SEED PRIMINGENHANCES DROUGHT STRESS TOLERANCE IN WHEAT SEEDLINGS BY MODULATING ANTIOXIDANT DEFENSE AND GLYOXALASE SYSTEMS

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CERTIFICATE

*This is to certify that thesis entitled, "**SEED PRIMING ENHANCES DROUGHT STRESS TOLERANCE IN WHEAT SEEDLINGS BY MODULATING ANTIOXIDANT DEFENSE AND GLYOXALASE SYSTEMS**" submitted to the Institute of Seed Technology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in SEED TECHNOLOGY**, embodies the result of a piece of bona fide research work carried out by **Mazhar Ul Alam**, Registration No. **09-03511** 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.

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Dated:

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DEDICATED TO MY BELOVED PARENTS AND ELDER BROTHER

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ABSTRACT

An experiment was conducted at the Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Japanto investigate the protective effect of priming with some phytopotectants [50 µM salicylic acid (SA), 4 mM, ascorbic acid (AsA) and 2.5 mM NaCl]under drought stress condition [induced by 15 % (m/v) polyethylene glycol, PEG-6000] in wheat. Drought stress caused higher proline (Pro) accumulation, lower relative water content (RWC), chlorosis and growth inhibition. Enhanced levels of the malondialdelyde (MDA) and hydrogen peroxide (H_2O_2) were evident with the overproduction of reactive oxygen species (ROS) by disrupting the antioxidant defense system under drought stress condition.The activities of antioxidant enzymes viz. catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), and glutathione peroxidase (GPX) declined under 15% PEG induced drought stress condition. Compared to control seedlings, drought also increased methylglyoxal (MG) formation which also induced oxidative damage by facilitating ROS production. However, priming of wheat seeds with SA, AsA and NaCl decreased the ROS production by decreasing AsA and increasing glutathione content and upregulating antioxidant defense system. Additionally, increased activities of glyoxalase I and glyoxalase II reduced the levels of MG in drought stressed-wheat seedlings. Seed priming helps to recover the seedlings from chlorosis and growth inhibition whereas decreased the MDA and Pro content and increased the activity of antioxidant enzymes. Therefore it can be concluded that seed priming with SA, AsA and NaCl confers drought stress tolerance by upregulating antioxidant defense and glyoxalase system and helps to better seedling establishment.

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LIST OF ACCRONYMS AND ABBREVIATION

ABBREVIATION	ELABORATION
SAU	Sher-e-Bangla Agricultural University
et al.	and others
SA	Salicylic acid
AsA	Ascorbic acid/ Ascorbate
IPCC	Intergovernmental Panel on Climate Change
ROS	Reactive Oxygen Species
GSH	Glutathione
SOD	Super Oxide Dismutase
CAT	Catalase
APX	Ascorbate peroxidase
MDHAR	Monodehydroascorbate reductase
DHAR	Dehydroascorbate reductase
GR	Glutathione reductase
GPX	Glutathione peroxidase
MG	Methylglyoxalase
Gly I	Glyoxalase I
Gly II	Glyoxalase II
H_2O_2	Hydrogen Peroxide
FAO	Food and Agricultural Organization
USDA	United States Department of Agriculture
BARI	Bangladesh Agricultural Research Institute
DAE	Department of Agricultural Extension
BBS	Bangladesh Bureau of Statistics
PS I	Photosystem I
PS II	Photosystem II
$O_2^{\bullet -}$	Superoxide radical
$^1O_2$	Singlet Oxygen
$OH^{\bullet}$	Hydroxyl radical

ABBREVIATION	ELABORATION
PEG	Poly Ethylene Glycol
MDA	Malondiadehyde
GSSG	Glutathione disulfide
SLG	S-lactoyl-glutathione
XOD	Xanthine Oxidase
µM	Micromolar
mM	Milimolar
Nm	Nanometer
POX/ POD	Peroxidase
CRD	Completely Randomized Design
ISTA	International Seed Testing Agency
NADPH	Nicotinamide adenine dinucleotide phosphate
NaN_3	Sodium Nitride
ANOVA	Analysis of Variance
LSD	Least Significant Difference
BAU	Bangladesh Agricultural University

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) the most important cereal belongs to the family Poaceae (Gramineae). In Bangladesh, after rice wheat is the second most important cereal crop. During the year 2014-2015, 13.31 million metric tons of wheat was produced from 0.42 million hectares of land with an average yield of 3.03 t ha^{-1} in the country (DAE, 2014).Unpredicting environmental conditions and increasing complexity of the environment, global climate change now become one of the most disastrous and calamitous threat to the world agriculture. Due to climate change world environment as well as agriculture fall in different biotic (Living organisms such as insects, pathogenic fungi, bacteria, and viruses) and abiotic stresses (water deficit or drought, salinity, high or low temperature, light, nutrient, heavy metals) and inhibits plant growth, development and productivity (Hasanuzzaman *et al.,* 2012a). Among the different abiotic stresses, drought stress itself can cause more than 50% yield losses. Drought casues detrimental effects on plant growth and development (Farooq *et al.,* 2009). However, situation now become more alarming due to changing behavior of global atmosphere (IPCC, 2007). At the initial stage of crop production, shortage of water may results in delayed and inconsistent germination with poor and abnormal seedling establishment (Almansouri *et al*., 2001; Kaya *et al*., 2006). Decrease in water uptake during imbibition phase of germination is the primary reason for this decline in stand establishment (Murillo-Amador *et al*., 2002). Drought also disturbs the plant growth owing to loss of turgor (Farooq *et al*., 2009; Taiz and Zeiger 2010), as water supply from the xylem to the surrounding elongating cells is interrupted (Nonami, 1998). Genetical, physiological, ecological, and morphological events and their complex interactions are seriously inhibited by drought stress. Under drought stress condition, biochemical processes in plants including stomatal conductance, membrane electron transport, carbon dioxide (CO_2) diffusion, carboxylation efficiency, water-use efficiency (WUE), respiration, transpiration, water loss, photosynthesis, and membrane functions are seriously affected. Crop growth, development and yield reduce with the disruption of these key functions (Hasanuzzaman *et al*., 2013).

Drought induces oxidative stress in plants by overproduction of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroxyl radicals ($OH^{\bullet}$), superoxide radical ($O_2^{\bullet-}$) and alkoxy radical ($RO^{\bullet}$). Over-production of ROS than their dousing is one of the key responses of plants to environmental stresses (Smirnoff, 1998; Farooq *et al.,* 2009). The ROS, thus generated, deteriorate the cellular membranes and several other vital substances and may even lead to cell death (Kratsch and Wise, 2000). However, plants have evolved several antioxidative defense mechanisms, by the production of non-enzymatic antioxidants such as ascorbate (AsA), glutathione (GSH), carotenoids, flavanones, and anthocyanins and upregulatingthe enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and glutathione -*S*- transferase (GST) (Gupta *et al.,* 2009; Hasanuzzaman *et al.,* 2012) to reduce ROS induced oxidative damages (Posmyk *et al.,* 2009a). Under drought stress the cytotoxic compound like Methylglyoxal (MG) production also increases (Hasanuzzaman *et al*., 2011a, 2012). In glyoxalase system (glyoxalase I, Gly I and glyoxalase II, Gly II) GSH act as a co-factor may detoxify MG (Yadav *et al.,* 2005a) and increase stress tolerance. However, plants improve drought stress tolerance through theamplification of antioxidant and glyoxalase systems (Hasanuzzaman *et al.,* 2011a; Alam *et al.,* 2013).

The cultivable area is decreasing day by day with the increasing population, and this problem will more acute in near future. Moreover global climate change causes drought, salinity, high or low temperature, uneven rainfall etc. Bangladesh is affected by the major country-wide droughts in five years interval. In Bangladesh very strong droughts hit in 1975, 1981, 1982, 1984, 1989, 1994, and 2000 (Dey, 2011). Soil water shortage or agricultural drought may occur at different stages of crop growth. Drought exposed more severely in northwestern part of Bangladesh. Severe water shortage during the *kharif* season can decrease the crop production more than 40%. In the *rabi*season, about 1.2 million ha of agricultural land face droughts of different magnitudes (Dey, 2011). Therefore, efforts to increase the drought tolerance of crop

plants are very important to ensure food security. Success in breeding for tolerance has been limited. Among various strategies, priming of seeds is easy, low cost, low damage and effective approaches to overcome the environmental stress problems in crop plants (Jisha *et al.*, 2013; Ahmad *et al*., 2012).

Seed priming is a controlled hydration technique that allows the pre-germination metabolisms without actual germination (Bradford, 1986). To conflict the effects of drought (Farooq *et al.*, 2010) and other environmental stresses (Jafar *et al*., 2012), seed priming is one of the most pragmatic and short-term strategy for increase germination and better seedlings establishment. Seed-priming techniques increase the activity of hydrolases and some other enzymes, which enhance the breakdown of reserve food. Seed priming simply reduce the delay of imbibitions (Brockle-hurst and Dearman, 2008), increases metabolite that relates to germination (Farooq *et al*., 2006), restore metabolite during imbibition (Bray *et al*., 1989) and osmotic adjustment (Bradford, 1986).

As an important signaling molecule SA adjusts physiological and metabolic processes in plants (Khan *et al.*, 2012). In addition, plants treated with SA improve drought stress tolerance (Al-Hakimi and Hamada, 2001). Salicylic acid improves plant water relations (Hayat *et al.*, 2010). Exogenous SA was found to be effective by upregulating both enzymatic and non-enzymatic components of antioxidant defense system (Kadioglu *et al.*, 2011). Ascorbic acid is an important non-enzymatic antioxidant involved in many cellular processes including cell division, cell wall development and other developmental processes (De Gara *et al.*, 2003; Pignochi and Foyer, 2003). Increasing cellular levels of ascorbic acid may reduce oxidative stress by scavenging ROS (Noctor and Foyer, 1998). Ascorbic acid may also act as a reaction substrate within the enzymatic cycle (Mittler, 2002). Ascorbic acid scavenges and controls the concentration of H_2O_2 in plants (Sairam *et al.*, 1998) with the help of an enzyme ascorbate peroxidase (APX) by converting H_2O_2 into dehydroascorbate and water (Raven, 2000). Exogenous AsA can increase the endogenous AsA which modulates the antioxidant defense system and confers abiotic stresses (Shalata and

Neuman, 2001). Exogenous AsA may increases drought stress tolerances by increasing nutrient uptake, leaf and root growth, photosynthesis and by decreasing lipid peroxidation; ultimately relief from the oxidative stress (Khan *et al.*, 2012). Seed priming with AsA improves the seedling establishment under drought condition (Farooq *et al.*, 2013). Plant biomass increases when seeds are primed with ascorbic acid (Razaji *et al*., 2014). Sodium chloride priming improves the energy of germination and lowered mean germination time and better seedling establishment under stress condition (Ruan and Xue, 2002). Seed priming with NaCl helps in osmotic adjustment.

Although the effects of priming with SA, AsA, NaCl on some seed crops has been studied, but little information is available on the antioxidant defense and glyoxalase systems on wheat crops under drought stress. Considering these strategies the present study was undertaken keeping in mind the following objectives:

i. To investigate the effect of drought on the physiology of wheat.
ii. To understand the role of seed priming with salicylic acid, ascorbic acid and sodium chloride in mitigating short-term drought stress.
iii. To investigate the mechanisms that improves drought tolerance of wheat induced by seed priming with SA, AsA and NaCl.

CHAPTER II

REVIEW OF LITERATURE

2.1 Wheat

Wheat (*Triticum aestivum* L.) is one of the first domesticated food crops belonging to the Poaceae family is the most important cereal, which is cultivatedin 17 percent of total aerable land of the world. Wheat, the major food grain of 35 percent population of the world, is also a supplement of rice **(**FAO**,** 2013) and grownin much land compered with any other cereal crop and gained the position of most important food source for human. Although wheat is a crop of temperate region, but varietal improvement through breeding facilitates it's cultivation in subtropical climatic region of the world now-a-daysis verysuccessful and potential yield is also high. The World wheat production was 734.05 million tons in 2015, from which Asia shares about 36 percent, while Europe and North America share 17 and 16 percent respectively (USDA, 2015). United States, China, India, Pakistan, Afganistan, France, and Russia were the top ranked producer of wheat in 2015 by the report of USDA. Approximately 80% of the total cropped area is covered by rice and wheat, where wheat covers only 7-9% of total grain production **(**BBS, 2014) in Bangladesh. Wheat cultivation generally takes part all over the Bangladesh but northern regions like Dinajpur, Thakurgaon, Kurigram, Rajshahi, Rangpur, Faridpur get emphasizes due to climatic conditions of at those parts.In 1971 wheat cultivation was an area of 0.126 million hectares with 0.103 million metric tons production, the area and production increased to 0.83 million hectares and 1.84 million metric tons respectively in 2000 (Hasan, 2006). Due to introduction of stress tolerant, early harvesting, insect resistance, hybrid varieties and cost of production compare to other crops now wheat growing area and production increases day by day. In 2014 total 0.429607 million hectares land was cultivated and production was 13.02998 million metric tons (DAE, 2014) where as it was 12.55 million metric tons in 2013 (DAE, 2013)**.** On an average every 100g of wheat supplies 0.33 Kilocalorie of energy, which is also an outstanding source essential food nutrients of the human body, for example high quality protein, dietary fiber, phosphorus manganese, and vitamin B_3 (FAO, 2013). Wheat is good source of

carbohydrate (71%) and protein (13%) with little amount of fat (1.5%)**.**For 8000 years wheat has been used as the staple food of North Africa, Europe, and West Asia. Flour from wheat is used to make bread, fine cakes, macaroni, spaghetti, and other pasta products. Vegetative plant parts of wheat make valuable livestock feed. Augmentation of modern develop varieties (i.e. salt tolerant, heat tolerant and early harvesting varieties) and hybrid varieties with farm mechanization, wheat production per hectare area now increases. In 2014 per hectare wheat production was 3.033 metric tons where it was 3.013 metric tons in 2013 (BBS, 2014).Wheat can be cultivatedin a broad range of climatic condition but in Bangladesh it is cultivated in winter season (November-February). Depending on variety and weather conditions, wheat requires 100-120 days from sowing to harvest. Generally wheat is grown in Bangladesh as a winter crop and in winter season there had scarcity of water, falling in drought stress is common phenomena at that time. Though Wheat is moderately drought tolerant crops but excessive drought stress can cause yield loss greatly (Ali *et al*., 2013). Sometimes farmers are not able to collect seeds at the proper time. Late sowing wheat can face heat stress. Salinity is one of the major constraints for crop growth, development and production (Iqbal *et al*., 2013). Southern region of Bangladesh facing salinity problem and wheat production is almost impossible because of unavailability of salt tolerant variety. Stress both biotic and abiotic greatly affects crop growth and yield. Drought stress occurs during the spike growth period can decreases grain number per spike, yield per plant and ultimately decreases the yield (Ali *et al*., 2013).Wheat suffers and a sharp decrease in grain yield and entire above-ground biomass is attributed due to heat stress, whilesoil salinity adversely effects the growthand development, and tillers viability, resulting inlower number of both primary and secondary tillers. Now-a-days the world specially developing countries like Bangladesh facing environmental problems like drought, uneven rainfall, high temperature, salinity problem etc due to global warming. So there have ample chances and crying needs to develop varieties to cope with this type of environmental stresses. Though diseases resistant and few heat sensitive wheat varieties like BARI Gom-27, BARI Gom-28, BARI Gom-29 and BARI Gom-30 are released by Bangladesh Agricultural Research Institute **(**BARI**)** but still there have no drought tolerant, salt

tolerant or other stress tolerant wheat variety is not released. Enhancing additional wheat yield may be achieved through the useof the fellow lands due to excess water deficit, salts and other abiotic stresses.

2.2 Drought stress and plant responses

2.2.1 Drought stress

Among different abiotic stresses, drought is the most complex and devastating on a global scale (Pennisi**,** 2008), and its frequency is expected to increase as a consequence of climate change (Ceccarelli *et al.*, 2010). Droughts rank first among all natural hazards as it affectsthe maximum number of people of the world (Obasi, 1994; Wilhite, 2000). Drought is one of the most acute abiotic stresses which adversely affect crop growth, yield and thus a main barrier for crop production (Jaleel *et al.*, 2009; Hasanuzzaman *et al.*, 2012a). Due to continuous climate change the extremity of drought stress is increasing day by day and global cropproduction will reduce up to 30% within 2025, compared with current yield if drought stress increases in similar trend as like as present (Zhang and Jia 2013).

2.2.2 Effect of drought stress on vegetative stage of wheat

Cell is the basic components for all living organisms. Growth is accomplished through cell division. Under drought stress or water deficit condition cell division reduces thus the growth of plant also reduces (Hasanuzzaman *et al.*, 2013). Drought stress hampers critical physiological as well as biochemical mechanisms in plants (Hasanuzzaman *et al.*, 2012a), which ultimately reduces crop yield (Shahbaz *et al.*, 2011).

Generally the growing period of wheat is divided under: vegetative and reproductive stages which are affected by drought stress (Shi *et al*., 2010). Ghodsi *et al***.**, (2006) reported that in wheat reproductive stage is more sensitive to drought stress than vegetative stage. Germination is the first step of plant establishment but drought reduces the germination percentage and results poor seedling establishment (Harris *et al*., 2002; Kaya *et al.*, 2006). It was shown earlier that drought stress decreases root

length (Nouri-Ganbalani *et al.,* 2009), relative water content, total chlorophyll content and photosynthesis rate (Abdoli *et al*., 2013). In wheat, under drought stress length and area of flag leaf decreases while the width did not significantly changes **(**Lonbani and Arzani, 2011). Drought stress inhibits root and shoot growth, increases transpiration rate and reduces CO_2 uptake during photosynthesis (Neumann, 2008). Water shortage at Crown root initiation stage of wheat causes 27% yield loss (Cheema *et al.,* 1973).

2.2.3 Effect of drought stress on reproductive stage and yield of wheat

In the past few decades drought stress drastically reduced production of wheat in many parts of Asia (IPCC, 2007). Crop yield is reduced by 70-80% due to a drought spell during the reproductive stage **(**Kulkarni and Deshpande, 2007). Spikelet of wheat became sterile due to water deficit at reproductive stage (Ji *et al.*,2010**).** Under drought stress number of grain per spike decreased **(**Chandler and Singh, 2008**)**. Wheat yield reduced by 21.8 % and 40.7 % due to 25 and 50% reduction of water consumption, respectively (Ramezanpoor and Dastfal, 2004). Watering at crown root initiation, tillering, jointing and flowering stage gives good yield **(**Banker *et al.,* 2008**)**. Upto 25% and 46% reduction of wheat grain yield was found if water deficit respectively after anthesis period and stem elongation stage (Keyvan, 2010)**.** Drought condition at tillering stage of wheat (when branching start) decreased 46% and during booting stage of wheat reduces 21% yield loss (Schneekloth *et al.,* 2012). Zhang *et al.,* (2006) concluded that drought stress should be avoided at the booting and heading of wheat to reduce yield loss.

2.2.4. Drought induced oxidative stressin wheat

Under various abiotic stress conditions ROS production increased greatly which hinders almost all feature of plants biochemistry and physiology (Hasanuzzaman *et al*., 2012a). Like other abiotic stresses, drought stress increased the ROS production manifold. Under normal conditions plants maintain a balance in ROS productions. Upon exposing to stress condition, there have an imbalance in ROS production. ROS

production beyond the plant's quenching capability is often defined as a disruption of redox signaling and redox control (Jones, 2006), which can cause oxidative stress by damaging membrane lipids, proteins, photosynthetic pigments, and nucleic acids through oxidation process, and these are considerably increased under drought stress (Hasanuzzaman and Fujita, 2011; Sorkheha *et al.,* 2011; Faiz *et al*., 2011). Drought stress impair plants water uptake. So under drought stress condition plants reduce the stomatal conductance. In that condition, abscisic acid carries stress signal from root to leaves. When leaves accept the signal, it activates closure of stomata (Reddy *et al.*, 2004; Cruz de Carvalho 2008). Plants eventually achieved water-saving approach by regulating stomatal opening, which decreases transpiration rate but the entrance of CO_2 also lessen and consequently internal CO_2 concentration decreased. Therefore, the rate of CO_2 reduction by the Calvin cycle becomes slow down which decrease regeneration of $NADP^+$, thus provoking excess reduction of the photosynthetic electron transport chain (Reddy *et al.,* 2004; Cruz de Carvalho, 2008; Hasanuzzaman *et al.,* 2013). That means drought stress hinders carbon fixation by reducing the availability of CO_2. Reducing CO_2 availability and inhibiting carbon fixation results chloroplast to excessive excited energy from PS I and enhance the production of different toxic ROS (Gill and Tuteja, 2010 and Hasanuzzaman *et al.*, 2013). Impaired electron transport procedures in the chloroplasts and mitochondria of the plant cell generate excess ROS production throughout the period of drought stress. On the other hand, decrease activity in PS II results in a disproportion between the generation and utilization of electrons, resulting in an alteration in yield of quantum. The resulted photochemial modification of the chloroplasts in drought-stressed plants leaves harvested excess light energy in the PS II and producevariousactive free radicals or ROS like $O_2^{\bullet -}$, $^1O_2$, H_2O_2 and $OH^{\bullet}$, which are very much hazardous for plants for generating oxidative damages (Hasanuzzaman *et al*., 2013; Apel and Hirt, 2004). ROS are off very active nature and harm to any organism whenproduced at a high concentration, causing lipid peroxidation, protein oxidation, DNA damage, inhibitionof vital enzymes, activating programmed cell death (PCD) pathway, leading to cell (Mishra *et al.,* 2011; Srivastava and Dubey, 2011**).** Presence of ROS as low/ moderate concentration can act as a second messenger by stimulating antioxidant

system and defending the plants from injury casued by overproduced ROS**.** Plants are well equipped with non-enzymatic (AsA, GSH; phenolic compounds; alkaloids; and α-tocopherol non-protein amino acids) and enzymatic (SOD, CAT, APX, GR, MDHAR, DHAR, GPX and GST) antioxidants which give protection against oxidative stress (Hasanuzzaman *et al*., 2012a).

Under various abiotic stress conditions, Methylglyoxal (MG), a cytotoxic compound, can also create oxidative stress by over production of $O_2^{\bullet-}$ (Yadav *et al.,* 2005a, b). Like other abiotic stresses, drought stress also increased the MG production which ultimately increases the ROS production (Kaur *et al.,* 2015). Glyoxalase system in living organism is well established always on the go to detoxify MG throughtwo very important enzyme glyoxalase I (Gly I) and glyoxalase II (Gly II).

The antioxidant defense and glyoxalase systems cannot work properly to detoxify ROS and MG that produces under drought stress condition. As a result plants cell death. Different research findings demonstrated that drought stress considerably increases oxidative damage by overproducing of ROS and altering the antioxidant defense system based on drought intensity and plant growing stages.

Tan *et al.,* (2008) reported that imposition of 15% Polyethylene glycol (PEG-6000) in wheat seedlings for 24 hours significantly increased oxidative stress by increasing MDA content, $O_2^{\bullet-}$ production and decreasing antioxidant enzymes like SOD, CAT etc.

Nayaar and Gupta, (2006) recorded that wheat plant (cv. C306) under PEG (-1.5 MPa) for 7 d increased the stress injury by 330% in roots and 495% in leaves with the drastic increase of MDA and H_2O_2. Beside these, drought stress decreased the activities of AsA, GSH, GR, APX, and DHAR both leaf and root.

Not only short term water shortage but also long term water shortage can significantly increase oxidative stress. According to Ibrahim (2014), withholding irrigation from

late tillering to the early flowering stage of *T. aestivum* L. (Giza 168) for 20 days increased oxidative stress markers MDA, H_2O_2 content 194% and 193% and decreased ROS scavenger enzymes activity (CAT, SOD). He also observed that membrane stability index and root viability decreased by 40 and 58%, respectively.

In 2013, Farooq *et al*. reported that maintenance of 35% water-holding capacity in soil decreased the membrane stability index by 23% and increased MDA content by 37% compared to control wheat plants. They also observed soluble phenolics and leaf free Pro content increased by 30 and 57%, respectively.

Nahar *et al.*, (2016) reported that 15% PEG (PEG-6000) induced drought stress rose the amount of MDA and H_2O_2, and $O_2^{\bullet-}$ production rate compared to control mung bean plants. CAT which is well known as a ROS scavenger, also decreased under drought stress condition.Besides these, drought stress also increased cytotoxic MG production by decreasing gly II enzyme activity.

2.3 Antioxidant defense system in plants

Under normal conditions potentially toxic oxygen metabolites are produced at a low level and there is a steady state balance between ROS productions and scavenges. The balance between ROS production and scavenge system breaks down under various stresses (i.e: drought, salinity, high temperature, low temperature etc) which gives rise to increases the intracellular ROS levels and tempts oxidative damage of lipids, breakdown of proteins and DNAs (Hasanuzzaman *et al*., 2011a). To balance between the productions and scavenge of ROS, plants possess antioxidant defense system which is composed of both enzymatic and nonenzymatic components. Non-enzymatic antioxidant consists of Ascorbate (AsA), Glutathione (GSH), Tocopherol, Phenolic compounds, alkaloids and non-protein amino acids. Furthermore, enzymatic antioxidant are Catalase (CAT),Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR), Glutathione reductase (GR), Glutathione peroxidase (GPx), Glutathione S-Transferases (GST) (Gill and Tuteja, 2010; Hasanuzzaman *et al.*, 2012).

Ascorbate

Ascorbate is an important non enzymatic, low molecular weight antioxidant in plant tissues that plays a major role in defense against oxidative stress caused by increased level of ROS. AsA has been appearing to assume essential part in physiological procedures in plants including growth, differentiation, and metabolism. Most of the AsA present in cytoplasm except unlike other antioxidants, a reasonableamout is transfered to apoplast, where its concentration is as low as millimolar. AsA plays a vital role to confer different abiotic stress tolerance (Hossain *et al.*, 2009; Hasanuzzaman *et al*., 2011a). Under typical physiologic situation, AsA mostly resideat chloroplast in reduced form, also a cofactor for the enzyme violaxanthin de-epoxidase, and role in excess energy dissipation (Smirnoff, 2000). It provides membrane protection by directly reacting with $O_2^{\bullet -}$, H_2O_2 and regenerating α-tocopherol from tocopheroxyl radical and preserves the activities of the enzymes that contain prosthetic transition metal ions (Noctor and foyer, 1998). AsA has a key role in removal of H_2O_2 *via* AsA-GSH cycle (Pinto *et al.*, 2003). Exogenous application or seed priming with AsA influences the activity of many enzymes and minimizes the oxidative damage through combining function with other antioxidants (Shalata and Neumann, 2001).

Glutathione

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is non protein thiol tripeptide component that plays direct role in ROS scavenging and reduce ROS induced oxidative damage. It is a molecular weight peptide and found in most cellular components, for example chloroplasts, mitochondria, endoplasmic reticulum, apoplast, peroxisomes, and also even in the vacuoles and cytosol. It functions in various biological processes like cell growth and division, differentiation of cells, sulfate transportregulation, xenobioticsdetoxification, metabolitesconjugation etc.. Beside these enzymatic activityregulation, translation of proteins and nucleotides biosynthesis, and expression of stress responsive genes are also the common activity of GSH. Glitathionperforms in animportant role in the antioxidtive defense mechanismby recycling AsA through the AsA-GSH cycle (Foyer and Halliwell,

1976). It can react chemically with ROS (O_2•−, OH• and H_2O_2) and, therefore, can function directly as a free radical scavenger. Through detoxification of ROS the reduced form of GSH is converted to oxidized glutathione (GSSG). The GSSG thus generated is converted back to GSH, either by de novo synthesis or enzymatically by GR (Gill and tuteja, 2010). Without the proper equilibriumof GSH and glutathione disulfide (GSSG) cellular redox state can not be sustained. The GSH/ GSSG determine the intracellular redox potential that indicates the ability of scavenging ROS as well as stress tolerance to a great extent. Glutathione (GSH) keeps or recycles some components of AsA- GSH cycle in reduced form that's help in ROS quenching. Apart from the ROS detoxification, GSH participates in methylglyoxal (MG) detoxification (Hasanuzzaman *et al.*, 2017). Numerous studies have confirmed that an increase in endogenous GSH levels enhances protection to various abiotic stresses including drought stress by ameliorating ROS-induced oxidative damages (Hasanuzzaman and Fujita 2011; Alam *et al.*, 2013; Nahar *et al.*, 2015a; Rahman *et al.*, 2016)

Tocopherols

Tocopherols (*α, β, γ,* and *δ*) are the set of lipophilic antioxidants which are involved in scavenging process of oxygen free radicals, lipid peroxy radicals, and $^1O_2$ (Diplock *et al.*, 1989). Tocopherols are considered as very efficient antioxidant so that one molecule of α-tocopherol can deactivate upto 120 $^1O_2$ molecules by resonance energy transfer (Munne-Bosch, 2007). Tocopherol are known as the protector of lipid and other cell membrane apparatus by physical quench as well as chemically reactwith O_2pesent in chloroplasts, thus protecting the structure and function of PS II (Ivanov and Khorobrykh, 2003). Accumulation of α-tocopherol has been shown to induce stress tolerance in different plant species (Munne-Bosch *et al.*, 1999; Guo *et al.*, 2006).

Enzymatic antioxidants

Superoxide Dismutases (SOD)

Superoxide dismutase (SOD, 1.15.1.1) defended aerobic organismsagainst oxidative stress (Scandalios, 1993). The enzyme SOD is basically metalloenzyme dismut O_2•−

to O_2 and H_2O_2. It is present in most of the sub cellular compartments that are the source of reactivated oxygen. In plants mostly three isozymes of SOD copper/ zinc SOD (Cu/ Zn-SOD), manganese SOD (Mn-SOD), and iron SOD (Fe-SOD) are found (Fridovich, 1989; Racchi *et al.,* 2001). MnSOD is mostly found in mitochondria, whereas, Fe-SOD is functions in chloroplasts of cells (Jackson *et al.,* 1978). Cu/Zn-SOD is of three isoforms, and likely to be found in cytosol, chloroplast and peroxisome, and mitochondria (Bueno *et al.,* 1995; Del Río *et al.,* 1998). Eukaryotic cell Cu/Zn-SOD is sensitive to cyanide and be as dimer, while other two (Mn-SOD and Fe-SOD) are insensitive to cyanide and may be present as dimer or tetramers (Del Río *et al.,* 1998). In various environmental stresses, SOD activity has been reported to increase, including drought and metal toxicity (Sharma and Dubey, 2005; Misra *et al.,* 2011). Overproduction and increased SOD activity is often correlated with increased environmental stresses and oxidative stress tolerance in plants (Gupta, 1993).

Catalase (CAT)

Catalase (CAT, 1.11.1.6), the antioxidant enzyme that was first discovered and characterized. It is a heme-containing enzyme, ubiquitous and tetrameric in nature with the potentiality to directly catalyze the dismutation of two molecules of H_2O_2 to one molecule of water and one molecule of oxygen and important for detoxification ROS at stress (Garg and Manchanda, 2009). CATs having a fast turnover rate, and has a much lower affinity for H_2O_2 compared to APX. In plants CAT scavenges H_2O_2 generated in peroxisomes at the time of photorespiration, fatty acid β-oxidation (Del Río *et al.,* 2006; Scandalios *et al.,* 1997; Corpas 2008). Although CAT may be present in other cell organels like cytosol, chloroplast, and mitochondria, CAT activity is insignificant in these sites (Mhamdi, 2010). When cells suffered for energy and H_2O_2 generation is rapid through catabolic processes, CAT degrad H_2O_2 in an energy efficient manner (Mallicand Mohn, 2000). Environmental stresses may enhance or deplete CAT activity, which depends on intensity, period, and stress type (Moussaand Abdel-Aziz, 2008). Simova- Stoilova *et al.,* (2010) reported increased CAT activity under drought in wheat while CAT activity decreased in rice drought stress (Sharma and Dubey, 2005). CAT activity shows variable trends under different abiotic stresses

(Singh *et al*., 2008; Hasanuzzaman and Fujita, 2011). Generally, reduced CAT activity was observed in those stresses that lessen the protein turnover rate. On the other hand Guan *et al.,* (2009) found overexpression of a CAT gene introduced to tobacco from *Brassica juncea*, exhibites more tolerance to oxidative stress induced by Cd.

APX (Ascorbate peroxidase)

Ascorbate peroxidase (APX, EC 1.1.11.1), an indispensable constituent of AsA-GSH cycle, is one of the important member of Class I heme peroxidases super family (Welinder, 1992). Ascorbate peroxidases are essential for controlling intracellular ROS levels, where APX converts two molecules of AsA to two molecules of MDHA while reducing H_2O_2 to water. The APX family coomprises of no less than five different isoforms, such as tAPX (thylakoid APX), gmAPX (glyoxisome membrane APX), sAPX (chloroplast stromal APX), cAPX (cytosolic APX) etc. (Peng *et al.,* 2005). Different APX isoforms have much affinity for H_2O_2 than CAT, and thus made APXs an efficient scavenger of H_2O_2 under stressful conditions (Wang *et al.*, 1999). In plant system APX activity enhanced subjected to abiotic stresses like drought, salinity, heavy metal toxicity (Maheshwari and Dubey, 2009; Hefiny and abdel kader, 2009; Boo and Jung, 1999). Sharma and Dubey (2005) found that in mild drought stress plants, APX activity gets higher in chloroplast compared to unstressed plants, but at severe stress the activity declined. Overexpression of APX in *Nicotiana tabacum* chloroplasts enhanced plant tolerance to salt and drought stress (Badawi *et al.,* 2004). It was also observed that APX overproduction, increase POD activity, which boosten the scavenging of ROS, and leads to stress tolerance (Sarowar *et al.*, 2005).

MonodehydroAscorbate Reductase (MDHAR)

Monodehydroascorbate reductase (MDHAR, 1.6.5.4) is a NADPH dependent flavin adenin dinucleotide (FAD) enzyme, which catalyzes the AsA regeneration from the MDHA radical (Hossain and Asada, 1985). The MDHAR isoenzymes have been reported to be occured in different cellular organels such as chloroplasts, mitochondria

and peroxisomes or even in cytosol (Jim´enez, 1997; Dalton *et al.*, 1993). In chloroplasts, MDHAR plays two physiological functions, firstly AsA the regeneration from MDHA the beneficial one, and secondly in absence of MDHA mediate photoreduction of oxygen to $O_2^{\bullet-}$ (Miyake, 1998). The involvent of MDHAR in generating $O_2^{\bullet-}$ in pea leaf peroxisomes. Many scientists also found increased MDHAR activity in plants at the time of environmental stresses (Sharma and Dubey, 2005; Maheshwari and Dubey, 2009; Sharma and Dubey, 2007; Boo and Jung, 1999). Overexpression of MDHAR confers enhanced tolerance to various ROS induced oxidative and osmotic stresses (Eltayeb, 2007).

Dehydroascorbate reductase (DHAR)

In plant system dehydroascorbate reductase (DHAR, EC 1.8.5.1) a monomeric thiol enzyme, generally found in dry seeds, roots and etiolated as well as green shoots in abundance, and plays an important role by catalyzing the reaction of DHA to AsA using GSH as a reducing substrate and, thus, maintaining AsA balance (Ushimaru *et al*., 1997; Sharma and Dubey, 2007). Despite the possibility of enzymatic and nonenzymatic regeneration, AsA directly from MDHA, at the time of AsA oxidation some DHA is always produced in leaves and other tissues. Dehydroascorbate is a very short-living, and can be hydrolyzed to 2, 3-diketogulonic acid or recycled back to AsA by the action of DHAR. In general, DHAR activity in plants, are upregulated by environmental stresses; for example, drought, metal toxicity, and chilling (Maheshwari and Dubey, 2009; Boo and Jung, 1999). The upregulation of DHAR helps to AsA recycling in the apoplast which improves tolerance in different crop species under various ROS induced stresses (Hasanuzzaman *et al*., 2011a, b).

Glutathione Reductase (GR)

Glutathion reductase is a NADPH dependent enzyme, occured in both prokaryotic and eukaryotic calls (Romero-Puertas *et al.,* 2006). It is an important enzyme of AsA-GSH cycle and roles in ROS defense system in various abiotic stresses by maintaining GSH status.Chloroplast is the main site of occurance of GR, but a small amount may also been present in mitochondria and cytosol (Edwards *et al.,* 1990; Creissen *et al.,* 1994).

Actually, GR convertes GSSG, consisting of two GSH molecules linked with a disulphide bond. Glutathion reductase involves in oxidative stress defense, where, GSH acts an important role, including AsA-GSH cycle participation, maintenance of the sulfhydryl group and a substrate for GSTs (Reddy *et al.,* 2004). Glutathion reductase also plays a crucial role in giving plants tolerance under various stresses by upregulating the antioxidant defense system (Hasanuzzaman *et al*., 2011 a, b). GR activity found to be increased in wheat, rice under drought stress condition (Sharma and Dubey, 2005).

Glutathione peroxidase (GPX)

Glutathione peroxidase (GPX) consists of multiple isoenzymes. Their subcellular locations are distinct and which exhibit tissue-specific expression patterns in abiotic stress. Besides scavanzing H_2O_2 and hydroperoxides, GPX The occurrence of thiol-dependent activities of plant GPX involved in cellular redox homeostasis by maintaining the balance between thiol and disulfide, or NADPH and $NADP^+$. Link between the glutathione and the thioredoxin system is also representes by the GPXs activity. GPX expression was found to be highly up regulated to maintain redox homeostasis under oxidative stress (Sugimoto *et al.*, 2014).

2.4 Methylglyoxal and Glyoxalase system

Plants possess a well-established enzymatic and non-enzymatic antioxidant defense system which enables it to render protection against various abiotic stress conditions by mainly scavenging the reactive oxygen species (ROS) produced due to oxidative damage. This oxidative stress conditions also result in production of some other toxic components like methylglyoxal (MG). Methylglyoxalis an α-oxoaldehyde, produced through various enzymatic and non-enzymatic reactions, are cytotoxic and very highly reactive in nature. Cell or cell components may be impaired or even DNA may be destroyed causing mutation by MG (Wang *et al.,* 2009; Desai *et al.,* 2010). In normal growing condition MG concentration in various plant species varies between 30-75 µM and 2- to 6-times more in response to different stresses including drought, salinity and cold etc (Yadav *et al.*, 2005a). Increased activity of MG synthase, conversion of

acetone into MG and metabolism of aminoacetone are the main reasons of MG production (Yadav *et al.,* 2009).

However, plants have glyoxalase system to detoxify MG, in collaboration with two vital enzymes Gly I and Gly II with the help of GSH (Yadav *et al.,* 2009). Glyoxalase I is present in cytosol and peroxisome and Gly (II) is present in cytosol and mitochondria. The first step of detoxification MG reacts with GSH catalyzed by Gly I forming hemithioacetal, which is later converted to *S*-D-lactoylglutathione (SLG). In the second step SLG is hydrolyzed to form GSH and D-lactate catalyzed by Gly II. If D-lactate, also considered as toxic compound, is over accumulated, D-lactate dehydrogenase convertes it into pyruvate.Therefore, pyruvate is the major catabolic product of MG but GSH is recycled at the end of total reaction, as the availability of GSH is an important for MG detoxification by the glyoxalase system. So, the glyoxalase system not only involved in detoxifing MG but also acts in an important role in oxidative stress tolerance by recycling GSH and thereby maintaining glutathione homeostasis. Beside the alleviation of stress damage, GSH can play some other beneficial role such as, modulating some other enzymatic and nonenzymatic antioxidants, interacting with redox molecules and hormones, participating in stress-induced signal transduction, and so on. In recent studies, both Gly I and Gly II, and exogenously applied GSH, all these components of glyoxalase system have been shown to have positive effects for plants under different abiotic stresses including drought by improving antioxidant defense system (Hasanuzzaman and Fujita, 2011).

Transgenic tobacco and rice plants with overexpressed glyoxalase pathway has been found to reduce ROS and MG under drought stress conditions by maintaining glutathione homeostatsis and antioxidant enzyme, glyoxalase enzyme levels. Gly I activity significantly increase was observed in response to drought stress. In addition to glyoxalase, aldose reductase pathway can also detoxify MG in plants (Yadav *et al.*, 2005a).

The performance of SA application to mitigate drought stress in *Brassica juncea* L (cv. BARI sharisha 11) was observed by (Alam *et al.,* 2013). Drought stress significantly increased MDA content, H_2O_2 content which is indicators for oxidative stress. The glyoxalase system components Gly I and Gly II activities decreased due to drought stress. Spraying seedlings with 50 µM salicylic acid increased the glyoxalase system components, reduced oxidative stress and improved physiological parameters and thus improved drought tolerance.

Aldesuquy and Ghanem, (2015) reported that exogenously application of SA and Tre in drought sensitive wheat cultivar Gemmieza-7 and drought tolerant Sahel-1. Drought stress reduced membrane stability by increasing oxidative damage. The glyoxalase system components Gly I and Gly II altered under drought stress. However, exogenously application of SA and tre give a hand to the plants to be tolerant to drought stress by upgrading the membrane character and improving antioxidant defense and glyoxalase systems.

2.5 Seed priming and tolerance against drought stress

2.5.1 Seed priming

Seed priming is a pre-sowing treatment of seed that regulates and increases pre-germinative metabolic activity while preventing radicle projection because it indicates completing germination **(**Bewley*et al.,* 2013). Seed priming is a controlled hydration activity that stimulates metabolic processes during early germination stage but before radical projection (Hussain *et al.,* 2016).Stress tolerant variety development is time consuming and economical process. It is wise to develop a technique which is rapid and cost effective against stresses, seed priming may be one of the substitute strategies because it is easy, cheap and effective against various abiotic and biotic stresses (Iqbal and Ashraf, 2007). In general, seed priming increased germination rate, uniform germination and good seedlings establishment and ultimately better yield (Jisha *et al.,* 2013). Metabolism of energy, osmotic regulation, embryo enlargement, upregulating enzyme activity, and fast cellular defense reaction under abiotic and biotic stresses

made seed priming technique as an effective and practical ones (Jisha *et al.*, 2013). Seed priming is one of the most pragmatic and short term approaches which resists the drought (Kaya *et al.*, 2006; Farooq *et al.*, 2010) and other environmental stress by increasing enzymatic antioxidants stresses (Farooq *et al.*, 2008b; Jafar *et al.*, 2012). Primed wheat seed improved stand establishment and better yield than non primed wheat seed (Farooq *et al.*, 2008b). Various seed priming techniques including hydropriming, osmopriming or halo-priming, Chemical priming, Hormone priming, Solid matrix priming etc. are practiced under various abiotic stresses (Jisha *et al.*, 2013; Paparella *et al.*, 2015). Hydropriming means priming with water under optimal condition with or without aeration (Paparella *et al.*, 2015). Under adverse environmental condition like- drought, high temperature etc; hydropriming is one of the suitable technique (McDonald, 2000). Osmopriming or halopriming means priming with osmotic solutions like Polyethylene glycol (PEG), glycine betaine, prolin, ascorbic acid, inorganic salt of sodium, potassium and magnesium (most commonly used NaCl, $NaNO_3$, $MnSO_4$, $MgCl_2$, K_3PO_4 and KNO_3) etc at low water potential that facilitates the control of water uptake and limit the ROS-mediated oxidative damage **(**Paparella *et al.*, 2015). Priming with phyto-hormone like salicylic acid (SA), abscisic acid (ABA) or gibberellic acid (GA) can regulate biochemical processes during germinationand improves antioxidant defense system to cope with environmental stresses (Radhakrishnan and Lee, 2013).

2.5.2 Seed priming and plant responses

Seed priming with SA and plant responses

Salicylic acid is an endogenous phenolic phytohormone, important signaling molecule and protects plant against abiotic stresses by adjusting vital growth and physiological mechanisms, such as RWC, photosynthesis, antioxidant defense, and metabolismnitrogen, Pro and GB (Syeed *et al.*, 2011; Nazar *et al.*, 2011; Khan *et al.*, 2012.).

SA plays a vital role against biotic stress as well as plays an important role in mitigating different abiotic stress including the salinity, temperature, UV radiation or

ozone water and heavy metal stress (Gunes *et al.,* 2007; Belkhadi *et al.,* 2010; Hayat *et al.,* 2010).

SA not only plays a key role in establishing and signaling a defense response against various pathogenic infections, but also plays an important role in mediating plant response to some abiotic stresses.

Yusuf *et al.* (2012) reported that antioxidant enzymes like SOD, CAT and POX are upregulated with the application of SA in both control and stressed conditions. Low concentrations of SA are able to improve plant's antioxidant capacity, but may be toxic for cell or enhance susceptibility to abiotic stresses at high concentration (Hara *et al.,* 2012). However, SA treated plants perform better in drought stress with upregulated antioxidant system (Al-Hakimi and Hamada 2001 and Kadioglu *et al.,* 2011).

Generally plants responses to water stress by developing long roots or closing stomata.SA also has a role in stomatal closure via the production of ROS, which is mediated by a peroxidase-catalyzed reaction (Dong *et al.,* 2001 and Mori *et al.,* 2001).

It was investigated that priming with SA of wheat genotypes (Al-Hakimi and Hamada, 2006) or application of SA through rooting medium of wheat (Arfan *et al*., 2007) mitigated the abiotic stresses greatly. Alam *et al.,* (2013) reported that Exogenous SA improved short-term drought stress in mustard by up regulating antioxidant defense and glyoxalase system. Exogenous SA reduced the adverse effects of drought stress and played a key role against drought stress by decreasing water loss and modulating the antioxidant system in plants with leaf rolling, an alternative drought protection mechanism (Saruhan *et al.,* 2012).

Seed priming with AsA and plant response

Ascorbic acid is a small, water soluble well recognized antioxidant molecule that protects plant by suppressing oxidative damage. AsA plays the most important role in

AsA-GSH cycle, where it functions coordinately with glutathione and several enzymatic antioxidants to scavenge ROS which is produced by the Mehler reaction and photorespiration in chloroplast and cytosol (Noctor and Foyer, 1998; Asada, 1999). Increase of endogenous biosynthesis of AsA or exogenously AsA supply are well established for improving abiotic stress tolerance (Guo *et al.,* 2006; Razaji *et al.,* 2014; Farooq *et al.,* 2013). Cell division and photosynthesis has also been regulated by ascorbic acid.Several research studies have described that using of AsA as a priming agent helps to improve abiotic stress tolerance in different crops. Bakry *et al.,* (2012) reported that application of different levels of AsA (100, 200 and 300 mg L^{-1}) to observe the beneficial role of drought affected wheat seedlings. Drought stress decreased germination percentage, better seedlings establishment, yield components and total yield of wheat. Different levels of AsA significantly improved those parameters which were affected by drought stress imposing to wheat plants.

Farooq *et al.* (2013) reported that seed priming with 2mM ascorbate helps to improve seedling emergence and early growth, emergence of leaf, leaf elongation, increasing in total and specific leaf area, chlorophyll quantity, root and shoot growth and seedling dry weight, better seedling establishment under drought stress condition in wheat plants. Increase in endogenous level of AsA by seed priming helped in scavenging the ROS as has been indicated by decrease in MDA contents. Seed priming with AsA improved the phenolics which helped in decreasing the oxidative damage.

Drought stress significantly reduced germination percentage and growth parameters of wheat plants. Seed priming with 1mM of AsA reduce oxidative damage by sustaining growth, RWC, membrane solidity, and osmotic balance through accumulation of proline and upregulating antioxidant enzymes (Malik and Ashraf, 2012).

Seed priming with AsA increased the root and shoots length, seedlings growth, upregulating the antioxidant enzymes viz. CAT, SOD, APX and GR. Lower levels of MDA and H_2O_2 was also found in the seedlings grown from primed seed, which

showed role of AsA priming in mitigation of drought induced oxidative damage (Singh and Bhardwaj, 2016).

Shafiq *et al.* (2014) investigated that even 60% field capacity caused drought stress with significantly reduction in growth parameters and leaf chlorophyll content. The inmpect was on also P content of shoot and root, as well as root K^+, and CAT activity. Drought stress affected plants showed increase of MDA content, free proline content, phenolics. Seed pretreatment with different doses of AsA decreased MDA content, increased root and shoot length, root and shoot fresh and dry weight, chlorophyll content and activity of enzymatic antioxidants such as CAT, POX in drought affected canola plants.

Seed priming with NaCl and plant responses

Halopriming or seed soaking in inorganic salts is an easy to use, low cost and low risk technique, and it is being successfully applied to improve the tolerance level of plants under various abiotic stress conditions (Jamal *et al.*, 2011). Many reports had shown that considerable enhancement in seed germination, emergence, seedlings establishment, antioxidant defense and glyoxalase systems upregulation of different crops under various abiotic stress conditions with seed halopriming.

NaCl used as halopriming agents showed positive impacts on seedlings growth under PEG induce drought stress. Drought stress decreased growth attributes such as shoot length, fresh weight, and dry weight, and also results in physiological disorders by reducing chlorophyll and metabolites in leaves of *Vigna radiata* seedlings. As chlorophyll and metabolite contents decreased, photosynthetic ablity and mitochondrial activity also reduced. PEG stress also attributed in MDA over production, H_2O_2generation, proline content, and decreased antioxidant enzymes (POD, APX) activity. However seed priming with NaCl decreased MDA, H_2O_2 and proline content and increased the antioxidant enzymes activity in mung bean seedlings under PEG stress (Jisha and Puthur, 2014).

Iseri *et al.* (2014) reported that NaCl priming reduced mean germination time, and increased final germination percentage together with energy of germination. Increased root and hypocotyls lengths as well as increases in fresh weights supported enhanced seedling vigor under salt stress conditions. Considering growth and stress parameters such as chlorophyll (chl) content, chlto carotenoid (Car) ratios, and lipid peroxidation and electrolyte leakage were less affected in primed tomato seedlings. Moreover NaCl priming increased AsA content and the activity of CAT in salt affected tomato seedlings.

Iqbal and Ashraf (2007) studied the effect of halopriming on two spring wheat cultivars namely MH-97 (salt sensitive) and Inqlab-91 (salt tolerant). Seeds were soaked in 100 m mol of $CaCl_2$, KCl or NaCl. The results showed that $CaCl_2$ followed by KCl and NaCl decreased the effect of salinity on grain yield and biomass production of both cultivars.

According to Patade *et al.* (2009), seed priming in sugarcane with NaCl, increased the germination percentage, shoot length, shoot and root fresh weight when subjected to 15 days polyethylene glycol (PEG 8000; 20%, w/v) stress. NaCl priming also decreased ROS production by upregulating enzymatic antioxidants (CAT, SOD, and POD).

Mohammadi (2009) investigated the effect of priming with NaCl on seedling growth of canola (*Brassica napus* L.) in saline conditions, the results showed that NaCl primingincreased germination percentage by 25.57%, germination rate by 34.67%, and the dry weight of seedlings by 36.67%.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental location

The experiment was conducted at Laboratory of Plant stress responses,Kagawa University; Kagawa, Japan during the period from March 2016 to October, 2016.

3.2 Plant materials

Wheat (*Triticum aestivum* cv. BARI Gom-30) seeds collected from the Bangladesh Agriculture Research Institute, Gazipur. The variety is high yielding, early in maturity having good level of tolerance to terminal heat stress. Grains are white amber in colour and medium in size. Spikes are long with 45-50 average grains in each. Leaves are broad and recurved. The cultivar matures at 100-105 days after sowing. The cultivar givesan average yield of 4.5-5.5 ton ha^{-1}.

3.3 Priming treatments

The seeds were primed with three different priming agents and doses e.g.50 µM salicylic acid (SA), 4 mM Ascorbic acid (AsA) and 2.5 mM Sodium chloride (NaCl) after trail with different doses of mentioned priming agent. Primed seeds are incubated for 5 hours in dark condition. The seeds were then sown in Petri dishes (9 cm) lined with 6 layers of filter paper moistened with 10 ml of distilled water for germination for 48 hours in germination chamber. Germinated seedlings then were grown in Petri dishes that contained 10,000-fold diluted Hyponex solution (Hyponex, Japan) under controlled conditions in growth chamber (light, 100 µmol photon m^{-2} s^{-1}; temp, (25±2) °C; RH, 65%~70%). The 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.

3.4 Stress treatments

Afterwords the five days old seedlings with or without SA, AsA, NaCl primed were subjected to drought stress (induced by 15% polyethylene glycol PEG) and grown

under above conditions for 72 hours. Control plants were grown in Hyponex solution only. Data were taken after 72 hours of treatment.

3.5 Experimental treatments:

Therefore our experiment consisted of eight treatments as follows:

1. Control (C)
2. 50 µM SA priming (SA)
3. 4 mM AsA priming (AsA)
4. 2.5 mM NaCl priming (NaCl)
5. Drought (15% PEG) (D)
6. 50 µM SA+ Drought (SA+D)
7. 4 mM AsA+ Drought (AsA+ D)
8. 2.5 mM NaCl+ Drought (NaCl+D)

The experiment was laid out in a CRD design with three replications.

3.6 Collection of data:

3.6.1 Seed quality parameter

- Germination percentage
- Vigour index
- Coefficient of velocity

3.6.2 Crop growth parameter

- Plant height
- Fresh weight plant^{-1}
- Dry weight plant^{-1}

3.6.3 Physiological parameters:

- Relative water content (RWC)
- Photosynthetic pigment

3.6.4 Oxidative stress indicators:

- Lipid peroxidation
- H_2O_2 content
- Proline content

- Methylglyoxal content
- Ascorbate content
- Glutathione content
- Activities of antioxidant enzymes (CAT, APX, MDHAR, DHAR, GR, GPX, Gly I and Gly II)

3.7 Germination percentage

Thirty seeds from each of the treatments were selected randomly and placed in 90 mm diameter petri plates on 6 layers of filter papers moistened with 8 ml of distilled water. Seeds were kept at controlled condition 25±1°C under dark to facilitate germination for 40 hours. Germination was considered to have occurred measuring radicles at least 2 mm long (Akbari *et al.*, 2007). Germination was inspected and data were collected 48 hours after seed sowing. The seedlings with abnormalities like short, thick and spiral hypocotyls as well as stunted primary roots were discarded (ISTA, 2003). These types of abnormal seedlings and the dead seeds were excluded at the time of counting. Germination percentage was calculated using the following formula:

TG (%) = (No. of germinated seeds/ total no of seeds set for germination) × 100.

3.8 Vigour Index (VI)

Vigour Index (VI) was calculated according to Abdul- Baki and Anderson (1970) using the following formula.

$$\text{Vigour index}=\frac{\text{TG (\%) x seedlings length (mm)}}{100}$$

Here,

TG = total germination

3.9 Coefficient of velocity (CV)

Coefficient of velocity (CV) also called the number of germinated seeds each day was measured according to Kader and Jutzi (2004) using the following formula.

$$CV=(\Sigma Nx/100)\times\Sigma T Nx$$

Where, T denotes the number of days after sowing and Nx denotes the number of seeds germinated after xth day.

3.10 Fresh weight and dry weight of seedling

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

3.11 Measurement of lipid peroxidation

The level of lipid peroxidation was measured by estimating malondealdehyde (MDA) content according to Heath and Packer (1968) with slight modification by Hasanuzzaman *et al.* (2012b). Leaf samples (0.5 g) were homogenized in 3 mL 5% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 11,500 × g for 15 min. The supernatant (1 mL) was mixed with 4 mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95 °C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged again at 11,500 × g for 10 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. MDA content was calculated by using extinction coefficient 155 $mM^{-1}cm^{-1}$ and expressed as nmol g^{-1} FW.

3.12 Measurement of H_2O_2

H2O2 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.

3.13 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)] ×100

3.14 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. Two 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.

3.15 Determination of Chlorophyll Content

Chlorophyll (Chl) content was determined by taking fresh leaf samples (0.25 g) from randomly selected seedlings. 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).

3.16 Determination of Methylglyoxal Content

Methylglyoxal was measured following the method of Wild et al. (2012). Leaves were homogenized in 5% perchloric acid and centrifuged at 4 °C for 10 min at 11,000×g. The supernatant was decolorized by adding charcoal. The decolorized supernatant was neutralized by adding a saturated solution of sodium carbonate at room temperature. The neutralized supernatant was used to estimate MG 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.

3.17 Extraction and analysis of ascorbate and glutathione

Fresh wheat leaves (0.5 g) were homogenized in 3 mL ice-cold 5% meta-phosphoric acid containing 1 mM 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 determined following the method of Huang *et al.* (2005) with some modifications. The supernatant was neutralized with 0.5 M K-P buffer (pH 7.0), and 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. Aliquots (0.2 mL) of supernatant were neutralized with 0.3 mL of 0.5 M K-P buffer (pH 7.0). Based on enzymatic recycling, 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, and 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 derivatization. Standard curves

with known concentrations of GSH and GSSG were used. The content of GSH was calculated by subtracting GSSG from total GSH.

3.18 Histochemical detection of H_2O_2 and $O_2^{\bullet-}$

Localization of $O_2^{\bullet-}$ in leaf was detected following Chen *et al.* (2010) with slight modification. Leaves were stained in 0.1% 3-diaminobenzidine (DAB) and 0.1% nitrobluetetrazolium chloride (NBT) solution for 24 h under a dark condition for H_2O_2 and $O_2^{\bullet-}$ detection, respectively. Incubated leaves were then blenched by immersing in boiling ethanol. After that, brown spots appeared resulting from the reaction of DAB with H_2O_2 and dark blue spots appeared resulting from the reaction of NBT with $O_2^{\bullet-}$ (Thordal-Christensen *et al.*, 1997). Photographs were then taken by placing the leaves on glass.

3.19 Determination of protein

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

3.20 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 0−4ºC.

3.20.1 Ascorbate peroxidase (APX, EC: 1.11.1.11)

APX (EC: 1.11.1.11) activity was assayed following the method of Nakano and Asada (1981). The reaction buffer solution contained 50 mM K-P buffer (pH 7.0), 0.5 mM AsA, 0.1 mM H_2O_2, 0.1 mM EDTA, and enzyme extract in a final volume of 700 µl. The reaction was started by the addition of H_2O_2 and the activity was measured by observing the decrease in absorbance at 290 nm for 1 min using an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$.

3.20.2 Monodehydroascorbate reductase (MDHAR, EC: 1.6.5.4)

MDHAR (EC: 1.6.5.4) activity was determined by the method of Hossain et al. (1984). The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.5), 0.2 mM NADPH, 2.5 mM AsA, 0.5 unit of AO and enzyme solution in a final volume of 700 µl. The reaction was started by the addition of AO. The activity was calculated from the change in absorbance at 340 nm for 1 min using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$.

3.20.3 Dehydroascorbate reductase (DHAR, EC: 1.8.5.1)

DHAR (EC: 1.8.5.1) activity was determined by the procedure of Nakano and Asada (1981). The reaction buffer contained 50 mM K-P buffer (pH 7.0), 2.5 mM GSH, and 0.1 mM DHA. The reaction was started by adding the sample solution to the reaction buffer solution. The activity was calculated from the change in absorbance at 265 nm for 1 min using extinction coefficient of 14 $mM^{-1}cm^{-1}$.

3.20.4 Glutathione Reductase (GR, EC: 1.6.4.2)

GR (EC: 1.6.4.2) activity was measured 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. The reaction was initiated with GSSG and the decrease in absorbance at 340 nm was recorded for 1 min. The activity was calculated using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$.

3.20.5 Catalase (CAT, EC: 1.11.1.6)

CAT (EC: 1.11.1.6) activity was assayed following the method of Hasanuzzaman et al. (2012) by monitoring 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 $M^{-1}cm^{-1}$.

3.20.6 Glyoxalase I (Gly I, EC: 4.4.1.5)

Glyoxalase I (EC: 4.4.1.5) assay was carried out according to Hasanuzzaman *et al.* (2011a). Briefly, 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 $mM^{-1}cm^{-1}$.

3.20.7 Glyoxalase II (Gly II, EC: 3.1.2.6)

Glyoxalase II (Gly II; EC: 3.1.2.6) activity was determined according to the method of Principato *et al.* (1987) by monitoring the formation of GSH at 412 nm for 1 min. The reaction mixture contained 100 mM Tris–HCl buffer (pH 7.2), 0.2 mM DTNB and 1 mM S-D-lactoylglutathione (SLG) in a final volume of 1 ml. The reaction was started by the addition of SLG and the activity was calculated using the extinction coefficient of 13.6 $mM^{-1}cm^{-1}$.

3.20.8 Glutathione peroxidase (GPX: EC: 1.11.1.9)

GPX (EC: 1.11.1.9) activity was assayed using the method of Elia *et al.* (2003). The reaction mixture consisted of 100 mM K-P buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide (NaN_3), 0.12 mM NADPH, 2 mM GSH, 1 unit GR, 0.6 mM H2O2 (as a substrate), and 20 µL of sample solution. The oxidation of NADPH was recorded at 340 nm for 1 min and the activity was calculated using extinction coefficient 6.62 $mM^{-1}cm^{-1}$.

3.21 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and the mean differences were compared by Fisher's LSD using XLSTAT v.2015 software (Addinsoft, 2015). Differences at $P \leq 0.05$ were considered significant.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Germination percentage

Germination percentage decreased by 55% under drought stress (Table 1). Seed priming with SA, AsA and NaCl increased germination percentageby 62%, 58%, and 55% respectively under drought stress condition. Seed priming with SA showed best result in respect of recovery of germination percentage compared with other priming agent (AsA and NaCl). However, seed priming did not show any significant differences in wheat seed germination under control condition.

Table 1: Effect of seed priming on germination percentage, vigour index and coefficient of velocity of wheat seedlings under drought and control condition

Treatment	Germination (%)	Vigour Index	Co-efficient of velocity
Control	94.00±1 a	15.01±0.42a	15.01±0.42a
SA	94.3±1.1a	15.35±0.24a	53.69±0.51ab
AsA	92.00±2a	15.14±0.28a	42.44±3.87c
NaCl	92.33±0.5a	14.35±1.16a	49.69±1.73b
Drought (D)	51±4.04d	8.01±0.69d	28.34±2.39e
SA+ D	83.3±1.5b	12.21±0.61b	44.1±0.77c
AsA+ D	81±1 bc	12.12±0.84bc	37.85±2.40d
NaCl+ D	79.6±1.52c	10.97±0.87c	42.4±0.95cd

Here, SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. D indicates 15% PEG induced drought stress

Means (±SD) were calculated from three replications for each treatment. Values with different letters are significantly different at P≤0.05 applying the Fisher's LSD test

Plants life cycle start with germination which is considered as the most critical stage.Plant growth, development and yield is greatly affected when germination stage falls under adverse environmental conditions like drought, high temperature, low temperature, salinity etc. Among them, Drought stress markedly inhibited germination of seeds by creating low osmotic potential preventing water uptake (Kaya *et al.*, 2006; Wei *et al.,* 2013). In the present study, seed priming with SA, AsA, and NaCl improve germination percentage under drought stress condition. These finding are supported by earlier work on seed priming with SA (sharafizad *et al.*, 2013), priming with AsA (Farooq *et al.*, 2013; Malik and Ashraf, 2012) in wheat; priming with Salt in sugarcane (Patade *et al.,* 2009) under drought stress. The increased rate of germination in primedseeds was found to be associated with an increased wateruptake potential (Srivastava *et al.,* 2010). Moreover stimulation of metabolic activities involved in the early phase of seed germination may results increased germination percentage even under drought stress (Bradford, 1986; Taylor and Harman, 1990).

4.2 Vigor index

There have no significant differences between SA, AsA and NaCl primed seedlings in contrast with control seedlings. Exposure of drought stress drastically decreased seed vigor index in germinating wheat seeds. Vigor index decreased by 46% under drought stress compared with control one. However priming with SA, AsA and NaCl increased vigour index by 52, 51 and 36% respectively as compared to drought stress alone (Table 1).

Similar results were also reported in previous studies (Afzal *et al.*, 2005; Sallam and Ibrahim, 2015; Heydariyan *et al.*, 2014; Delian and Lagunovschi-Luchian, 2015) where reported that SA, AsA, NaCl priming showed higher vigor index under abiotic stress condition. Seed priming enhanced vigor index as indicated by seedling length, seedlings fresh weight, dry weight etc. (Khan *et al.*, 2011). The possible reason for increased vigor index is due to the increased cell division within the apical meristem of seedling root.

4.3 Coefficient of velocity

Drought stress decreased coefficient of velocity by 52% compared with control seedlings (Table 1). Seed priming with SA, AsA and NaCl increased coefficient of velocity under drought stress condition. Ansari *et al.,* (2013) reported that seed priming with hormone improved germination characteristics (germination percentage, vigour index, coefficient of velocity etc.) in mountain rye under drought stress condition. Increased cell division in primed seeds may increase coefficient of velocity.

4.4 Plant Growth

Plant height

Drought stress significantly reduced the plant height and growth of the wheat seedlings compared with control.Seed priming with SA, AsA and NaCl increased plant height under drought stress condition. In contrast, Seed priming have no significant effect on plant height under control condition.

Shoot FW and DW

Drought stress sharply decreased FW of shoots by 40% as compared to control. Drought stress decreased the dry weight of seedlings by 29% compared to control seedlings. However, DW of seedlings increased compared with drought-stressed seedlings when primed with SA, AsA and NaCl respectively (Table 2).

Table 2.Effect of seed priming on Plant height, FW and DW of wheat seedlings under drought and control condition.

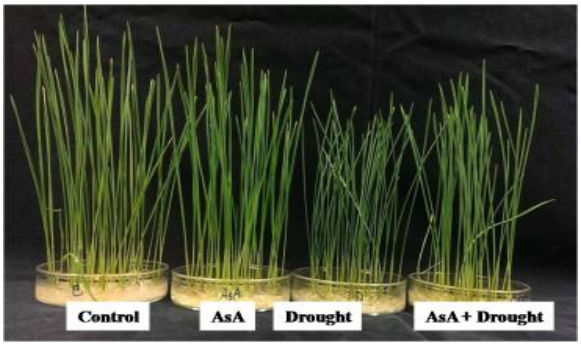
Treatment	**Plant height**	**FW (mg seedling^{-1})**	**DW (mg seedling^{-1})**
Control	16.23±0.2a	0.13±0.001a	0.0210±0.001a
SA	16.43±0.4a	0.12±0.003ab	0.0200±0.001abc
AsA	15.9±0.09ab	0.12±0.002bc	0.0211±0.001a
NaCl	15.60±0.08b	0.11± 0.002 c	0.0206±0.001ab
Drought (D)	11.9±0.1e	0.07±0.02e	0.014±0.001 d
SA+ D	14.1±0.4cd	0.10±0.02d	0.0182±0.009c

AsA+ D	14.3±0.3c	0.09±0.008d	0.0186±0.001bc
NaCl+ D	13.66±0.3d	0.10±0.005d	0.0180±0.001bc

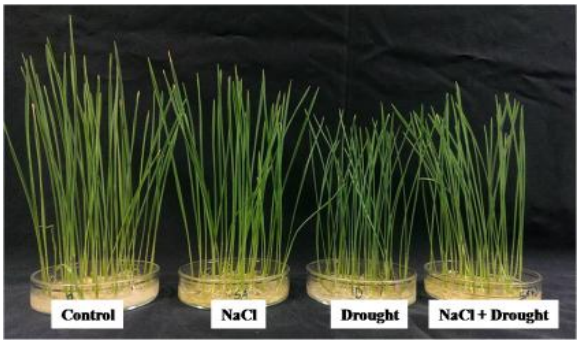
Here, SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. D indicates 15% PEG induced drought stress

Means (±SD) were calculated from three replications for each treatment. Values with different letters are significantly different at P≤0.05 applying the Fisher's LSD test

A



b



C

Plate 1. Effect of (a) SA, (b) AsA and (c) NaCl priming on plant growth under drought stress condition in eight days old wheat seedlings

Plant growth is characterized by some morphological traits like plant height, Plant FW and DW, shoot length, root length etc. In the following experiment drought affected wheat seedlings exhibited growth retardation in terms of plant height, seedlings FW and DW. Cell is basic components of all living organism and water is the basic requirement for cell growth and development. Drought stress hinders cell expansion and reduces stomatal opening and carbohydrate supply ultimately affects growth and development of plants (Kang *et al.*, 2012; Hasanuzzaman *et al.*, 2013). However, the present study indicated the improvement of plant growth in terms of seedling length,

FW, DW by seed priming with SA, AsA, and NaCl under drought stress. This results is consistent with the previous findings in which seed priming with SA, AsA, NaCl improved growth of plants under different abiotic stress condition (Farooq *et al.*, 2008; Razajii *et al.*, 2014; Patade *et al.*, 2009; Saha *et al.*, 2010).

4.5 Relative water content

Drought stress significantly decreased relative water content of wheat seedlings compared with the control seedlings. Seed priming with SA, AsA and NaCl increased RWC by 63%, 55%, and 52%, respectivelyunder drought stress condition. However, non stressed seedlings which were primed with SA, AsA and NaCl did not exhibit any differences in RWC compared to control (Figure 1).

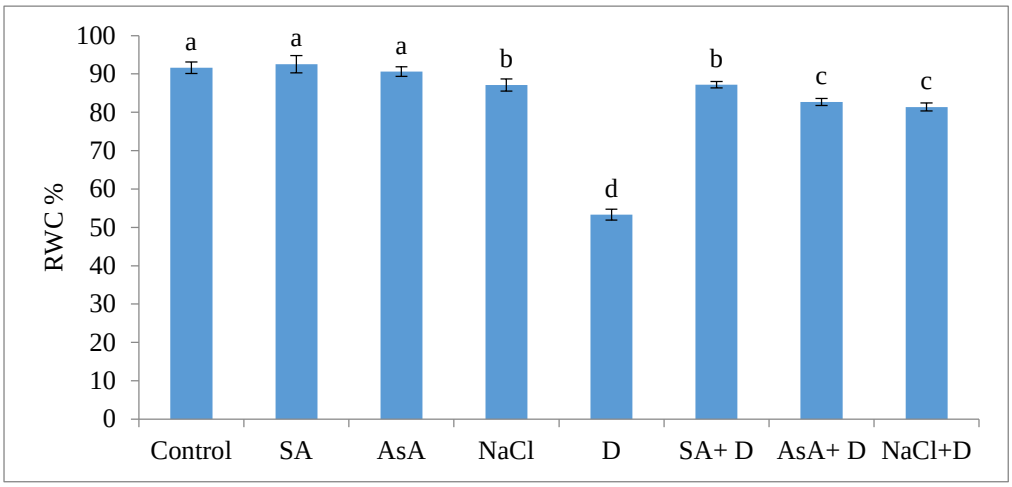


Figure 1: Effect of seed priming on RWC of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. D indicates 15% PEG induced drought stress. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test.

Water is the most important component for the survival of all living organism. Under drought stress condition, water lost from the cell of plants that result in decreased RWC. Leaf RWC is inversely correlated with drought stress (Hasanuzzaman *et al.*, 2013). For evaluating plants for tolerance to drought stress, leaf RWC is considered as an effective parameter. But SA, AsA and NaCl primed seedlings helped in balancing

and increasing RWC in the leaf tissue under drought stress condition. Similar results also observed by Kang *et al.*, (2012); Atreya *et al.*, (2009).

4.6 Oxidative stress markers

4.6.1 Levels of MDA

Drought stress leads to higher accumulation of ROS which disrupts cellular redox homeostasis and result in oxidative damage. Drought stress significantly increased MDA content (the product of lipid peroxidation) which ultimately results in membrane damage. In our study, drought stress increased MDA content by49%compared to controlwhereseed priming decreased MDA content under drought stress condition. Priming with SA, AsA and NaCl decreased MDA content in drought-stressed seedlings by 22, 20 and 19%, respectively(Figure 2).

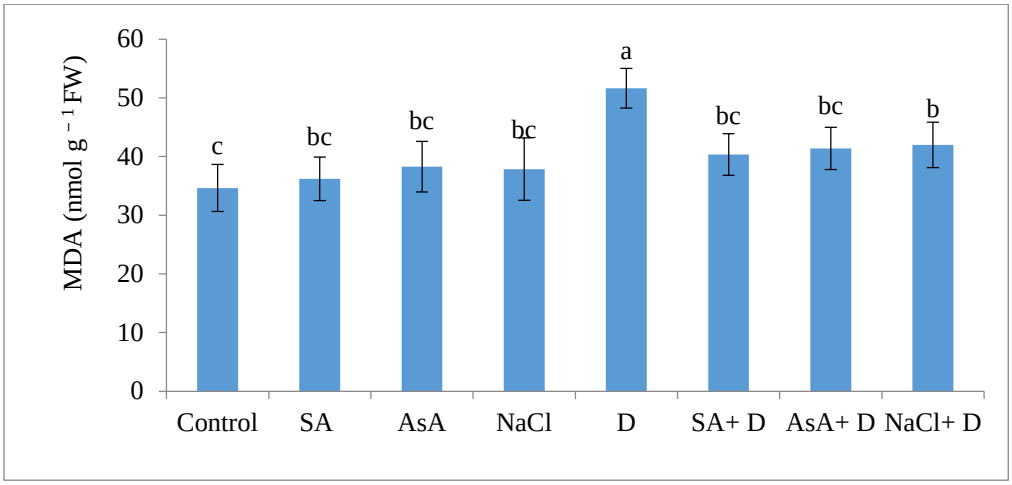


Figure 2: Effect of seed priming on MDA content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment.Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

MDA is a major cytotoxic compound which is produced through lipid peroxidation. MDA act as an indicator of oxidative damage due to increasing production of ROS (Mittler *et al.*, 2010). ROS production increases under various abiotic stresses

including drought stress. In our study, levels of MDA content increased markedly under drought stress condition where decreased levels of MDA content was observed in seedlings which were primed with SA, AsA, and NaCl and grown in drought stress condition.Similar protective effect of SA priming was observed by (Fayez and Bazaid, 2014), AsA priming was observed by (Yan *et al.*, 2015), and NaCl priming was observed by (Jisha and Puthur, 2014). Improving membrane repairing and inducing responses of antioxidant enzymes (SOD, APX) helps to decrease the MDA content, which helps to provides protection against oxidative damage.

4.6.2 Levels of H_2O_2

Increased production of H_2O_2 due to various abiotic stresses including drought stress is also a marker of oxidative damage. Under drought stress condition, the levels of H_2O_2 increased compared to control seedlings. However, seedling grown with SA, AsA and NaCl priming significantly decreased H_2O_2 content as compared to drought induced seedlings (Figure 3).

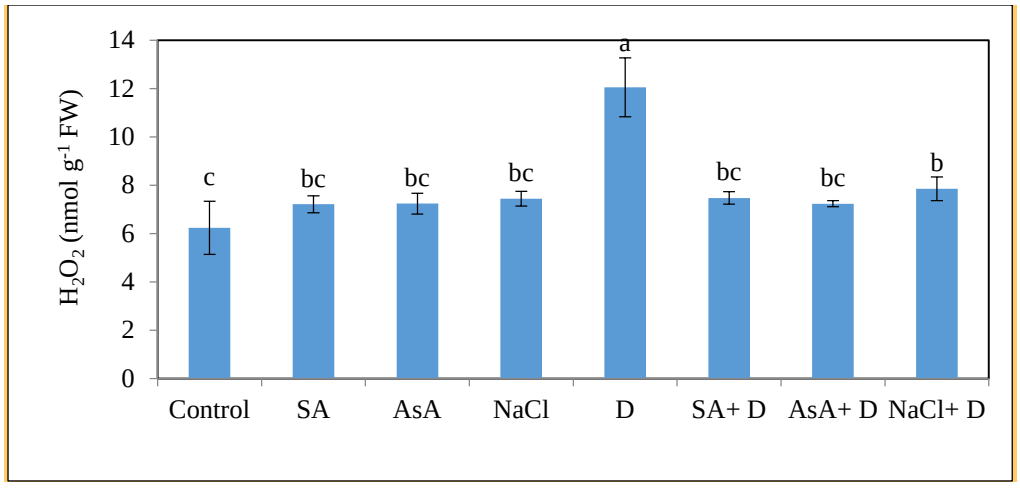


Figure 3: Effect of seed priming on H_2O_2 of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

H_2O_2 is a ROS, which is formed when oxygen acknowledges two electrons. Its metabolism determines formation of exceptionally toxic species; nonetheless, at the physiological concentration it is not dangerous. H_2O_2 converted to cytotoxic HO• by the fenton or Haber–Weiss reactions. H_2O_2 content increased remarkably when plants falls under various abiotic stresses (Tian and Lei, 2007 and Hasanuzzaman *et al.*, 2014). Higher H_2O_2 concentration may cause oxidative stress. In the following experiment, drought stress caused a surprising increase of H_2O_2 which is clear sign for oxidative stress. Seed priming with SA, AsA and NaCl reduced the generation of H_2O_2 in stressed plants by upregualting the enzymes such as APX, CAT and GPX. This result is well agreed with Alam *et al.*, (2013), Farooq *et al.*, (2013) and Ali, (2015).

4.7 Proline content

Proline content was markedly increased in wheat seedlings upon exposure to drought stress compared to control seedlings. Seed priming with SA, AsA and NaCl decline Pro content by89, 88 and 85% respectively under drought stress condition(Figure4).

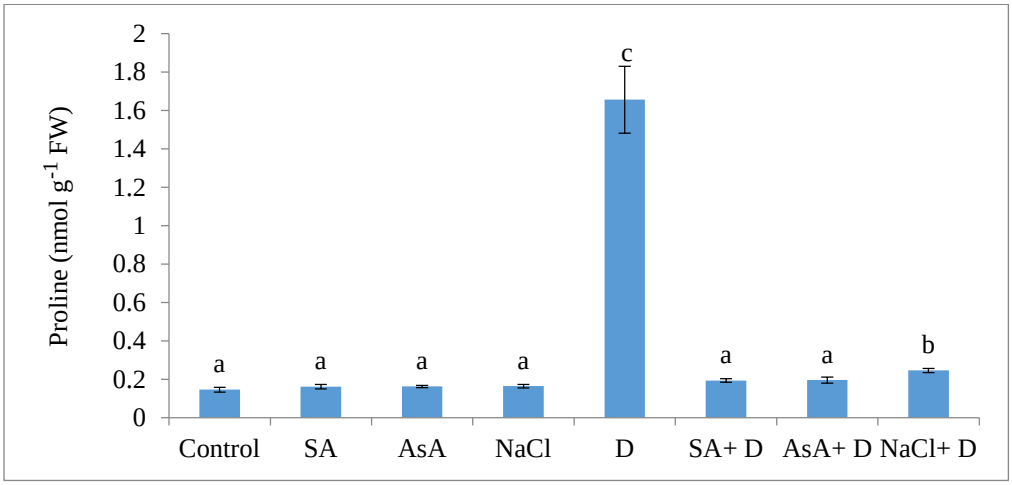


Figure 4: Effect of seed priming on proline content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. D indicates 15% PEG induced drought stress. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

Proline is a most important among different osmolyte which plays a vital role in abiotic stress tolerances (Hasanuzzaman *et al.,* 2012; Nahar *et al.,* 2016). Under stress condition proline interact with macromolecules of cell to maintain their biological activity (Srivastava *et al*., 2010); Proline is well established as an osmotic stress marker and when plants exposed to various abiotic stresses including drought stress accumulation of proline content increases manifold (Alam *et al*., 2013). Although under the following experiment proline content increased under drought stress condition but. Seed priming with SA, AsA, and NaCl under drought stress condition reduced the proline content but increased compared to control. The Similar effect of SA priming AsA priming and NaCl priming under drought stress were found by El tayeba *et al.* (2010); Alam *et al.* (2013); Farooq *et al.* (2013); Razaji *et al.* (2014); Srivastava *et al.* (2010) where they reported that Seed priming with SA, AsA and NaCl reduced proline accumulation under stress conditions.

4.8 Chlorophyll content

Chlorophyll a content of the leaves decreased significantly under drought stress conditions. Compared to drought stress alone, the seedlings with SA, AsA and NaCl showed higher chla content. Under drought stress condition, chl b content also decreased significantly. Consequently, chl (a+b) content significantly decreased under drought stress compared to controls seedlings. However, seedlings exposed to drought stress after priming withSA, AsA, NaCl priming showed significantly higher amount of chl (a+b) content (Table 3).

Table 3 Effect of seed priming on chlorophyll content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. D indicates 15% PEG induced drought stress

Treatment	**Chl a**	**Chl b**	**Chl (a+b)**
Control	0.264±0.02 a	0.264±0.02 a	0.264±0.02 a
SA	0.221±0.016 bc	0.221±0.016 bc	0.221±0.016 bc
AsA	0.240±.006 ab	0.240±.006 ab	0.240±.006 ab
NaCl	0.231±0.013bc	0.231±0.013bc	0.231±0.013bc
Drought (D)	0.185±0.005 d	0.185±0.005 d	0.185±0.005 d
SA+ D	0.209±0.013cd	0.209±0.013cd	0.209±0.013cd
AsA+ D	0.216±0.014 c	0.216±0.014 c	0.216±0.014 c
NaCl+ D	0.215±0.013c	0.215±0.013c	0.215±0.013c

Means (±SD) were calculated from three replications for each treatment. Values with different letters are significantly different at P≤0.05 applying the Fisher's LSD test

Plants produce their own food and later converted this food into energy by the process of photosynthesis. Afterall for survival of plants photosynthesis is the most important factor. In this experiment photosynthetic pigments like chl a, chl b, and total chl content decreased under 15% PEG induced drought stress which is corroborated with the result of previous studies. The possible reason for decreasing chlorophyll content under drought stress might be due to the changes in chloroplast structure (Plesnicar and Lei, 1997) or the increased degradation of chlorophyll pigments due to stress-induced metabolic imbalance (Ashraf *et al.* 1994). However SA, AsA, NaCl primed increased chlorophyll content under PEG- induced drought stress which was similar with the result of previous studies with different plant species like chickpea (Kaur *et al.*, 2002), rice (Li and Zhang, 2012).

4.9 Antioxidant defense system

4.9.1 Ascorbate content

Ascorbate content decreased significantlyunder drought stress condition compared to control. Seed priming with SA, AsA and NaCl increased AsA content under drought stress condition. Priming with AsA showed higher endogenous ascorbate content than other priming agents (SA and NaCl) under drought stress condition(Figure 5).

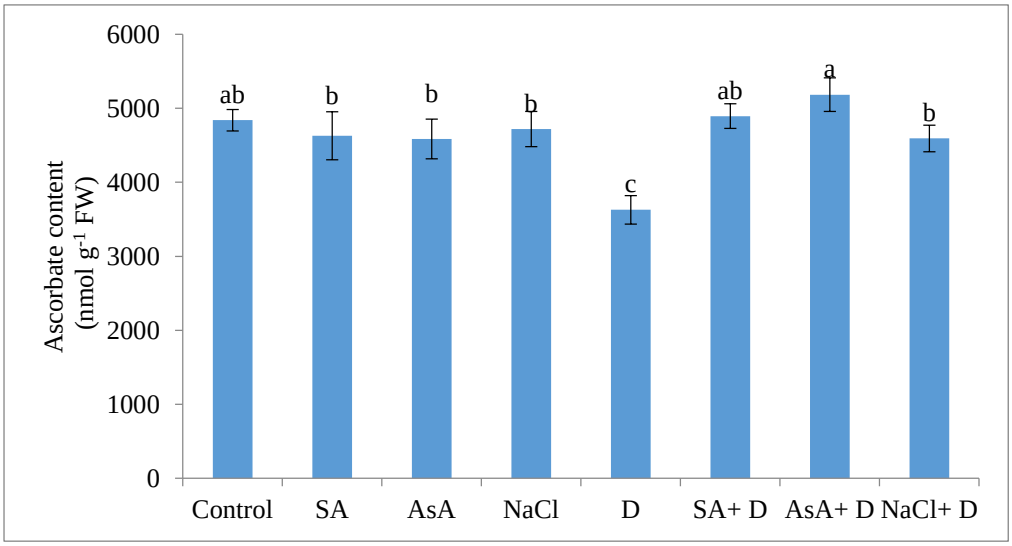


Figure 5: Effect of seed priming on ascorbate content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

4.9.2 GSH content

Compared to control seedlings, GSH content increased by 9% under drought stress.Priming with SA, AsA and NaCl further increased GSH content by20%, 18%, and 17% respectively in drought-stressed wheat seedlings(Figure 6).

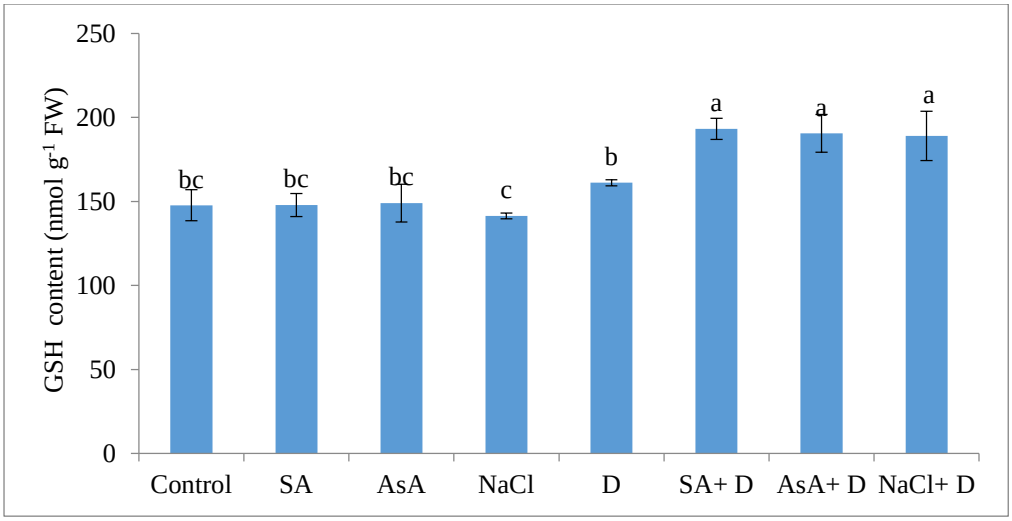


Figure 6: Effect of seed priming on GSH content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

4.9.3 GSSG content

A significant increased in GSSG content (247%) was observed under drought stress condition. In contrary, seed priming (SA, AsA and NaCl) decreased r GSSG level in drought-stressed seedlings (Figure 7).

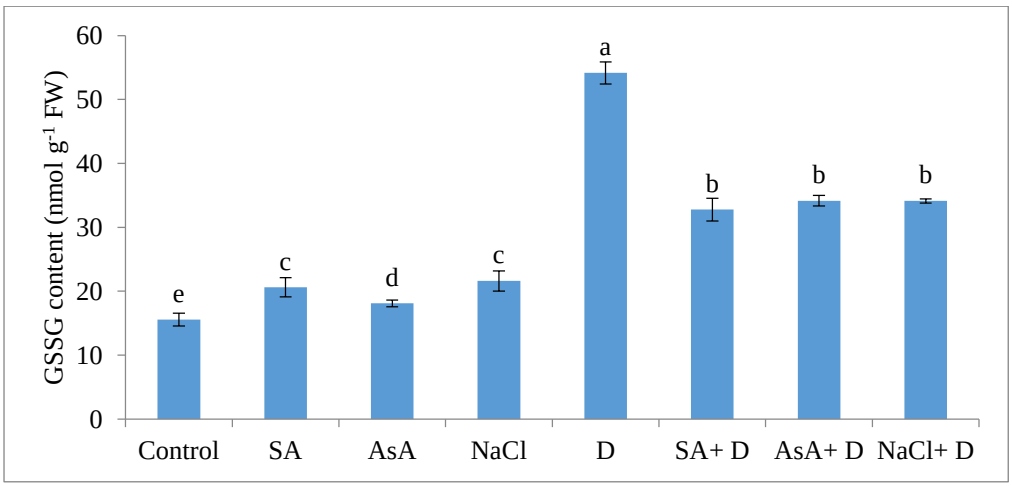


Figure 7: Effect of seed priming on GSSG content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and

2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

4.9.4GSH/ GSSG ratio

The ratio of GSH/GSSG decreased significantly by68% compared to control seedlings. However, seed priming recovered GSH/GSSG ratio in drought-stressed seedlings (Figure 8).

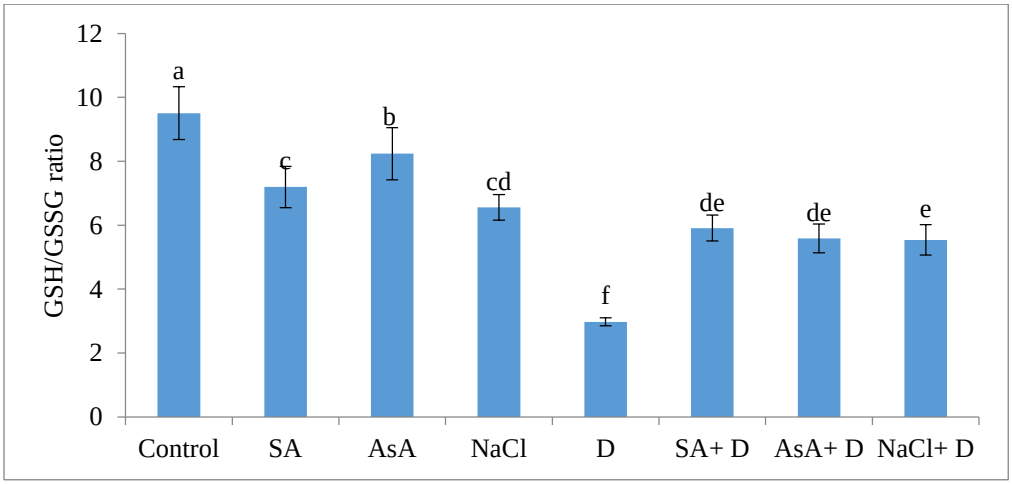


Figure 8: Effect of seed priming on GSH/ GSSGratio of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

GSH is another important nonezymatic antioxidant which involves in the reduction of most ROS (Noctor and Foyer, 1998). GSH also plays a key role by regenerating AsA via AsA- GSH cycle. To scavenge ROS, GSH is a substrate for GPX; as GPX is well known ROS scavenger enzyme (Noctor *et al.*, 2002a). In this study, the endogenous GSH level increased under drought stress in wheat seedlings which in accordance with earlier studies of Alam *et al.* (2013) and Nahar *et al.* (2015b). However seed priming with SA, AsA, NaCl further increased GSH levels which might be possible reason for quenching free radicals that's produced under various abiotic stress condition.

Reduced Glutathione is oxidized to GSSGand increased the level of GSSG during the scavenging reaction of ROS (Asada, 1992; Rahman *et al.*, 2015). The results of this study showed that drought stress markedly increase GSSG content. Similar reports were also found in Alam *et al.* (2013) and Nahar *et al.* (2015). SA, AsA and NaCl primed seedlings decreased GSSG content under drought stress conditions.

GSH/GSSG ratio has immense functions in cell redox potential and stress signaling processes (Forman *et al.*, 2009). Higher GSH/GSSG ratio is an indicator for higher stress-tolerances. Under drought stress condition, the GSH/GSSG ratio was greatly reduced compared with control. However SA, AsA and NaCl primed seedlings under drought stress showed increase GSH/GSSG ratio.

4.9.5 APX activity

Exposure of drought stress increased APX activity by14%control seedlings. Priming with AsA further increased APX activity in drought-stressed seedlings. But Priming with AsA showed higher APX activity compared to two other priming agents under drought stress condition (Figure 9).

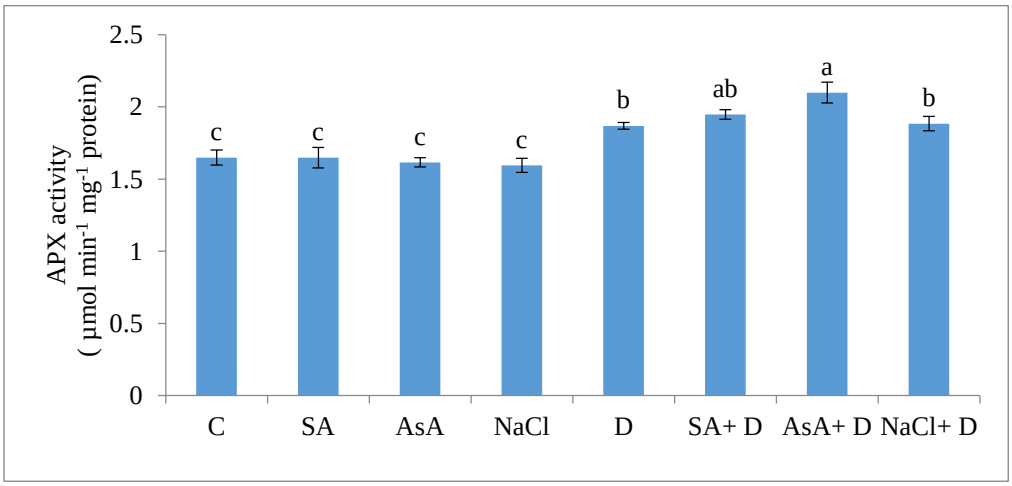


Figure 9: Effect of seed priming on APX activity of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

APX is one of the most important enzymes of AsA-GSH cycle which converts H_2O_2 into water. APX used AsA as a reducing equivalent during conversion of H_2O_2 into water. In this study, under drought stress APX activity increased compared to control seedlings. Similar trends were also observed by Alam *et al.* (2013) and Nahar *et al.* (2016). APX activity increased under drought stress might be due to higher H_2O_2 and lower AsA content. However APX activity further increased in SA, AsA and NaCl primed drought stressed seedlings. These results are supported by Nazar *et al.* (2011), Hameed *et al.* (2013) and Islam *et al.* (2015).

4.9.5 MDHAR activity

MDHAR activity decreased by 80% after exposing the wheat seedlings to drought stress, compared with control but seed priming with SA, AsA and NaCl increased MDHAR activity under drought stress condition (Figure 10).

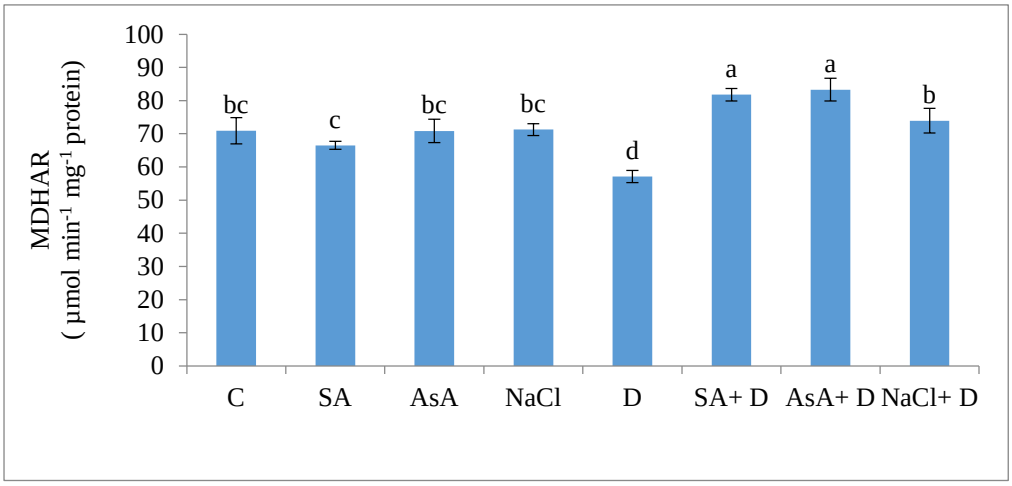


Figure 10: Effect of seed priming on MDHAR content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

4.9.6 DHAR activity

To adjust the level of AsA and its redox state under stress condition, DHAR is an equally important enzyme as MDHAR (Eltayeb *et al.*, 2007). Decreased activity of DHAR was observed under drought stress condition compared with controls. Seed priming with SA, AsA and NaCl showed a significant increased (16%, 14%, and 13% respectively) under drought stress condition (Figure 11).

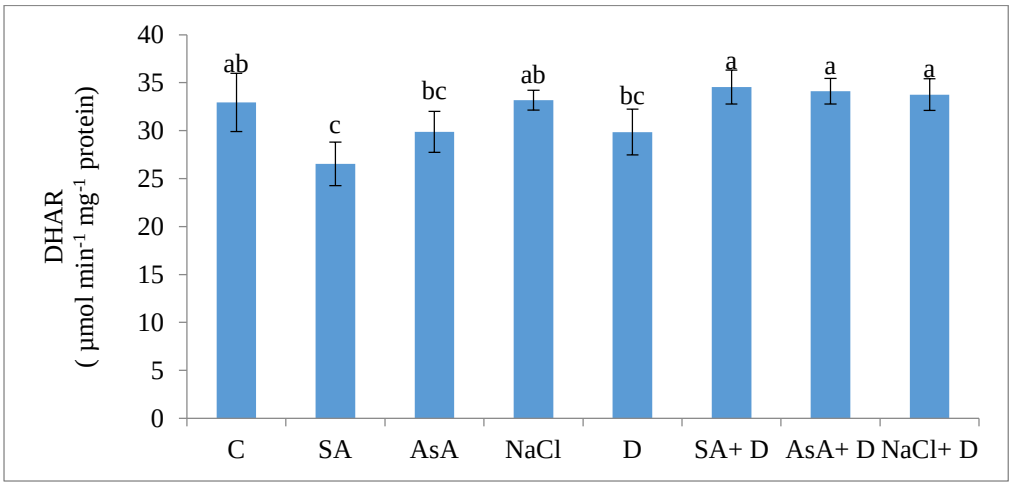


Figure 11: Effect of seed priming on DHAR content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively. Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

4.9.7 GR activity

The seedlings exposed to drought stress reduced GR activitycompared to control seedlings. Comapare with control, seed priming also decreased GR acivity in wheat seedlings. Importantly, under drought stress condition, seed priming with SA, AsA, NaCl upregulated GR acivity by 18%, 15% and 13%, respectively, compared to drought stress alone (Figure 12).

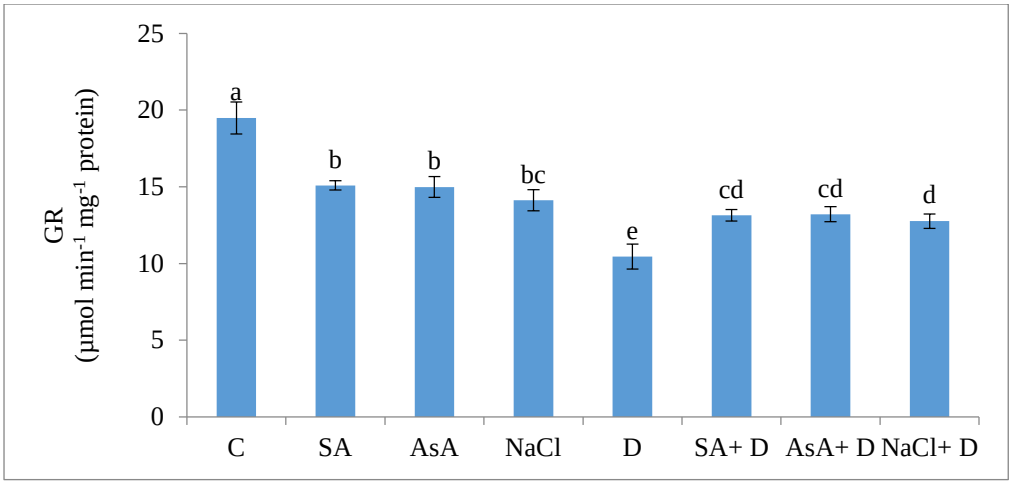


Figure 12: Effect of seed priming on GR activity of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

4.9.8 CAT activity

Catalase is considered as a vital enzyme for ROS detoxification. The present study resultedin a decreased CAT activity under drought stress condition comparedto control seedlings. Seed priming with SA, AsA and NaCl increased CAT activity under drought stress condition compared to drought stress alone (Figure 13).

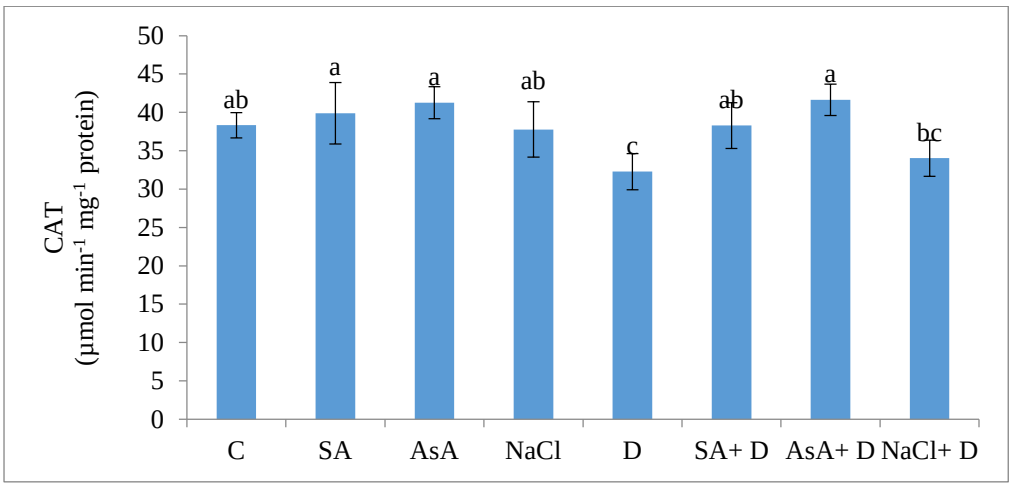


Figure 13: Effect of seed priming on CAT activity of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and

2.5 mM NaCl respectively. Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

Catalase is one of the major enzymes that convert excess H_2O_2 into water. Several earlier reports suggested that higher activity of this enzyme helps to reduce excess level of H_2O_2 (Hasanuzzaman *et al.*, 2012). In this study, under exposure to drought stress CAT activity decreased. Increased production of H_2O_2 or ineffective synthesis of enzymeunder drought stress may result decreased activity of CAT (Zhang and Kirkham, 1994; Gupta *et al.*, 2009). However SA, AsA and NaCl seeds primed drought stressed seedlings showed enhance CAT activity than those under drought stress without seed priming. This trend was supported by Ahamad *et al.*(2012), Azooz, (2009) and Yan *et al.*(2015) who reported that SA, AsA and NaCl priming enchance the CAT activity under abiotic stress conditions.

4.9.9 GPX activity: Compared with the control seedlings GPX activity decreased in the drought stressed seedlings by 34%. However seedlings which were primed with SA, AsA, and NaCl showed an increase in GPX activity by 32%, 24%, and 16%, respectively, under drought stress condition (Figure 14).

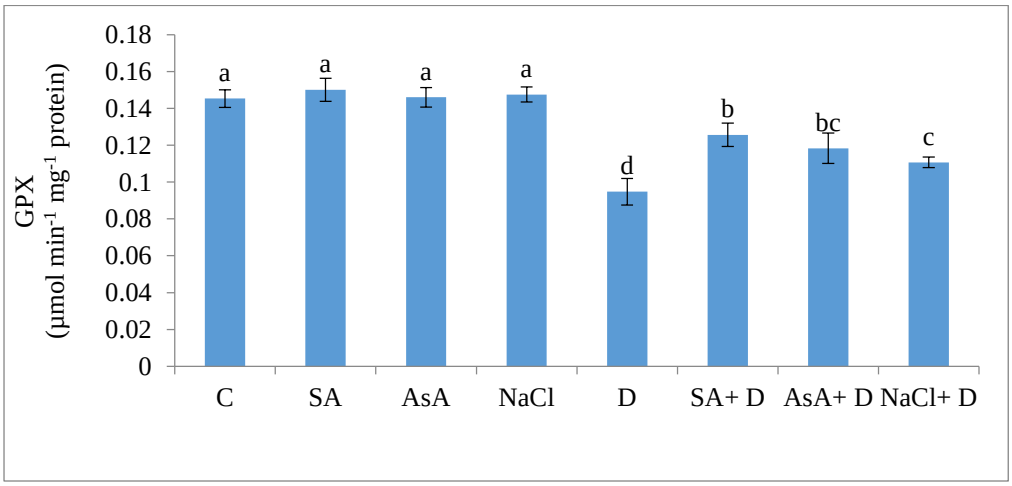


Figure 14: Effect of seed priming on GPX activity of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and

2.5 mM NaCl respectively. Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

To protect plants from oxidative stress GPX plays crucial role by utilizing GSH as a substrate to reduce H_2O_2 and lipid hydroperoxide (Noctor *et al.*, 2002). In the present study, under drought stress generation of H_2O_2 may decrease GPX activity compared to control seedlings. It is consistent with Alam *et al.* (2013), who reported that drought stress decreased GPX activity. Seed priming with SA, AsA and NaCl increased GPX activity in the drought stressed seedlings. This finding is an agreement with Singh and Bhardwaj, (2016), Gowsami *et al.* (2013) who showed that AsA and NaCl priming increased GPX activity in drought stressed seedlings.

4.10 Glyoxalase system and methylglyoxal detoxification

The Glyoxalase system, comprising the Gly I and Gly II enzymes, effectively eliminates cytotoxic MG**.**

4.10.1 Methylglyoxal (MG) content

Exposure of drought stress significantly increased MG content by 94% compared to control seedlings. However, seed priming with SA, AsA and NaCl significantly decreased the MG content under drought stress condition (Figure 15).

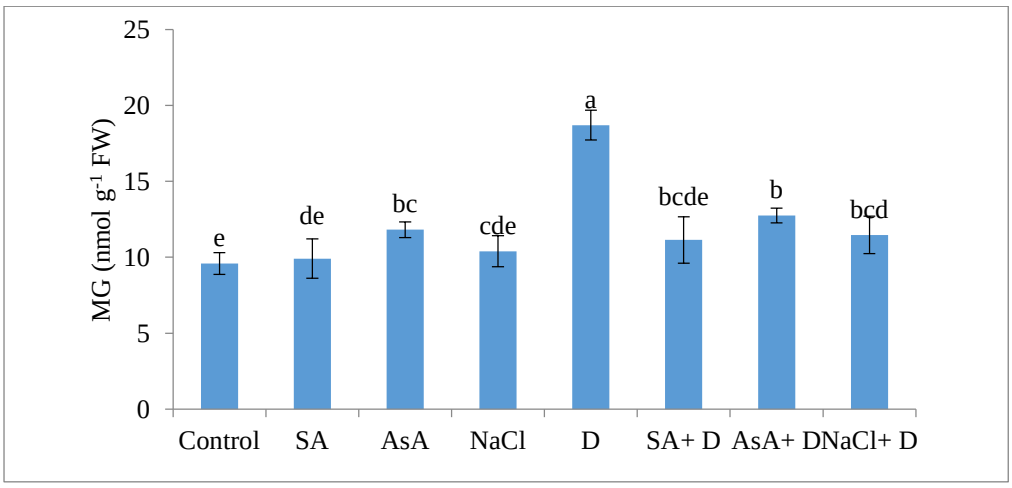


Figure 15: Effect of seed priming on MG content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

4.10.2 Gly I

The activity ofGly I decreased by 46% under drought stress condition compared with control seedlings. However, seed priming with SA, AsA and NaCl increased Gly I activity 50%, 35%, and 34%, respectively compared to drought stressalone (Figure 16).

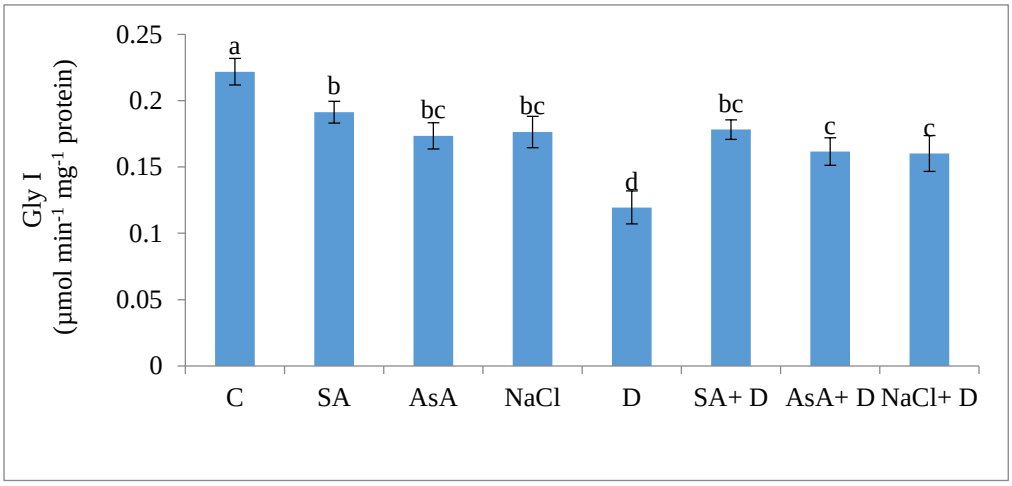


Figure 16: Effect of seed priming on Gly I content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

4.10.3 Gly II

Comparing with the control seedlings, the activity of Gly II decreased by 39% under drought stress condition.Exposure of wheat seedlings to drought stress after priming with SA, AsA and NaCl increased Gly II activity by16%, 25%, and 23% respectively compared todrought stress alone (Figure 17).

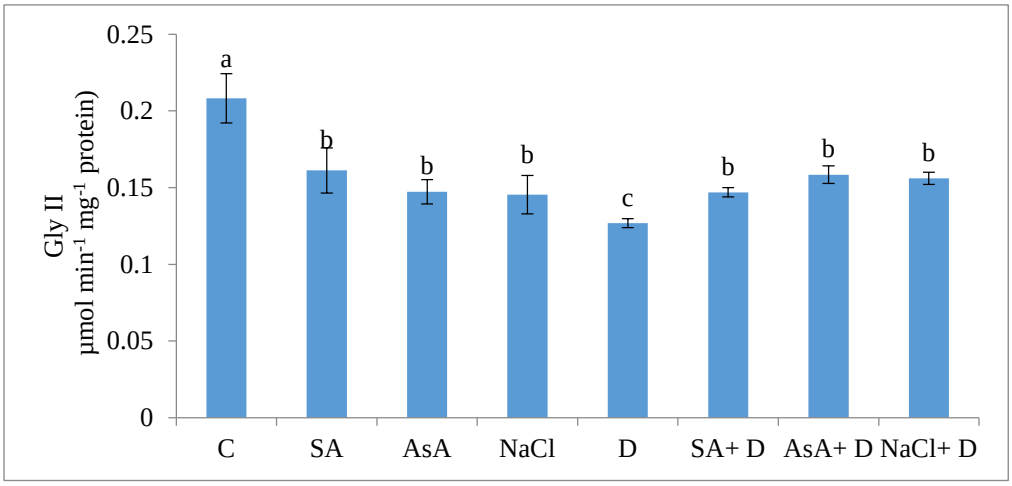


Figure 17: Effect of seed priming on Gly II content of wheat seedlings under drought and control condition. Here SA, AsA and NaCl indicate 50 µM SA, 4mM AsA and 2.5 mM NaCl respectively Mean (SD) was calculated from three replicates for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

Under abiotic stress condition cytotoxic MG production aggrevated which can enchance the ROS production. Rapid accumulation of endogenous MG has been observed in plants under various abiotic and biotic stresses condition. MG detoxification is one of the potential systems for actuating tolerance in plants against various abiotic and biotic stresses (Yadav *et al.,* 2005a; Rahman *et al.,* 2016; Hasanuzzaman *et al.,* 2011b; Nahar *et al.,* 2016). To detoxify MG, Glyoxalase system plays pivotal roles which is includes Gly I and Gly II enzymes. Upregulation of Gly I and Gly II can limit the over accumulation of MG. In our study, it was observed that drought stress increased MG production with a decreased activity of Gly I and Gly II.

Similar results were reported by Alam *et al.* (2013) and Hasanuzzaman *et al.*(2011). However, Seed priming with SA, AsA and NaCl considerably enhanced the activities of Gly I and Gly II under drought stress condition. Enhanced Gly I and Gly II activities detoxify the elevated level of MG.

4.11 Histochemical detection of H_2O_2 and $O_2^{\bullet-}$ generation

Leaves from each treatment were subjected to staining by DAB and NBT to see the generation of ROS. DAB staining resulted in brown spot of H_2O_2 and NBT staining resulted in dark blue spots of $O_2^{\bullet-}$. Drought stress increased H_2O_2 and $O_2^{\bullet-}$ generation in the leaves compared with control. Seed priming with SA, AsA and NaCl decreased the spots on the leaves produced by H_2O_2 and $O_2^{\bullet-}$ underdrought stress condition, indicating a reduction in ROS generation compared with drought stress alone (Plate 2).

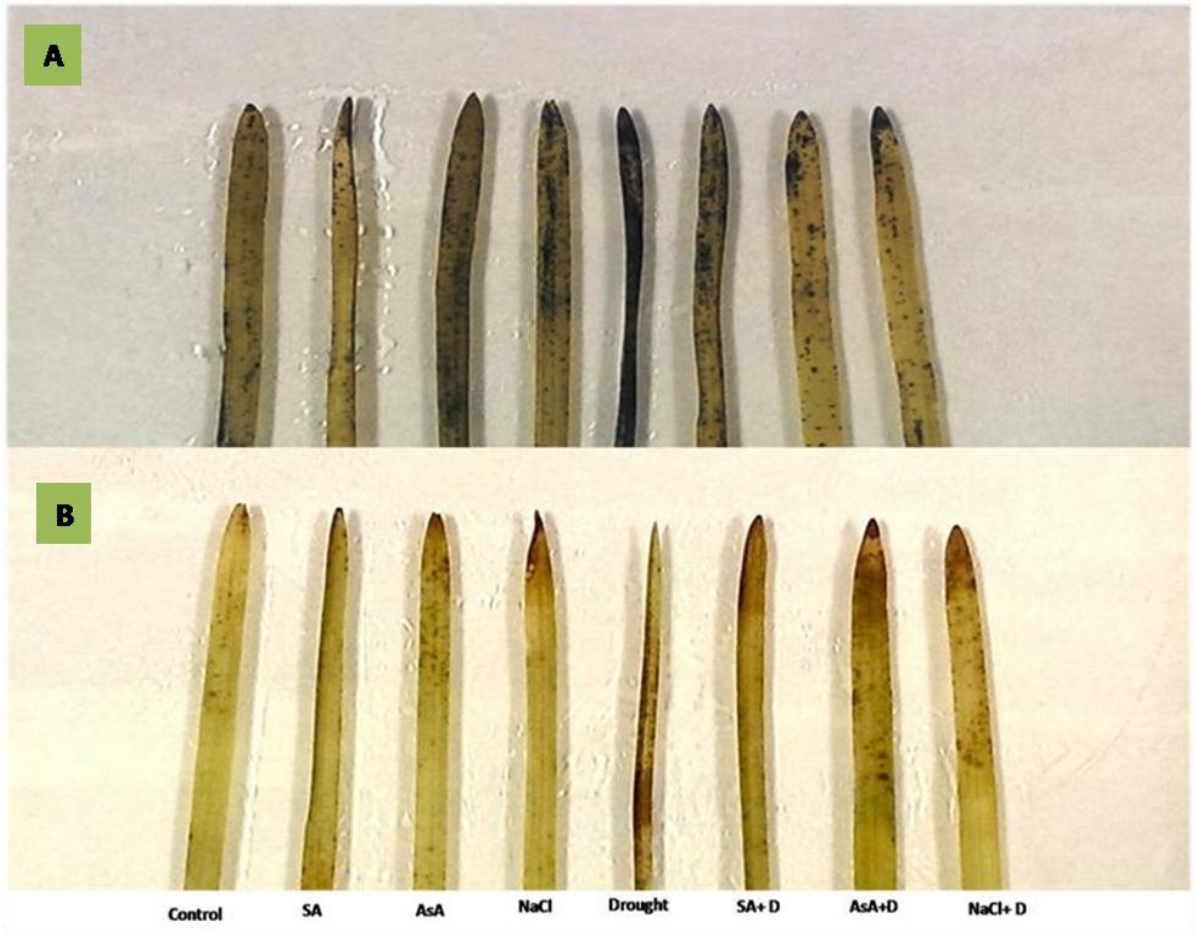


Plate 2. Histochemical detection of (A) superoxide ($O_2^{\bullet-}$) and (B) hydrogen peroxide (H_2O_2) in the leaves of wheat seedlings under drought stress

CHAPTER V
SUMMARY AND CONCLUSION

Exposure of drought stress caused germination reduction, growth inhibition, chlorosis and reduction of water content. Drought stress also induced oxidative damages by disrupting antioxidant defese system through overproduction of ROS. Glyoxalase system also disrupted due todrought stress followed by higher production of MG, which induced oxidative stress. However, priming with SA, AsA and NaCl recovered germination reduction, growth inhibition and water loss under drought stress condition. Seed priming alos confer drought stress tolerance by reducing ROS and MG production through upregulationg antioxidant and glyoxalase system, respectively. Priming with SA showed better result in recovering germination loss in terms of germination percentage and vigour index compared with other priming agents (AsA and NaCl). In contrast, conferring in drought stress tolerance AsA showed better result than other priming agents (SA and NaCl) by reducing ROS production and lipid peroxidation through upregulating antioxidant defense and glyoxalase system.

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APPENDICES

Appendix I.Mean square values of germination %, vigour index, coefficient of velocity, plant height,fresh weight,dry weightof BARI Wheat-30 seedlings as influenced by seed priming with SA, AsA and NaCl under drought stress condition

Source of variation	df	Mean square value					
		Germination (%)	Vigour index	Coefficient of velocity	Plant height	Fresh weight	Dry weight
Treatment	7	614.381	19.835	223.547	7.269	0.001	0.001
Error	16	3.583	0.500	6.904	0.127	0.009	0.001

Appendix II.Mean square values of RWC (%), Chl *a*, Chl *b*and Chl *(a+b)*, MDA, H_2O_2, Prolineof BARI Wheat-30 seedlingsas influenced by seed priming with SA, AsA and NaCl under drought stress condition

Source of variation	df	Mean square value						
		RWC	Chl a	Chl b	Chl (a+b)	MDA	H_2O_2	Proline
Treatment	7	125.099	0.002	0.001	0.002	82.015	8.698	0.819
Error	16	1.993	0.001	0.001	0.000	16.009	0.316	0.004

Appendix III.Mean square values of MG, Gly I, Gly II, AsA, GSH, GSSG, GSH/GSSG of BARI Wheat-30 seedlings as influenced by seed priming with SA, AsA and NaCl under drought stress condition

Source of variation	df	Mean square value of						
		MG	Gly I	Gly II	AsA	GSH	GSSG	GSH/GSSG
Treatments	7	25.386	0.003	0.002	888.285	1477.94	481.21	11.591
Error	16	1.070	0.000	0.001	592.427	80.879	1.638	0.318

Appendix IV.Mean square values of APX, MDHAR, DHAR, GR, CAT, GPXof BARI Wheat-30 seedlings as influenced by seed priming with SA, AsA and NaCl under drought stress condition

Source of variation	df	Mean square value of					
		APX	MDHAR	DHAR	GR	CAT	GPX
Treatments	7	0.079	110.477	23.545	20.358	32.609	0.001
Error	16	0.012	8.177	4.157	0.422	7.548	0.009