant height, shoot fresh weight, dry weight chlorophyll content was reduced due to drought stress at 48 h and 72 h of stress but GA restore the plant growth under drought stress. Lipid per oxidation, H_2O_2, proline content was increased in drought stress but in case of using GA on drought stress, they were in lower amount. After 48h stress there was not so much difference but after 72h the difference was highly significant. Ascorbate content was lower in drought stress after 48h but at 72 h it was increased. Although in every cases GA played an effective role to keep the ascorbate content at an optimum level. GSH and GSSG were increased due to drought stress after 48h and 72h respectively but GSH/GSSG ratio of wheat under drought stress condition was decreased where as GA increased it. CAT and APX activities was also reduced in drought stress but GA restore that. Under drought stress, after 48h and 72 h MDHAR activity was increased by GA. DHAR activity was increased under drought stress but GA reduced it. GA prominently worked on glyoxalase system. It was observed that under drought MG content was increased at 48h and 72h but GA protected the wheat as the MG production was reduced in these treatments. Gly I activity was increased and GLY II activity was reduced under 48 h and 72 h of drought stress but GA in every cases protected the wheat plants from the damage of drought stress. In every cases water played very negligible role to reduce oxidative damage with some exceptions.

From the experiments some conclusion may be drawn

- Drought stress reduced the growth and yield of wheat drastically whereas GA increased the growth and yield both in stress and irrigated condition and it protected the crop from drought stress.
- CRI stage was very critical for optimum growth. Drought stress at CRI stage reduced plant height, biomass production, leaf number, tiller number and other growth parameters but in GA treated wheat plants showed lower effect of drought stress at CRI stage that means GA protected wheat seedlings appreciably from drought stress as GA was applied at CRI stage.
- In case of yield consideration, flowering and grain development stage was more critical as stress at this two stages reduced the yield attributes significantly.
- Here another thing was also observed that if soil moisture is available after drought stress at CRI stage, wheat sometimes can overcome the damage that occurred at CRI stage to some extent but if drought stress is in flowering or grain development stage the yield reduced remarkably.
- If we consider about the sensitive stage to yield then flowering and grain development was more susceptible to drought to reduce yield. Sometimes spike length or husk weight may increase but due to unfilled spikelets or lower no. of spikelets because of drought, yield may reduce.
- Drought stress affects the wheat seedlings through oxidative damage and it depends on the duration of stress as with the increase of duration, damage will also increased.
- GA protected the wheat seedlings by upregulating the antioxidant defense and glyoxalase system but water played a negligible role. So the effect of GA spraying was very much clear.

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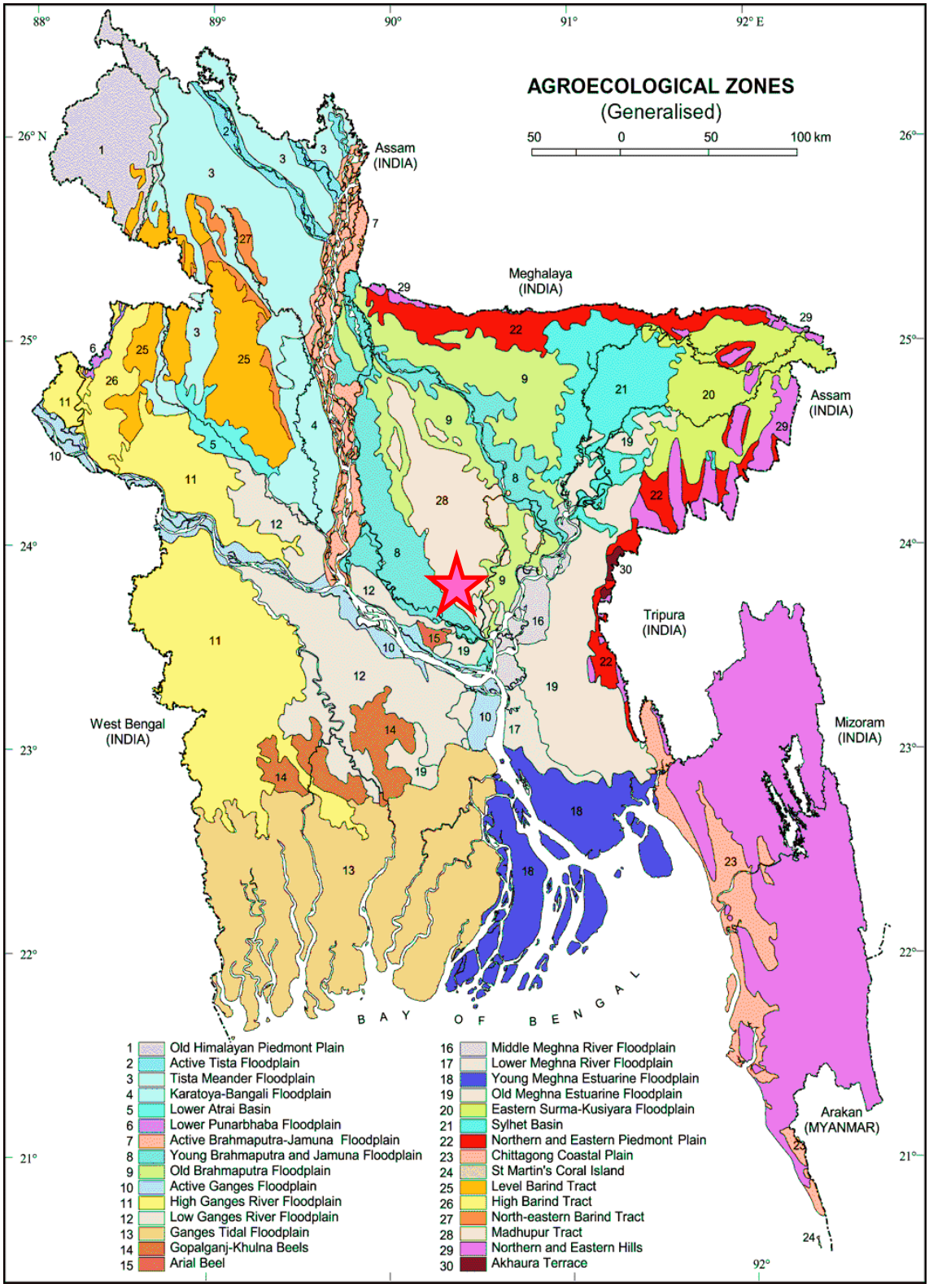
APPENDICES

Appendix I. Map showing the location of experiment-1



★ Shows the experimental site.

Appendix II. Soil characteristics of experimental field as analyzed by Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka

A. Morphological properties of the soil

Morphological features	Characteristics
Location	Agronomy field , SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled

B. Physical properties of the soil

Particle size analysis	**Results**
Sand (%) (0.0-0.02 mm)	21.75
Silt (1%) (0.02-0.002 mm)	66.60
Clay (%) (<0.002 mm)	11.65
Soil textural class	Silty loam
Colour	Dark grey
Consistency	Grounder

Source: Soil Resources Development Institute (SRDI), Dhaka.

Appendix III. Monthly record of air temperature, relative humidity and rainfall of the experimental site during the period from November 2014 to March 2015

Month	*Air temperature (^{0}C)		*Relative humidity (%)	Rainfall (mm) (total)
	Maximum	Minimum		
November, 2016	25.82	16.04	78	00
December, 2016	23.4	14.5	74	00
January, 2017	26.5	16.4	68	00
February, 2017	29.5	19.7	67	00

* Monthly average

* Source: Bangladesh Meteorological Department (Climate & weather division), Agargoan, Dhaka.

Appendix IV. Analysis of variance (mean square) of the data for plant height at different days after sowing

Source of variation	df	Mean Square Values of Plant height at			
		20 DAS	**45 DAS**	**70 DAS**	**harvest**
Replication	2	0.05201	0.515	7.664	84.139
GA (A)	1	0.09363*	265.880**	152.304**	127.466**
Error of Replicati*GA	2	0.00253	1.390	0.522	0.646
Drought stress	7	0.02826	48.730**	66.304**	80.902**
GA* Drought stress	7	0.00841	4.333*	16.296*	7.872**
Error of Replicati*GA* Drought stress	28	0.07424	1.387	4.378	0.611

*Significant at 5% level

** Significant at 1% level

Appendix V. Analysis of variance (mean square) of the data for number of leaves/plant at different days after sowing

Source of variation	df	Mean Square Values of number of leaves/plant at		
		20 DAS	**45 DAS**	**70 DAS**
Replication	2	0.01521	0.0822	0.09730
GA	1	0.00187	12.3627**	0.22413**
Error of Replicati*GA	2	0.01688	0.1263	0.01213
Drought stress	7	0.00664	0.6237**	0.13213**
GA* Drought stress	7	0.01330	0.0878*	0.04444*
Error of Replicati*GA* Drought stress	28	0.00604	0.0291	0.02650

*Significant at 5% level

** Significant at 1% level

Appendix VI. Analysis of variance (mean square) of the data for dry weight/plant at days after sowing

Source of variation	df	Mean Square Values of dry weight/plant at		
		25 DAS	**55 DAS**	**Harvest**
Replication	2	2.992E-05	0.00101	2.96358
GA	1	2.677E-05	0.46934*	5.16758**
Error of Replicati*GA	2	5.106E-05	0.00671	0.03066
Drought stress	7	2.062E-05	0.43637**	6.28196**
GA* Drought stress	7	1.361E-05	0.03837	0.30508**
Error of Replicati*GA* Drought stress	28	2.356E-05	0.02874	0.01144

*Significant at 5% level

** Significant at 1% level

Appendix VII. Analysis of variance (mean square) of the data for SPAD VALUE, Days of 50% flowering and Tiller number.

Source of variation	df	SPAD VALUE	Days of 50% flowering	Tiller number
Replication	2	0.6667	0.396	0.06437
GA	1	9.5676	102.083**	0.63021*
Error of Replicati*GA	2	0.9340	1.021	0.00396
Drought stress	7	51.0268**	2.988**	0.41378**
GA* Drought stress	7	11.8306**	2.607**	0.09164**
Error of Replicati*GA* Drought stress	28	0.6750	0.280	0.02083

*Significant at 5% level

** Significant at 1% level

Appendix VIII. Analysis of variance (mean square) for the data of Translocation percentage, Absolute growth rate, Stress intensity (SI) and Pedicle length.

Source of variation	df	Mean Square Values of			
		Translocation percentage	Absolute growth rate	Stress intensity (SI)	Pedicle length
Replication	2	184.72	96.862	81.823	4.77982
GA	1	11.51	298.930**	371.182**	0.65731**
Error of Replicati*GA	2	6.04	1.113	2.387	0.00620
Drought stress	7	1047.26**	81.857**	547.973**	3.0060**
GA* Drought stress	7	3.63	12.465**	24.701**	0.06248**
Error of Replicati*GA* Drought stress	28	14.92	0.571	0.616	0.05714

** Significant at 1% level

Appendix IX. Analysis of variance (mean square) for the data of Spike length, spikelets/spike, and grains / spike.

Source of variation	df	Mean Square Values of		
		Spike length	spikelets/ spike	grains / spike
Replication	2	1.9339	2.7527	0.05395
GA	1	16.5675**	26.7307**	0.39933**
Error of Replicati*GA	2	0.0557	0.1025	0.00106
Drought stress	7	15.6864**	13.7442**	0.72583**
GA* treatments	7	1.3294**	1.2300**	0.01152**
Error of Replicati*GA* Drought stress	28	0.0995	0.1414	0.00061

** Significant at 1% level

Appendix X. Analysis of variance (mean square) for the data of 1000-grain weight, Straw yield and Grain yield.

Source of variation	df	Mean Square Values of		
		1000 grain weight	Straw yield	Grain yield
Replication	2	24.975	0.02935	0.20456
GA	1	53.155*	0.90399**	1.10230*
Error of Replicati*GA	2	0.701	0.00535	0.00597
Drought stress	7	158.494**	0.38546**	1.52518**
GA* Drought stress	7	0.604*	0.06036**	0.06508**
Error of Replicati*GA* Drought stress	28	0.228	0.00365	0.00154

*Significant at 5% level

** Significant at 1% level

Appendix XI. Analysis of variance (mean square) for the data of Biological yield and Harvest index (HI).

Source of variation	df	Mean Square Values of	
		Biological yield	Harvest index (HI)
Replication	2	0.50042	8.5312
GA	1	4.67557**	6.5143**
Error of Replicati*GA	2	0.03286	0.0248
Drought stress	7	5.17151**	26.3733**
GA* Drought stress	7	0.27520*	2.5856**
Error of Replicati*GA* Drought stress	28	0.00802	0.3585

*Significant at 5% level

** Significant at 1% level

Experiment 2

Appendix XII. Analysis of variance (mean square) for the data of growth parameters.

Source of variation	df	Mean Square Values of			
		Plant height	Shoot fresh weight	Shoot dry weight	Relative water content (RWC)
Treatment	11	5.22181**	0.09867**	1.650**	98.6594**
Error	24	0.06496	0.00476	7.011	1.8918

** Significant at 1% level

Appendix XIII. Analysis of variance (mean square) for the data of Oxidative damage.

Source of variation	df	Mean Square Values of					
		MDA content	H_2O_2 Content	Proline Content	Chlorophyll		
					Chl a	Chl b	Chl(a+b)
Treatment	**11**	191.32**	5.393**	4.248**	1.007*	0.138*	1.624*
Error	**24**	1.98	0.283	0.019	0.419	0.063	0.68

*Significant at 5% level

** Significant at 1% level

Appendix XIV. Analysis of variance (mean square) for the data of Antioxidant enzyme activity-I.

Source of variation	df	Mean Square Values of				
		Ascorbate content	GSH content	GSSG content	GSH/GSSG ratio	CAT activity
Treatment	**11**	616523**	7449.26**	51.0526**	27.0630*	730.484**
Error	**24**	97248	988.16	1.4319	9.8255	95.121

*Significant at 5% level

** Significant at 1% level

Appendix XV. Analysis of variance (mean square) for the data of Antioxidant enzyme activity-II.

Source of variation	df	Mean Square Values of			
		APX activity	MDHAR activity	DHAR activity	GR activity
Treatment	**11**	0.29762**	153.189**	45565.6**	122.232**
Error	**24**	0.01228	28.208	1813.6	6.373

** Significant at 1% level

Appendix XVI. Analysis of variance (mean square) for the data of Glyoxalase system and methylglyoxal detoxification.

Source of variation	df	Mean Square Values of		
		Methylglyoxal (MG) content	Gly I	Gly II
Treatment	**11**	94.0335**	0.04462**	2.679**
Error	**24**	1.9624	0.00094	3.344E-04

** Significant at 1% level