EFFECT OF SALICYLIC ACID ON GERMINATION, GROWTH, PHYSIOLOGY, YIELD AND ANTIOXIDANT DEFENSE OF WHEAT UNDER SALINITY

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CERTIFICATE

This is to certify that thesis entitled, "EFFECT OF SALICYLIC ACID ON GERMINATION, GROWTH, PHYSIOLOGY, YIELD AND ANTIOXIDANT DEFENSE OF WHEAT UNDER SALINITY" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (MS) IN AGRONOMY, embodies the result of a piece of bona-fide research work carried out by JANNATUL FARDUS, Registration no. 09-03311 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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ABSTRACT

A pot experiment was conducted at the experimental shed of the Department of Agronomy, Sher-e Bangla Agricultural University, Bangladesh during winter season (2013-2014) with a view to find out the regulatory roles of exogenous salicylic acid (SA) in growth, yield and antioxidant defense systems of wheat under different salt stress condition. The experiment was carried out with two varieties i.e. BARI Gom 21 and BARI Gom 25 and ten salt stress treatments viz. control (without salt), SA (1 mM salicylic acid), S50 (50 mM salt stress), S50+SA (50 mM salt stress with 1 mM SA), S100 (100 mM salt stress), S100+SA (100 mM salt stress with 1 mM SA), S150 (150 mM salt stress), S150+SA (150 mM salt stress with 1 mM SA), S200 (200 mM salt stress) and S200+SA (200 mM salt stress with 1 mM SA).Seed germination percentage, number of normal seedling, length of shoot and root, fresh weight of shoot and root and dry weight were decreased under the stress condition but the number of abnormal seedling increased. Salt stresses significantly reduced the plant height, tiller hill^{-1}, fresh weight and dry weight of both varieties at all growth duration. Leaf relative water content (RWC) and chlorophyll (chl) content also reduced due to salt stress. The malondialdelyde (MDA) and H_2O_2 were increased under the stress condition. The ascorbate (AsA) content, reduced glutathione (GSH) and GSH/GSSG ratio were reduced by salt stresses (50, 100, 150 and 200 mM, respectively). But the glutathione disulfide (GSSG) amount increased with an increase in the all level of salinity. The ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and catalase (CAT) activities showed a significant reduction in response to salt stress but CAT increased only at 100 mM stress condition. The glutathione *S*-transferase (GST) and glutathione reductase (GR) activity increased significantly with severe salt stress (200 mM). But the activity of peroxidase (POD) was decreased with increasing salinity level. At harvest, salt stresses reduced the effective tiller hill^{-1}, 1000 grain weight, grain yield, straw yield and harvest index for both of varieties. However, number of non-effective tiller hill^{-1} significantly increased in response of salt stress. Exogenous 1 mM SA application with salt stress improved germination, crop growth parameters, physiological parameters, reduced oxidative damage and yield in both cultivars where BARI Gom 25 showed better tolerance. But, SA application could not improve germination, crop growth parameters, physiological parameters and yield at extreme level of salt stress (200 mM).

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LIST OF ABBREVIATIONS

AO	Ascorbate oxidase
APX	Ascorbate peroxidase
AsA	Ascorbic acid (ascorbate)
BARI	Bangladesh Agricultural Research Institute
CAT	Catalase
Chl	Chlorophyll
DHA	Dehydroascorbate
DHAR	Dehydroascorbate reductase
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione *S*-transferase
MDA	Malondialdehyde
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate reductase
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SRDI	Soil Resource Development Institute
USDA	United States Department of Agriculture

Chapter 1

INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to the family Poaceae (Gramineae) is the second largest cereal crop next to rice in Bangladesh. During the year 2014-2015, 1.3 million metric tons of wheat was produced from 0.42 million hectares of land with an average yield of 3.1t ha^{-1} in the country (USDA, 2015). It has more salt tolerance ability than rice.

Crop plants, as sessile organisms, encounter unavoidable abiotic stresses during their life cycles, including salinity, drought, extreme temperatures, metal toxicity, flooding, UV-B radiation, ozone, etc., which all pose serious challenges to plant growth, metabolism, and productivity (Hasanuzzaman *et al.*, 2012, 2013, 2014). From the abiotic stresses, salt stress is a major environmental threat to agriculture, and its adverse impacts are getting more serious problems in regions where saline water is used for irrigation (Türkan and Demiral, 2009). Therefore, efforts to increase the salt tolerance of crop plants are very important to ensure global food security, as well as for water and land conservation. A high salt concentration in the soil or in irrigation water can have a devastating effect on plant metabolism; that is, it can result in the disruption of cellular homeostasis and uncoupling of major physiological and biochemical processes. Plants can respond and adapt to salt stress by altering their cellular metabolism and invoking various defense mechanisms (Ghosh *et al.*, 2011). The survival of plants under this stressful condition depends on their abilities to perceive the stimulus, generate and transmit a signal, and initiate various physiological and biochemical changes (Tanou *et al.*, 2009a; El-Shabrawi *et al.*, 2010). Molecular and biochemical studies of the salt stress responses of plants have demonstrated significant increases in reactive oxygen species (ROS) such as, singlet oxygen ($1O_2$), superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) (Mittler, 2002; Tanou *et al.*, 2009a; Pérez-López *et al.*, 2010).

Presence of excess soluble salt in soil is one of the major factors that reduces the growth and development of cultivated crop plant in coastal areas of Bangladesh. Salts primarily

have two types of effects on the growing plants, specific effect due to rising of osmotic pressure of the soil solution in and around the root regime of the crop. In the long run prolonged transpiration bring old leaves that causes its senescence. This process eventually limits the supply of assimilates to growing parts and limits yields of the crop. It has been reported that there are some plant that have their capability of developing adaptive mechanism to salinity (Flower *et al.*, 1977; Greenway and Munns, 1980) which in turn induces the plant to have better growth and yield under saline conditions.

The present population of Bangladesh is around 160 million. Bangladesh will have to grow food for an estimated 201 million people by 2050 (Worldometers, 2015). Owing to population pressure, the cultivable area is decreasing day by day, and this problem will gradually but soon be acute. Food shortage and land scarcity are driving. Asian Countries need to make an attempt to grow food crops on land that has been unutilized because of soil problems.

One of the most common soil problems is the salinity. Worldwide, around 17% of the cultivated land is under irrigation and irrigated agriculture contributes more than 30% of the total agricultural production (Hillel, 2000). It is estimated that at least 20% of total irrigated lands in the world is salt-affected (Pitman and Läuchli, 2002). In Bangladesh, more than 30% of the cultivable land is in the coastal area. Out of 2.86 million hectares (ha) of coastal and off-shore lands about 1.056 million ha of arable lands are affected by varying degrees of salinity (SRDI, 2010). In the last three decades about 170,000 ha of agriculture land has been degraded by increased salinity (Ministry of Agriculture and FAO, 2011).The reasons for salinity in Bangladesh are: a) intrusion of sea water due to river drying in the winter, b) cyclone in the coastal area and c) influx of salts from the deep and around to the surface through capillary movement during the dry season. The problem of salinity is severe in the winter though during summer the salt concentration decreases dramatically due to monsoon rains. Cropping intensity in saline area of Bangladesh is relatively low, mostly 170% (FAO, 2007). To feed the millions of people of Bangladesh food production must be increased in these areas.

Rice is the main crop in the saline area of Bangladesh. Some other crops like wheat, maize, mustard, barley, cotton, onion, beans are also being grown in the saline soil. The acreage and production of wheat in saline area is low. Wheat, ranking the second major cereal crop in Bangladesh, still is a minor crop in saline prone area. In order to increase cropping intensity of saline area and to increase food grain production in country, wheat could be fitted in the cropping pattern.

Now is the right time to be strategic: first by understanding the reasons – fundamental to complex – for yield reductions so that precise research planning can be brought about to cope with increasing salinity problems. With that view, plant scientists are now searching for ways to make the plants adaptive under saline conditions. Researchers are trying to understand the effects of salt stress on plants so that they can modify the plant's external growing condition as well as change the plant from within by applying different exogenous protectants including trace elements and phytohormones by molecular mechanisms.

Salicylic acid (SA) is a common plant-produced phenolic compound and a potential endogenous plant hormone that plays an important role in plant growth and development (Hasanuzzaman *et al.*, 2014). The role of SA is intensively studied in plant responses to biotic stress. In recent years, the involvement of SA in the response to abiotic stresses has come into light. Several studies support a major role of SA in plant adaptation to the changing environment, and induce plant tolerance to various abiotic stresses including elevated NaCl (Stevens *et al.*, 2006; Arfan *et al.*, 2007; Gunes *et al.*, 2007). It is a well observed fact that SA potentially generates a wide array of metabolic responses in plants and also affects plant water relations (Hayat *et al.*, 2010).

However, the response of plants to salt stress varies among the crop varieties and the dose and duration of stress. In addition, the role of exogenous protectants also variable in such conditions. Although there are several studies on the effect of salt stress on wheat but there is hardly any study regarding the role of exogenous protectants in mitigating salt stress in wheat. This study was designed to understand the physiological mechanisms of

salt stress tolerance mediated by exogenous SA on two high yielding wheat varieties such as BARI Gom 21 and a tolerant variety BARI Gom 25 which were grown in saline condition. Therefore, the present study was undertaken keeping in mind the following objectives:

i. To investigate the effect of salinity on the growth, physiology and yield of wheat.
ii. To understand the role of exogenous salicylic acid in mitigating salt stress
iii. To understand the biochemical mechanism of salt stress tolerance in wheat.

Chapter 2

REVIEW OF LITERATURE

2.1 Wheat

Wheat (*Triticum aestivum L.*) was a key factor enabling the emergence of city-based societies at the start of civilization because it was one of the first crops that could be easily cultivated on a large scale, and had the additional advantage of yielding a harvest that provides long-term storage of food. Wheat contributes to the emergence of city-states in the Fertile Crescent, including Babylonian and Assyrian empires. Wheat grain is a staple food used to make flour for leavened, flat and steamed breads, biscuits, cookies, cakes, breakfast cereal, pasta, noodles, couscous and for fermentation to make beer, other alcoholic beverages, or biofuel (Davies and Evans, 2009). Wheat is the most important cereal crop for the majority of world's populations. It is the most important staple food of about two billion people (35% of the world population). Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman and Graur, 1995). Wheat is a staple food in many parts of the world. Wheat is an annual grass of Poaceae family that can be grown in areas at sea level to altitudes over 3000m. It prefers a habitat with well-drained, clay-loam soils and with a temperate, arid or semi-arid environment (Wiese, 1977). Most plants grow up to about 1 meter in length and have more than two-thirds of their fibrous roots within 20 cm of the soil surface. However, certain species may reach up to two meters in length (Wiese, 1977). Wheat ranks third in the world's grain production (FAOStat, 2013) and accounts for more than 20% of the food calories consumed by human (USDA, 2014). This crop can be grown throughout temperate, Mediterranean and sub-tropical regions of the world. Wheat is the staple food of traditional farming communities throughout the Atlantic coast of Europe to the Northern parts of the Indian subcontinent and from Scandinavia and Russia to Egypt (Perrino *et al.*, 1995). Wheat is the primary and the cheapest source of protein and calories for the population (Anjum and Walker, 1991). Its portentous portion named as gluten, which assists to convert it into a variety of popular baked products.

2.2 Abiotic stress

World agriculture is facing a lot of challenges like producing 70% more food for an additional 9.7 billion people by 2050 while at the same time fighting with poverty and hunger, consuming scarce natural resources more efficiently and adapting to climate change (Wilmoth, 2015). However, the productivity of crops is not increasing in parallel with the food demand. The lower productivity in most of the cases is attributed to various abiotic stresses. Curtailing crop losses due to various environmental stressors is a major area of concern to cope with the increasing food requirements (Shanker and Venkateswarlu, 2011). The complex nature of the environment, along with its unpredictable conditions and global climate change, are increasing gradually, which is creating a more adverse situation (Mittler and Blumwald, 2010). Plants can experience abiotic stress resulting from the high concentrations of toxic or antagonistic substance. In some cases, such as the supply of water, too little (drought) or too much flood can both impose stress on plants. Abiotic stresses modify plant metabolism leading to harmful effects on growth, development and productivity. If the stress becomes very high and/or continues for an extended period it may lead to an intolerable metabolic load on cells, reducing growth, and in severe cases, result in plant death (Hasanuzzaman *et al.*, 2012a, b).

Plant stress may vary depending on the types of stressor and on the prevailing period. In nature, plants may not be completely free from abiotic stresses. They are expected to experience some degree of stress by any factor(s). Some environmental factors, such as air temperature, can become stressful in just a few minutes; others, such as soil water content, may take days to weeks, and factors such as mineral deficiencies can take months to become stressful (Taiz and Zeiger, 2006).

According to Araus *et al.* (2002) abiotic stresses not only limits crop productivity, but also influence the distribution of plant species in different types of environment. Wang *et al.* (2003) quoted that temperatures could rise by another 3-9^{0}C by the end of the century with far-reaching effects. Increased drought and salinization of arable land are expected to have devastating global effects. There is also growing evidence that all of these

stresses are inter connected, for instance during drought stress, plant also suffers nutrient deficiency as most of the nutrients in the soil are available to plant when dissolved in water. In case of heat stress drought stress occurred simultaneously. Ahmad and Prasad (2012) reported that abiotic stress cause changes in soil-plant-atmosphere continuum which is responsible for reduced yield in several of the major crops in different parts of the world. Abiotic stresses like heavy metals, drought, salt, low temperature, etc. are the major factors that limit crop productivity and yield. These stresses are associated with production of certain deleterious chemical entities called reactive oxygen species (ROS), which include hydrogen peroxide (H_2O_2), superoxide radical (O^{2-}), hydroxyl radical (OH^-), etc. (Choudhury *et al.*, 2013). In their review, Macedo (2012) concluded that plant abiotic stress has been a matter of concern for the maintenance of human life on earth and especially for the world economy. In their review, Keunen *et al.* (2013) concluded that plants suffering from abiotic stress are commonly facing an enhanced accumulation of reactive oxygen species (ROS) with damaging as well as signaling effects at organellar and cellular levels. The outcome of an environmental challenge highly depends on the delicate balance between ROS production and scavenging by both metabolic and enzymatic antioxidants. To meet these challenges, genes, transcripts, proteins, and metabolites that control the architecture and/or stress resistance of crop plants in a wide range of environments will need to be identified, in order to facilitate the biotechnological improvement of crop productivity.

The crop losses due to abiotic stress are estimated by many researchers. As per the report of Bray *et al.* (2000), abiotic stress is already the primary reason of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. Some recent reports showed that the major abiotic stresses negatively influence the survival, biomass production and yields of staple food crops up to 70% (Thakur *et al.*, 2010). However the loss due to abiotic stresses has been predicted to become even more severe as desertification will further increase and the current amount of annual loss of arable area may double by the end of the century because of global warming (Evans, 2005; Vinocur and Altman, 2005). Although all of the abiotic stresses which are devastating for crop production, dehydration stress imparted by drought, salinity and temperature severity has

been reported as the most prevalent abiotic stress that limits plant growth and productivity (Jaleel *et al.*, 2009; Thakur *et al.*, 2010). Collins *et al.* (2008) reported that the tolerance to abiotic stress is multigenic and quantitative in nature and thus a massive challenge exists to understand the key molecular mechanisms for advanced selective breeding purposes. Similarly, Patakas (2012) reported that the understanding abiotic stress responses in plants is difficult due to the complexity, interrelationship, and variability of mechanisms and molecules involved a fact that consist their evaluation an important and challenging topic in plant research. Mantri *et al.* (2012) also reported that the yield of food crops worldwide become reduced severely because of drought, cold, high-salinity and heat which are major abiotic stresses. Traditional plant breeding approaches to improve abiotic stress tolerance of crops had limited success due to multigenic nature of stress tolerance.

2.3 Salt stress

Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil. A considerable amount of land in the world is affected by salinity which is increasing day by day. More than 45 million hectares (M ha) of irrigated land which account to 20% of total land have been damaged by salt worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in the soil (Pitman and Läuchli, 2002; Munns and Tester, 2008). On the other hand, increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% loss of cultivable lands by the middle of the twenty- first century (Mahajan and Tuteja, 2005).

Most of Bangladesh's coastal region lies on the southwest coastal region of the country. Approximately 30% of the crops land of Bangladesh is located in this region (Mondal *et al.*, 2001) and continuous to support crops productivity and GDP growth. But in the recent past, the contribution of crops to GDP has decreased because of salinity. In total, 52.8% of the cultivable land in the coastal region of Bangladesh was affected by salinity in 1990 (Karim *et al.*, 1990) and the salt affected area has increased by 14600 ha per year

(SRDI, 2001). SRDI had made a comparative study of the salt affected area between 1973 to 2009 and showed that about 0.223 million ha (26.7%) of new land has been affected by varying degrees of salinity during the last four decades and that has badly hampered the agro-biodiversity (SRDI, 2010). Farmers mostly cultivate low yielding, traditional rice varieties. Most of the land kept fallow in the summer or pre-monsoon hot season (March-early June) and autumn or post-monsoon season (October- February) because of soil salinity, lack of god quality irrigation water and late draining condition. In the recent past, with the changing degree of salinity of southwest coastal region of Bangladesh, crop production becomes very risky and crop yields, cropping intensity, production levels of crop and people's quality of livelihood are much lower than that in the other parts of the country. Cropping intensity in saline area of Bangladesh is relatively low, mostly 170% ranging from 62% in Chittagong coastal region to 114% in Patuakhali coastal region (FAO, 2007).

In most of the cases, the negative effects of salinity have been attributed to increase in Na^+ and Cl^- ions in different plants hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. Although both Na^+ and Cl^- are the major ions produce many physiological disorders in plant, Cl^- is the most dangerous (Tavakkoli *et al.*, 2010). Salinity at higher levels causes both hyperionic and hyperosmotic stress and can lead to plant demise. The outcome of these effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leading to plant death (Mahajan and Tuteja, 2005; Hasanuzzaman *et al.*, 2012a).

The available literature revealed the effects of salinity on the seed germination of various crops like *Oryza sativa* (Xu *et al.*, 2011), *Triticum aestivum* (Akbarimoghaddam *et al.*, 2011), *Zea mays* (Carpici *et al.*, 2009; Khodarahampour *et al.*, 2012), *Brassica spp.* (Ibrar *et al.*, 2003; Ulfat *et al.*, 2007), *Glycine max* (Essa, 2002), *Vigna spp.* (Jabeen *et al.*, 2003) and *Helianthus annuus* (Mutlu and Bozcuk, 2007). It is well established that salt stress has negative correlation with seed germination and vigor (Rehman *et al.*, 2000).

Higher level of salt stress inhibits the germination of seeds while lower level of salinity induces a state of dormancy (Khan and Weber, 2008).

Hasanuzzaman *et al.* (2009) observed a significant reduction in germination rate of 4 rice cultivars when exposed to various concentration of salt (30-150 mM). However, the sensitive cultivars were more prone to germination reduction under salt stress. In *Vigna radiata*, germination percentage decreased up to 55% when irrigated with 250 mM NaCl (Nahar and Hasanuzzaman, 2009). In a recent study, Khodarahmpour *et al.*, (2012) observed drastic reduction in germination rate (32%), length of radicle (80%) and plumule (78%), seedling length (78%) and seed vigour (95%) when *Zea mays* seeds were exposed to 240 mM NaCl.

One of the most initial effects of salt stress on plant is the reduction of growth rate. Salinity can affect growth of plant in various ways. First, the presence of salt in the soil reduces the water uptaking capacity of the plant, and this quickly causes reduction in the growth rate. This first phase of the growth response is due to the osmotic effect of the soil solution containing salt, and produces a package of effects similar to water stress (Munns, 2002a, b).

Some crops are most sensitive under saline condition during vegetative and early reproductive stages, less sensitive during flowering and least sensitive during the seed filling stage. Seed weight is the yield component in all these studies, but similar conclusions regarding growth stage sensitivity were obtained with both determinate crops (the grain crops) and indeterminate (cowpea) crops (Läuchii and Grattan, 2007). Dolatabadian *et al.* (2011) observed that salinity stress significantly decreased shoot and root weight, total biomass, plant height and leaf number but not affected leaf area while studying with *Glycine max*.

A high concentration of Na^+ and/or Cl^- accumulation in chloroplasts is also inhibited photosynthesis. As photosynthetic electron transport is relatively insensitive to salts, either carbon metabolism or photophosphorylation may be affected due to salt stress

(Sudhir and Murthy, 2004). In fact, the effect of salinity on photosynthetic rate depends on salt concentration as well as plant species or genotypes.

Fisarakis *et al.* (2001) reported a positive growth inhibition caused by salinity associated with a marked inhibition of photosynthesis. There is evidence that at low salt concentration salinity sometimes stimulate photosynthesis. For instance, in *B. parviflora*, Parida *et al.* (2004) observed that rate of photosynthesis increased at low salinity while decreased at high salinity, whereas stomatal conductance remained unchanged at low salinity and decreased at high salinity.

The alteration of photosynthetic pigment biosynthesis is one of the most notable effects of salt stress (Hasanuzzaman *et al.*, 2012b). The decrease in chlorophyll (chl) content under salt stress is a commonly reported phenomenon and in various studies and the chl concentration were used as a sensitive indicator of the cellular metabolic state (Chutipaijit *et al.*, 2011).

Saha *et al.* (2010) observed a linear decrease in the levels of total Chl, Chl *a*, Chl *b* Car and xanthophylls as well as the intensity of Chl fluorescence in *Vigna radiata* under increasing concentrations of NaCl treatments. Compared to control, the pigment contents decreased on an average, by 31% for total Chl, 22% for Chl *a*, 45% for Chl *b*, 14% for carotene and 19% for xanthophylls (Saha *et al.*, 2010). Associated with the decline in pigment levels, there was an average 16% loss of the intensity of Chl fluorescence as well. In the study of Hasanuzzaman *et al.* (2011) observed that a higher chlorosis in wheat and rapeseed leaves when subjected to salt stress.

In *O. sativa* leaves, the reduction of Chl *a* and *b* contents of leaves was observed after NaCl treatment (200 mM NaCl, 14 d) where reduction of the Chl *b* content of leaves (41%) was affected more than the Chl *a* content (33%) (Amirjani, 2011). In another study, *O. sativa* exposed to 100 mM NaCl showed 30, 45 and 36% reduction in Chl *a*, Chl *b* and carotenoids (Car) contents compared to control (Chutipaijit *et al.*, 2011) which retarded the growth efficiency.

According to Romero-Aranda *et al.* (2006) increase of salt in the root medium can lead to a decrease in leaf water potential and, hence, may affect many plant processes. Osmotic effects of salt on plants are the result of lowering of the soil water potential due to increase in solute concentration in the root zone. At very low soil water potentials, this condition interferes with plants' ability to extract water from the soil and maintain turgor. However, at low or moderate salt concentration (higher soil water potential), plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water. Salt treatment caused a significant decrease in relative water content (RWC) in sugar beet varieties (Ghoulam *et al.*, 2002).

A decrease in RWC indicates a loss of turgor that results in limited water availability for cell extension processes (Katerji *et al.*, 1997). Steudle (2000) reported that in transpiring plants, water is thought to come from the soil to the root xylem through apoplastic pathway due to the hydrostatic pressure gradient. However, under salt stressed condition, this situation changes because of the restricted transpiration. Under these situations, more of the water follows the cell-to-cell path, flowing across membranes of living cells (Vysotskaya *et al.*, 2010).

Salt stress significantly reduced the yield of crops as indicated by many researchers. As reported by Greenway and Munns (1980), after some time in 200 mM NaCl, a salt-tolerant species such as sugar beet might have a reduction of only 20% in dry weight, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might be dead. On the other hand, a halophyte such as *Suaeda maritime* might be growing at its optimum rate (Flowers *et al.*, 1986).

Murty and Murty (1982) reported that the severe inhibitory effects of salts on fertility may be due to the differential competition in carbohydrate supply between vegetative growth and constrained supply of these to the developing panicles. Grain yield reduction of rice varieties due to salt stress is also reported earlier by Linghe and Shannon (2000) and Gain *et al.* (2004). In *O. sativa* varieties, grain yield, which is the ultimate product of yield components greatly influenced by salinity levels. The loss of grain yield due to 150

mM salinity are 50%, 38%, 44% and 36% over control for the cultivars BR11, BRRI dhan41, BRRI dhan44 and BRRI dhan46, respectively (Hasanuzzaman *et al.*, 2009).

Nahar and Hasanuzzaman (2009) also reported that different yield components of *V. radiata* were significantly affected by salinity stress. Numbers of pods per plant, seeds per pod and seed weight were negatively correlated with salinity levels. The reproductive growth of *V. radiata* was also affected by salinity as the number of pods per plant substantially decreased with increasing salinity levels. An application of 250 mM NaCl reduced 77%, 73% and 66% yield in *V. radiata* cv. BARI mung-2, BARI mung-5 and BARI mung-6, respectively over control (Nahar and Hasanuzzaman, 2009).

2.4 Abiotic stress-induced oxidative stress

The chloroplast is the main source of ROS in plants. Insufficient energy dissipation during photosynthesis can lead to the formation of a Chl triplet state that can transfer its excitation energy onto O_2 to make $^1O_2$ (Logan, 2005). $O_2^{\cdot -}$ is produced by the photosynthetic electron transport chain (ETC) via the reduction of O_2 (Apel and Hirt, 2004), which is subsequently converted to H_2O_2 by SOD (Foyer and Noctor, 2000). Under stress conditions CO_2 fixation impaired in the chroloplast, the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) increases and glycolate that is produced moves from chloroplasts to peroxisomes (Takahashi and Murata, 2008). In peroxisomes, the generation of H_2O_2 involves glycolate oxidation catalyzed by glycolate oxidase (GO), the β-oxidation of fatty acids and catabolism of lipids (Halliwell, 2006). On the other hand, the generation of $O_2^{\cdot -}$ involves both the reaction of xanthine oxidase (XO) in the organelle matrix and a small electron transport chain is also an important source of ROS production in plant cells and consists of several dehydrogenase complexes that reduce a common pool of ubiquinone (Q). ROS production is likely to occur mainly in complex I (NADH dehydrogenase) and the Q zone (Møller, 2001; Blokhina *et al.*, 2003). Although mitochondrial ROS production is much lower compared to chloroplasts, mitochondrial ROS are important regulators of a number of cellular processes, including stress adaptation and PCD (Robson and Vanlerberghe, 2002). In glyoxysomes, acyl-CoA oxidase is the primary enzyme responsible for the generation of

H_2O_2. Plasmamembrane-bound NADPH oxidases (NADPHox) as well as cell-wall associated peroxidases (POX) are the main sources of $O_2^{\cdot-}$ and H_2O_2 producing apoplastic enzymes activated by various forms of stress (Mittler, 2002; Mhamdi *et al.*, 2010). Additional sources of ROS in plant cells include the detoxifying reactions catalyzed by cytochromes in both cytoplasm and the endoplasmic reticulum (Urban *et al.*, 1989).

At the metabolic level abiotic stress induced signal transduction triggers the generation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radicle ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicle ($OH^{\cdot}$), which consequently indirectly promotes oxidative stress by diminished antioxidant cell capacity, leading to oxidative damage, which could be at least partially responsible for stress induced damages (Yadav, 2010; Hasanuzzaman *et al.*, 2012a). Certain environmental stresses or genetic defects cause the production of ROS to exceed the Environmental stresses such as salinity, drought, extreme temperatures, metal toxicity lead to enhanced generation of ROS in plants due to disruption of cellular homeostasis and are extremely harmful to organisms at high concentrations (Hasanuzzaman *et al.*, 2012a, b; Hasanuzzaman and Fujita, 2012a). When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of "oxidative stress". The enhanced production of ROS during environmental stresses can pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells (Mishra *et al.*, 2011).

As per the report of Tanou *et al.* (2009a), it is not possible to determine the concentration of all sources to the generation of ROS under salt stress. Enhanced ROS production under salt stress induces phytotoxic reactions such as lipid peroxidation, protein degradation, and DNA mutations. Several reports showed the overproduction of ROS in plants under saline conditions and ROS-induced membrane damage is a major cause of cellular toxicity by salinity (Mittova *et al.*, 2004; Hasanuzzaman *et al.*, 2011a, b; Hossain *et al.*, 2011).

According to Vinocur and Altman (2005), Reactive oxygen species produced in response to oxidative stress can cause permanent damage to the cellular apparatus. Reactive oxygen intermediates (ROI) typically result from the excitation of O_2 to form singlet oxygen ($^1O_2$) or the transfer of one, two, or three electrons to O_2 to form superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), or a hydroxyl radical ($OH^{\cdot}$), respectively. The enhanced production of ROIs during stresses can pose a threat to plants because they are unable to detoxify effectively by the ROI scavenging machinery. The unquenched ROIs react spontaneously with organic molecules and cause membrane lipid peroxidation, protein oxidation, enzyme inhibition, and DNA and RNA damage.

Shalata and Tal (1998) reported that an unfortunate consequence of salinity stress in plants is the excessive generation of ROS. The excess production of ROS under salinity stress resulted from impaired electron transport processes in chloroplast and mitochondria as well as from pathways such photorespiration causing membrane damage and chlorophyll degradation and responsible for the development of leaf chlorosis and necrosis (Choi *et al.*, 2002).

According to Asada and Takahashi (1987), ROS are a group of free radicles, reactive molecules, and ions that are derived from O_2. It has been estimated that about 1% of O_2 consumed by plants is diverted to produce ROS in various subcellular loci such as chloroplasts, mitochondria, depending on their concentration in plants.

2.5 Antioxidant defense system

In general, plant cells are adequately equipped to keep ROS within the limits that are generated as a consequence of normal cellular metabolic activities. Under different stress conditions, however, ROS generation often exceeds the overall cellular antioxidative potential leading to stress-induced adverse effects on plant growth and physiology. A steady state balanced is required to protect plant cells from oxidative damage (Hasanuzzaman *et al.*, 2011a). Plants possess an efficient non-enzymatic (AsA, GSH, α-tocopherol, phenolic compounds, alkaloids and non-protein amino acids) and enzymatic (SOD, CAT, APX, MDHAR, DHAR, GR, GPX, GST and POD) antioxidant defense

systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging ROS (Gill and Tuteja, 2010). These antioxidant defense systems are found in almost all cellular compartments, demonstrating the importance of ROS detoxification for cellular survival (Gill and Tuteja, 2010).

Ascorbate is an important antioxidant in plant tissues which is synthesized in the cytosol of higher plants primarily from the conversion of D-glucose to AsA. It reacts with a range of ROS such as H_2O_2, $O_2^{\cdot-}$, $^1O_2$ and $OH^{\cdot}$ at diffusion-controlled rates (Smirnoff, 2005). AsA is also responsible for keeping prosthetic metal ions in a reduced form, thereby maintaining the activity of various antioxidant enzymes (De Tullio, 2004). AsA plays an important role in plant stress tolerance (Hossain *et al.*, 2010, 2011; Hasanuzzaman *et al.*, 2011a). Exogenous application of AsA influences the activity of many enzymes and minimizes the damage caused by oxidative processes through synergic function with other antioxidants (Shalata and Neumann, 2001).

Glutathione acts as an antioxidant and is involved directly in the reduction of most ROS (Noctor and Foyer, 1998). Additionally, GSH plays a key role in the antioxidative defense system by regenerating other potential water-soluble antioxidants like AsA via the AsA-GSH cycle (Foyer and Halliwell, 1976). GSH is a substrate for GPX and GST, which are also involved in the removal of ROS (Noctor *et al.*, 2002a). Other functions for GSH include the formation of phytochelatins (PCs), which have an affinity to HM and are transported as complexes into the vacuole, thus allowing plants to have some level of resistance to HM (Sharma and Dietz, 2006). GSH also takes part in the detoxification of xenobiotics and acts as a storage and transport form of reduced sulfur (Srivalli and Khanna-Chopra, 2008). The role of GSH in the antioxidant defense system provides a strong basis for its use as a stress marker. The change in the ratio of its reduced (GSH) to oxidized (GSSG) form during the degradation of H_2O_2 is important in certain redox signaling pathways (Li and Jin, 2007). GSH acts as a redox sensor of environmental cues, and an increase in GSH provides resistance to plants against oxidative stress. Recent

reports suggest that an increase in GSH content enhances protection to various abiotic stresses (Hossain *et al.*, 2010, 2011; Hasanuzzaman *et al.*, 2011a, b).

Antioxidant enzymes are located in different sites of plant cells and work together to detoxify ROS. The major antioxidant enzymes are SOD, CAT, GPX, GST and AsA-GSH cycle enzymes. The AsA-GSH cycle involves 4 enzymes (APX, MDHAR, DHAR and GR) as well as AsA, GSH and NADPH which work together to detoxify H_2O_2 in a series of cyclic reactions and further regenerate AsA and GSH (Hasanuzzaman *et al.*, 2012a).

Catalases (CATs) are tetrameric heme-containing enzymes that use H_2O_2 as a substrate and convert it to H_2O and O_2, thus preventing cells from oxidative damage (Sanchez-Casas and Klesseg, 1994). CATs are present in peroxisomes, glyoxysomes, and related organelles where H_2O_2-generating enzymes are located (Agarwal *et al.*, 2009). CAT has one of the highest turnover rates of all enzymes: one molecule of CAT can convert around six million molecules of H_2O_2 to H_2O and O_2 per minute. Thus, CAT is important in removing H_2O_2, which is generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration, and purine catabolism (Gill and Tuteja, 2010). It has also been reported that apart from its reaction with H_2O_2, CAT also reacts with some hydroperoxides (Ali and Alqurainy, 2006). CAT activity shows variable trends under different abiotic stresses (Singh *et al.*, 2008; Hasanuzzaman *et al.*, 2011a, b; Hasanuzzaman and Fujita, 2011a).

APX are heme-containing enzymes involved in scavenging H_2O_2 in water-water and AsA-GSH cycles using AsA as the substrate, catalyzing the transfer of electrons from AsA to H_2O_2, producing DHA and water (Pang and Wang, 2010). The APX family consists of at least five different isoforms including mitochondrial (mAPX), thylakoid (tAPX) and glyoxisome membrane forms (gmAPX), as well as chloroplast stromal soluble form (sAPX), cytosolic form (cAPX) (Noctor and Foyer, 1998). APX activity is enhanced in plants in response to during different abiotic stress conditions (Singh *et al.*, 2008; Hasanuzzzaman and Fujita, 2011a, b).

The univalent oxidation of AsA leads to the formation of MDHA. If MDHA is not reduced again to AsA by MDHAR, it will spontaneously disproportionate into AsA and DHA. DHA is then reduced to AsA by DHAR in a reaction requiring GSH (Chen *et al.*, 2003). Rapid regeneration is necessary in order to maintain the antioxidative capacity of AsA. The regeneration of AsA could be regulated in this cycle mainly by NADPH-dependent MDHAR activity (Mittova *et al.*, 2000) and thus it is crucial for AsA regeneration and essential for maintaining a reduced pool of AsA (Martínez and Araya, 2010). Although there are also a few reports about MDHAR activity in other physiological processes those are related to oxidative stress, research on different crops under environmental stresses revealed the regulatory role of MDHAR during oxidative stress tolerance and acclimation (Hasanuzzaman *et al.*, 2011a, b). MDHAR and DHAR are equally important in regulating the level of AsA and its redox state under oxidative stress (Eltayeb *et al.*, 2006, 2007). DHAR is also a key component of the AsA recycling system (Martínez and Araya, 2010) which regenerates AsA from the oxidized state (DHA) and regulates the cellular AsA redox state. It is thus crucial for tolerance to various abiotic stresses leading to the production of ROS. Increased DHAR activity was reported in response to various ROS-inducing stresses (Lee *et al.*, 2007; Hossain *et al.*, 2010; Hasanuzzaman *et al.*, 2011a and Hasanuzzaman *et al.*, 2014).

Glutathione reductase (GR) is a potential enzyme of the AsA-GSH cycle which catalyzes the reduction of GSH, involved in many metabolic regulatory and antioxidative processes in plants where GR catalysis the NADPH-dependent reduction of disulphide bond of GSSG and is thus important for maintaining the GSH pool (Chalapathi Rao and Reddy, 2008). Pang and Wang (2010) reported that GR also maintains a high ratio of GSH/GSSG in plant cells, also necessary for accelerating the H_2O_2 scavenging pathway, particularly under stress conditions. GR plays a crucial role in determining the tolerance of a plant under various stresses by maintaining the antioxidant machinery of the cell, conferring stress tolerance (Hasanuzzaman *et al.*, 2011a, b).

Plant GSTs are a superfamily of multifunctional enzymes which catalyse the conjugation of electrophilic xenobiotic substrates with GSH (Dixon *et al.*, 2010). Among the enzymes

related to GSH metabolism, GST isoenzymes account for approximately 1% of a plants total soluble protein (Marrs, 1996). GSTs catalyse the binding of various xenobiotics (including numerous pesticides) and their electrophilic metabolites with GSH to produce less toxic and more water-soluble conjugates (Edwards *et al.*, 2000). Besides catalyzing the conjugation of electrophilic compounds to GSH, GST isoenzymes also exhibit POX activity (Gullner and Kömives, 2001). Various abiotic stresses are powerful inducers of GST activity in plants (Dixon *et al.*, 2010). Plant GSTs are also associated with responses to various forms of abiotic stress (Hossain *et al.*, 2006; Dixon *et al.*, 2010; Hasanuzzaman *et al.*, 2011a, b) and confer stress tolerance in plants.

The activity of ROS-0scavanging enzymes is highly correlated with antioxidant stress defense and abiotic stress tolerance. However, the activities vary with plant cultivar, stress duration and dose.

The generation of ROS and increased activity of many antioxidant enzymes during abiotic stress have been reported in different plant studies with several reports indicating that the activity of antioxidant enzymes of tolerant genotypes increased in response to abiotic stress whereas the sensitive species failed to do so (Hasanuzzaman *et al.*, 2012a).

El-Bastawisy (2010) concluded that salt tolerance was related to the endogenous levels of the enzymatic and the non-enzymatic antioxidants in wheat seedlings. Among the three wheat cultivars (H 168, Gimmeza 7 and Beni swif 1) under observation, the activities of SOD, CAT, APX and GR as well as the non-enzymatic antioxidants (AsA and GSH) increased mostly in H 168, but declined in Gimmeza 7 and particularly in Beni swif 1. H 168 had a superior antioxidant defense system and was more tolerant to NaCl than the other two cultivars due to the higher enzymatic and non-enzymatic antioxidants.

2. 6 Effect of salinity on wheat

Turki *et al.* (2014) conducted an experiment with thirty-six highly tolerant and 16 highly susceptible wheat varieties which were evaluated in the saline area in the field. The results showed that tolerant varieties could grow and develop biomass under saline conditions. In contrast, susceptible varieties could not even emerge in the stressed

condition. They also showed that at seedling stage 100 mM NaCl decreased chlorophyll content, leaf length, number of tillers per plant, number of leaves per plant, shoot length and shoot fresh and dry weights, while at maturity stage plant height, the number of fertile spikes per plant and the number of seeds per spike were affected by at seedling stage 100 mM NaCl. The shoot fresh and dry weights were the most affected traits at seedling stage; however the number of fertile spikes and the number of seeds per spike were the most affected traits at maturity stage.

A field experiment was conducted by Jiang *et al.* (2013) to study the effects of deficit irrigation with saline water on spring wheat growth and yield in an arid region of Northwest China. They applied nine treatments included three salinity levels S1, S2 and S3 (0.65, 3.2, and 6.1 dSm^{-1}) in combination with three water levels W1, W2 and W3 (375, 300, and 225 mm). For most treatments, deficit irrigation showed adverse effects on wheat growth; meanwhile, the effect of saline irrigation was not apparent. At 3.2 and 6.1 dSm^{-1}, the highest yield was obtained by W1 treatments, however, the weight of 1,000 grains and wheat yield both followed the order W2> W1 > W3. They showed that, spring wheat was sensitive to water deficit, especially at the booting to grain-filling stages, but was not significantly affected by saline irrigation and the combination of the two factors. The results demonstrated that 300-mm irrigation water with a salinity of less than 3.2 dS/m is suitable for wheat fields in the study area.

A pot experiment was conducted to study the effect of different salinity levels, i.e. ECe= 3 dSm^{-1} (control), 8, 12 and 16 dSm^{-1} on four wheat grain yield, yield components and leaf ion uptake. Result revealed that higher grain yield production, higher leaf K^+ concentration, K^+:Na^+ ratio and lower leaf Na^+ and Cl^- concentration were observed in Kouhdasht, followed by Attrak, Rasoul and Tajan, respectively (Asgari *et al.*, 2012).

Kumar *et al.* (2012) was conducted an experiment on eight genotypes of wheat with varying in their salt tolerance level, to evaluate effect of salinity on germination, growth, and yield related parameters. Lower salinity (3dSm^{-1}) did not affect the germination, growth and yield attributing parameters. Higher salinity levels reduced germination,

growth and yield attributing parameters. Genotypes K9644 and K9465 showed maximum reduction in all these regards. Genotypes K9006, K8434, KRL1-4, K88 and HD2733 showed hardness against higher levels of salinity.

An experiment has been carried out by Akbari ghogdi *et al.* (2012) on four cultivars of wheat (Neishabor and Sistani as salt tolerant and Bahar and Tajan as salt sensitive) were exposed to four salinity levels (1.3 dSm^{-1} as control, 5, 10, 15 dSm^{-1}) via calcium chloride and sodium chloride with 1:10 (Ca^{2+}:Na^{+} ratio). Chlorophyll content (CHL), Leaf relative water content (RWC), sodium and potassium contents, and also K^{+}/Na^{+} ratio were measured at tillering and flowering stages, Total grain yield and yield components were determined. Salinity stress decreased relative water content (RWC), K^{+} content, K^{+}/Na^{+} ratio and grain yield; however Na^{+} content in all the genotypes and in both stages were increased. CHL content increased at tillering stage while it is decreased at flowering stage. Sistani and Neishabour cultivars had more amounts of K^{+} content, K^{+}/Na^{+} ratio and RWC under salt conditions, at tillering stage Bahar and Tajan cultivars recorded higher CHL and sodium content at both stages. Results showed that the salinity tolerance in tolerant cultivars as manifested by lower decrease in grain yield is associated with the lower sodium accumulation and higher K^{+}/Na^{+} compared to the sensitive cultivars.

A pot experiment was carried out by Al-Musa *et al.* (2012) to study the performance of some BARI wheat varieties under the coastal area of Patuakhali. Four wheat varieties viz. BARI ghom 23, BARI ghom 24, BARI ghom 25 and BARI ghom 26 were planted in the field to evaluate their comparative performance in respect of germination percentage, growth, yield and yield attributing characters. Among the four varieties, BARI ghom 26 showed superior performance irrespective of all parameters studied except total dry matter content (TDM) and yield reduction percentage. Among the BARI varieties, BARI ghom 26 produced greater germination (61.00%) at 13 days judge against to other varieties. The taller plant (47.91 cm), higher LAI (1.84), maximum TDM (17.37 g $plant^{-1}$) and effective tillers $hill^{-1}$ (18.08) were also obtained with the similar variety. BARI ghom 26 was also most effective to produce the maximum grains $spike^{-1}$ (38.52), higher

weight of 1000-grains (49.38 g), higher grain (3.35 t ha^{-1}) and straw (8.50 g $plant^{-1}$) yield and greater HI (4.03%).

Sadat Noori *et al.* (2010) conducted an experiment to examine the morpho-physiological effects of eight wheat genotype (Cajema × Sette Cerros, Cajema × HO_2 and Cajema × Lermaroja as hybrid; Sette Cerros, HO_2, Lermaroja, Cajema as parent and Axona as a control) with the application of four saline solutions (0, 150, 200 and 250 mM NaCl) As salinity levels increased, yield and 1000 grain weight and K^+ concentration declined. Based on Na/K ratio, the best physiological characteristic for recognizing sensitive and tolerant genotypes, Cajema was the most tolerant genotype. Hybrids produced in this study weren't good for salinity condition and the hybrids didn't show more feature than their parents.

Khajanchi *et al.* (2010) conducted a hydroponic experiment, effects of 0, 40, 80 and 160 mM NaCl applied for 4 and 7 days were studied on root morphology of 19 days old wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). The 80 mM NaCl treatment significantly reduced the fresh yield, relative plant growth rate, root length and root surface area of wheat by 42, 62, 45 and 51%, respectively measured 4 days after salt application. The deleterious effects of salinity on wheat were recorded even at of 40 mM NaCl concentration when applied for longer duration of 7 days. In general barley could tolerate 80 mM of NaCl without any adverse effect on the parameters studied except the plant biomass obtained 7 days after salt application. The adverse effects were prominent at 160 mM NaCl both in wheat and barley and more so when applied for longer duration. Under similar levels, NaCl stress was found to be more harmful to wheat than barley. A negative plant growth rate was recorded in wheat 7 days after application of 160 mm NaCl. Majority of the roots of wheat and barley were found in the 0.0 to 0.5 mm diameter category.

Iqbal (2010) conducted a pot experiment on the leaf extension growth of wheat cv. wembley having salinity levels NaCl (at 0, 50, 100 and 200 mM) and Na_2SO_4 (at 0, 50 and 100 mM). The extension growth of leaf 4 to leaf 9, and the flag leaf decreased with

increasing Na^+ concentration. NaCl inhibited the growth of leaf and shorter. On the other hand Na_2SO_4 increased leaf growth of leaf 9 and growth continued up to 43 days after transplanting.

An experiment has been carried out by Hassan (2010) on Egyptian cultivar of wheat (*Triticum aestivum* cv. Giza 63) were exposed to salinity levels (0 and 50 mM NaCl) are found that significantly decreased stomatal conductance, net photosynthetic rate and chlorophyll content by 20, 25, and 21 %, respectively. This reduction resulted in a change in assimilate allocation in favour of shoot growth, leading to a decrease in root to shoot ratio and eventually to a decrease in relative growth rate of both root and shoot. As a result there was a large reduction in yield parameters, especially in the number of ears per plant and 1000 grain mass.

Noaman (2010) conducted a pot experiment with four durum wheat (*Triticum turgidum*, *Triticum durum*) lines (133, 146, 56 and 83) transferred from *Triticum aestivum* cv. Sakha-8 (control), *Hordeum vulgarae cv.* Giza (control), *Triticum turgidum* cv. Langdon (LDN) and recombinant DS4D (LDN4B) where grown at 3 levels of salinity (2, 4 and 8 g $liter^{-1}$). They reported that increasing salinity affected plant height most in line 56 (24.5% reduction). Increasing salinity levels had no significant effect on the number of days from planting to booting, heading or flowering, even though differences among genotypes were significant. Under saline condition DS4D (LDN 4B) had the highest biological yield and grain yield followed by the lines 13,146 and 83. *Triticum turgidum* cv. Langdon (LDN) showed the greatest sensitivity to salinity.

Abdel-Ghani (2009) was carried out an experiment to determine the effects of salinity levels (control, 6, 12 and 18 dS m^{-1}) on germination, seedling growth, some agronomic traits and proline accumulation in leaves of nine wheat varieties adapted to semi-arid areas of Jordan. Final germination percentage, shoot and seminal root length, and all growth and yield parameters were significantly decreased by increasing salinity level. Proline content was significantly increased by increasing salinity.

Goudarzi and Pakniyat (2008) also conducted an experiment with Fifteen Iranian wheat cultivars (*Triticum aestivum* L.) were compared for salt tolerance using three treatments: 1.26 (control), 6.8 and 13.8 dSm^{-1} in a greenhouse. During vegetative growth, shoot Na^+, K^+, K^+:Na^+ ratio and agronomic traits were measured. In general, tolerant cultivars (Kavir, Niknejad, Chamran and Falat) with better agronomic performance, contained low Na^+ and higher K^+ and K^+:Na^+ ratio compared to non-tolerant ones (Ghods, Bayat, Cross Adl and Zarin). Shoot Na^+ content was negatively correlated with grain yield.

Moud and Maghsoudi (2008) conducted an experiment of thirty wheat cultivars under salt stressed condition which were examined at germination and seedling growth stages. Seeds were germinated and grown in long dark cups using distilled water as control and two levels of salt stress imposed by 9 and 15 dSm^{-1} NaCl solution for 48 hours. Coleoptile and root growth was measured as the response of cultivars to salinity. Seedling respiration was expressed as the difference between initial seed weight and seedling dry weight after 48 hours. Significant differences were found among cultivars in terms of coleoptile and root growth under salt stress condition. They were also found that seedling respiration was decreased as salinity level was increased. Salt stress inhibited coleoptile growth more than root growth.

Gawish *et al.* (2008) studied the responses of status and translocation of Na, Cl, N and production for both shoots and roots of two wheat varieties differing in salt tolerance, Giza-164 a relatively salt tolerant and Sakha-69 a relatively salt sensitive variety. The plants were treated with NaCl, $CaCl_2$ or their mixture at a level of 50, 750, 1500 or 3000 ppm, after the first leaf had emerged. The status of Na and Cl positively responded in shoots. The rate of translocation for the different ions was higher under salinity conditions, particularly in relatively salt tolerant plants presumably due to osmotic adjustment and to reduce the adverse effect on root growth.

Tammam *et al.* (2008) conducted an experiment of one wheat cultivars (Banysoif 1) under salinity condition. Wheat cv. Banysoif 1 was grown in clay soil for 7 days in different pots. Then seedlings were irrigated by different saline waters (0, 60, 120, 180,

240 and 320 mM NaCl) near the field capacity. Plants were kept in the natural condition under these saline levels for 155 days. Fresh and dry weight of roots were measured unchanged up to the level of 120 mM NaCl then a significant reduction obtained at 240 and 320 mM NaCl. In shoots and spikes, dry matters were either unchanged or even stimulated to increase toward 180 mM NaCl then a quick reduction was observed. They also showed that, in shoots, the production of carbohydrates remained mostly unaffected even at the highest salinity level. In spikes, the soluble fractions were increased significantly by salt stress while the insoluble slightly reduced. Protein content reduced at high levels of salinity in roots while has been increased significantly in shoots and spikes. Amino acid content increased significantly towards 120 mM and 180 mM NaCl then a quick reduction about 55% and 45% recorded in roots and shoots respectively. In spike, there was a significant reduction in amino acids by increasing salt stress. In roots, there was a large accumulation of proline even at the lowest salinity level.

Bagci *et al.* (2010) The effect of increasing application of NaCl on root and shoot dry weight at early growth stage, and concentrations of K and Na was studied in 16 bread wheat genotypes grown in nutrient solution. NaCl was applied at 2, 55, 117, 194, and 287 mM. The genotypes showed a wide range of variation for the traits measured under the NaCl treatments. The salt tolerance index (STI) of the genotypes, expressed as the ratio of dry matter yield produced under the NaCl treatments compared to the control treatment, was a reliable criterion for ranking genotypes for their tolerance to NaCl. The very poor correlation between the shoot Na concentration and the STI values indicates that the root uptake capacity for K and the tissue tolerance (e.g. Na compartmentation) appear to be important physiological factors contributing to differential salt tolerance among the 16 bread wheat genotypes. This study also identified highly sensitive and tolerant genotypes to excess NaCl treatments (up to 287 mM) and these genotypes could be used in breeding programs and molecular physiological studies for development of high-yielding salt-tolerant bread wheat genotypes.

As per the experiment of Hossain *et al.* (2006), two wheat varieties (Aghrani and Kanchan) were grown in pots and subjected to 50, 100 and 150 mM NaCl till their

maturity. Water relations, chlorophyll content and mineral ions accumulation in wheat plants were analyzed. Water retention capacity and relative water content were decreased while water uptake capacity and water saturation deficit were increased with the increasing levels of salinity. Salinity increased diffusive resistance but decreased transpiration rate. Chlorophyll content was decreased due to salinity in both Aghrani and Kanchan. Accumulation of Mg^{2+}, Ca^{2+} and Na^{+} increased while that of K^{+} decreased in the salt treated plants. In general, Aghrani accumulated greater amount of Mg, Ca and Na ions than that of Kanchan. It is appeared that Aghrani possesses a better mechanism of salt tolerance than that of Kanchan.

Mandhania *et al.* (2006) studied the effect of salt stress on cell membrane damage, ion content and antioxidant enzymes in wheat (*Triticum aestivum*) seedlings of two cultivars salt-tolerant KRL-19 and salt-sensitive WH-542. Seedlings (4-d-old) were irrigated with 0, 50 and 100 mM NaCl. Observations were recorded on the 3rd and 6th day after salt treatment and 2nd day after salt removal. The relative water content declined with induction of salt stress, more in WH-542 than in cv. KRL-19. K^{+}/Na^{+} ratio in KRL-19 was higher than in WH-542. WH-542 suffered greater damage to cellular membranes due to lipid peroxidation as indicated by higher accumulation of H_2O_2, MDA and greater leakage of electrolytes than KRL-19. The activities of catalase, peroxidase and ascorbate peroxidase and glutathione reductase increased with increase in salt stress in both the cultivars, however, superoxide dismutase activity declined. Upon desalinization, partial recovery in the activities of these enzymes was observed in KRL-19 and very slows recovery in WH-542.

In the experiment of El-Bassiouny and Bekheta (2005), two wheat cultivars (Giza 168 and Gimeza 9) were investigated with 0-14 dSm^{-1} NaCl stress. Changes in relative water content (RWC), polyamines (putrescine, Put; Sepermidine, Spd; Spermine, Spm), amino acids, ethylene and lipid peroxidation were determined in both cultivars in absence and presence of NaCl. NaCl stress reduced the RWC in both cultivars, the reduction was more pronounced in Giza 168. Lipid peroxidation was increased with salinity in both cultivars, more so in Giza 168. Salt stress increased Spd and Spm level in Gimeza 9 while

the level of both polyamines was decreased in Giza 168. PUT was increased only by 2.1 dSm^{-1} NaCl in Giza 168 whereas its level was decreased by all NaCl treatments in Gimeza 9. Amino acid content was increased in Gimeza 9, while the content was decreased in Giza 168 in all NaCl treatments. The predominant amino acids in both cultivars were glutamic acid and proline. Salt stress increased proline level in both cultivars; greater increase was obtained in Gimeza 9. Ethylene level was increased in Gimeza 9, while it was decreased in Giza 168 with increasing salt level.

Hamdy *et al.* (2005) carried out in a green house on the application of supplemental irrigation to wheat and barley using brackish water with salinity (EC 3 to 9 dSm^{-1}). They observed that possibility of securing high yields with reductions of only 21 to 25% compared to the fully, fresh-water irrigated control through the application of limited amounts of brackish water.

Bhatti *et al.* (2004) conducted an experiment with 50 salt tolerant wheat lines using tissue culture technique in a greenhouse having salinity levels of EC 1.5 (control) 15 and 30 dS m^{-1}. They observed that increasing salinity levels drastically affected the seedling growth.

Keles and Oncel (2004) conducted an experiment on the soluble metabolites in several cultivars of *Triticum aestivum* and *T. durum* with exposed to water logging, drought and salinity (0.7% NaCl, w/w) stresses for six days. They found that root and shoot fresh weights, significantly decreased under water logging, drought and salt stress and proline content significantly increased in case of salt stress.

An experiment was conducted by Ismail (2003) to study the effect of different concentration of salinity (NaCl up to 250 mM) on the germination, dry matter suction and same relevant metabolic parameters of two lines (Sukha 69 and Sakha164, and one cultivar (Stork) of wheat *(Triticum aestivum).* He observed that during germination and seedling stages, the lines could be tolerated in lower and moderate doses of salinity, while the growth was significantly retarded at the lower and moderate levels and completely inhibited at higher levels of salinity.

An experiment was conducted by Iqbal (2003) to evaluate the effects of constant and variable salinity on spring wheat cv. Wembley. He found that salinity significantly decreased the number of tillers, leaf area, shoot and root dry weight per plant. These parameters were always higher at variable than that in constant salinity.

Husain *et al.* (2003) conducted an experiment with six durum wheat genotypes at salinity levels having 1, 75 and 150 mM NaCI, with supplemental Ca^{2+} and measured leaf chlorophyll content, ion concentration, plant height and dry biomass. They observed that the low Na^{+} genotypes showed much longer chlorophyll retention hen the high Na^{+} genotypes, the start of leaf senescence being prolonged by weed or more in the low Na^{+} genotypes. The difference was greatest at 75 mM NaCl.

An experiment was conducted by Sangwan *et al.* (2003) with the effect of salinity (control, 1.2, 4, 8, and 12 dSm^{-1}) on the performance of wheat cv. WH-291.They observed that increase in salinity levels decreased wheat dry matter production.

Zein *et al.* (2003) conducted two pot experiments under the wire proof greenhouse condtions with irrigation water salinity on yield and yield components of two Egyptian i.e. Sakha 8 and Sakha 92, and six Syrian wheat cultivars, i.e. Bohos 4, Bohos 5, Bohos 6, sham 1, sham 4 and sham 6 were irrigated with saline water. Hoagland solution in five water salinity levels 0.4 (control treatment), 4.8, 12 and 16 dSm^{-1} were used in the first season. Based on the results of the first season, the more tolerant wheat cultivars (Sakha 8, Sakha 69, Bohos 5, Bohos 6 and sham) were chosen for the second season study, which were irrigated with Hogland solution in five water salinity levels, 0.4 (control treatment.), 6, 8, 10 and 12 dSm^{-1}. Here, they observed that the wheat grain and straw yields as well as plant height, spike length and 1000 grain weight were significantly affected by increasing salinity and the Egyptian cultivars could tolerate up to 12 dSm^{-1} salinity while the salt tolerant Syrian cultivars Bohos 6 could tolerate up to 8 dSm^{-1} irrigation water salinity.

Sairam and Srivastava (2002) conducted an experiment with the application of long term, medium level of NaCl salinity in two wheat genotype one is tolerant Kharchia 65 and another one is susceptible HD 2687. NaCl salinity caused decrease in relative water content (RWC), chlorophyll (CHL), membrane stability index (MSI) and ascorbic acid (AA) content, and increased the contents of hydrogen peroxide (H_2O_2), thiobarbituric acid reactive substances (TBARS) (measure of lipid peroxidation) and activities of superoxide dismutase (SOD), its various isozymes, ascorbate peroxidase (APX) and glutathione reductase (GR) in wheat genotypes Kharchia 65 (tolerant) and HD 2687 (susceptible). Salinity tolerant wheat cv. Kharchia 65 showed fewer declines in RWC, CHL, and MSI estimated in whole tissue than salt sensitive HD 2687. Kharchia 65 also exhibited less decrease in AA content, less increase in H_2O_2, TBARS contents and higher increase in SOD and its isozymes, APX and GR in all sub cellular fractions than salt sensitive HD 2687.

Rajpar and Sial (2002) conducted a pot experiment with eight varieties of wheat such Kharchia-65, Anmol, NIAB-20 PAI-81,TW-161, Bakhtwar, KTDH-19 and SARC-1, They observed that plant height, shoot dry weight and root length were decreased salinity up to EC 19 dSm^{-1}.

Ashraf *et al.* (2002) conducted an experiment on the effect of salt stress on the growth, ion accumulation and photosynthetic capacity of two spring wheat cultivars and Barani-83 (Salt Sensitive) and SARC-1 (Salt tolerant). Three week old plants of both cultivars were exposed to 0, 100 and 200 mol m^{-3} NaCl in (Hogland nutrient) solution. The, observed that fresh weights of shoots and roots, plant height and leaf areas were decreased with increasing levels of salinity.

An experiment was conducted by Ashraf and Parveen (2002) with two wheat (*Triticum aestivum* L.) cultivars, salt tolerant SARC- I and salt sensitive Potohar. Eighteen-day-old plants of both the lines were grown in sand culture and irrigated with 0 (control) 80, 160 or 240 mM NaCl in full strength Hoagland's nutrient solution. Shoot fresh and dry masses, and leaf area per plant at the vegetative stage of SARC-1 were significantly

greater than those of cv. Potohar at higher salt concentrations. However, relative growth rate (CGR) of cv. Potohar was significantly higher than that of SARC-1. At the grain development stage, SARC-1 had significantly higher net photosynthetic rate (Pn) and stomatal conductance (gs) in the leaf than cv. Potohar under salinity. SARC-1 was superior to cv. Potohar with respect to number of grains per spike, number of grains per spikelet; mean grain mass and main yield per plant at all NaCl concentrations.

Akram *et al.* (2002) studied in a pot experiment the effect of salinity (10, 15 and 20 dSm^{-1}) on the yield and yield components of salt-tolerant (234/2), medium-responsive (243/1), and susceptible (Fsd 83) wheat varieties. They reported that salinity reduced the spike length, number of spikelets $spike^{-1}$, number of grains $spikelet^{-1}$, 1000- grains weight, and yield $plant^{-1}$ of all the varieties but the susceptible variety was affected the most adversely.

Shazia *et al.* (2001) examined the effect of foliar application of indole Acetic Acid on growth and yield of two lines of spring wheat, Kohistan-97, and Parwaz-94 under different levels (8.12 and 16 dSm^{-1}) of NaCl salinity. The results revealed that all the growth and yield parameters such as plant height, root length, number of leaves per number of fertile tillers, spike length, number of spikelets $spike^{-1}$, number grain $spike^{-1}$, 1000 grain weight and grain yield $plant^{-1}$ were decreased progressively with increasing salinity.

Mutawa and Katony (2001) conducted experiment with two wheat genotypes *(Triticuin aestivum* cv. Giza 157 and cv. Sakha 8). Plants were subjected to different levels of salinity viz. 0, 75 and 150 mM NaCl in nutrient solutions containing 12 mM N either from NH_4 or NO_3 as the sole nitrogen source. Growth of the two cultivars particularly Sakha 8 was better under nitrate than under ammonium nutrition. Ammonium fed plants was poorly developed with a distinctly lower root: shoot ratio and thick, short and highly branched roots compared with nitrate fed plants. The two cultivars exhibited greater salt (NaCl) tolerance under nitrate than under ammonium-N nutrition.

Singh *et al.* (2000) reported that 20 wheat varieties were subjected to salinity stress during seedling growth along with the control. The salinity levels used were 0.0% (control) and 0.5% with corresponding EC values of 2.8 and 20.8 dS m^{-1}, respectively. Seedling growth declined under salinity stress. The genotype Raj-3077 and Kharchina-5 were tolerant to salinity with respect to seedling vigour while Raj-4530 and Raj-3934 were most susceptible genotypes under salinity.

Flagella *et al.* (2000) evaluated the effect of salinity on grain yield and yield components of durum wheat cv. Duilio subjected to the salinity levels of 0.5, 6, 12, 18 and 24 dSm^{-1} in a growth chamber. The changes in photosynthetic activity were not slated to changes in leaf turgor. With regard to photosynthesis and grain yield, durum &heat was moderately resistant to salinity showing significant damages only when irrigation water with EC of 12 dSm^{-1} or higher was used.

A study was carried out by Bouaouina *et al.* (2000) with the salt tolerance durum wheat (*Triticum turgidum*). They observed decreased growth of whole plants, delayed emergence of new leaves and limited K^+ and Ca^{++} accumulation in these organs under NaCl treated soil salinity. Moreover, Na^+ accumulation decreased from older to younger leaves. Cellular dry matter production was not much affected in spite of a drop in cellular water content. Depressive effects of K^+ and Ca^{++} accumulation were evident while Na^+ cellular accumulation increased with NaCI concentration. These results suggest that wheat has mechanisms to restrict Na^+ transport and accumulation in younger leaves.

Chopra *et al.* (1997) conducted a field experiment with 6 wheat cultivars which were irrigated with water having salinity levels of 4.0 (control), 6.0, 7.0 and 12.0 dSm^{-1}. Grain yield decreased with increasing salinity level. The cv. Kharachia-65 and II D-2189 were found the most salt tolerant.

A pot experiment was conducted by Maliwal (1997) to study on a medium black calcareous clay soil, 5 wheat cultivars were exposed to salinities of 0.78, 15.4 dSm^{-1} with chloride or sulphate salts. Plant growth and yield decreased with increasing salinity. The

reduction in yield was the lowest in cv. Kharahia-65 and the highest of that was in cv. J-405. Yield was lower with chloride salt than that with sulphate salt.

2.7 Salicylic acid and crop productivity

Salicylic acid (SA) is a common plant-produced phenolic compound and a potential endogenous plant hormone that plays an important role in plant growth and development (Khan *et al.*, 2012; Alam *et al.*, 2013). The role of SA is intensively studied in plant responses to biotic stress. In recent years the involvement of SA in the response to abiotic stresses has widely been studied (El Tayeb 2005; Ahmad *et al.* 2011; Alam *et al.*, 2013; Hasanuzzaman *et al.*, 2014; Li *et al.*, 2014).

Gémes *et al.* (2011) suggested that, the cross-talk of signaling pathways induced by SA and high salinity may occur at the level of ROS and nitric oxide (NO) production. They observed that SA-induced generation of H_2O_2 and NO are considered to be functional links of cross-tolerance to various stressors. SA-stimulated pre-adaptation state was beneficial in the acclimation to subsequent salt stress in *Solanum lycopersicum* (Gémes *et al.*, 2011). At the whole plant level, SA-induced massive H_2O_2 accumulation only at high concentrations (1–10 mM), which later leads to death of the plant. Torabian (2011) reported that pre-treatment with SA induced adaptive responses in *Medicago sativa* plant under salinity stress and consequently, encouraged protective reactions in biotic membranes which improved the growth of seedlings. SA pre-treatment improved growth and resulted in higher resistance of plants to salinity, so that it increased germination percentage, seed vigor index and growth parameters of the seedlings. Also, salinity intensified electrolyte leakage, while SA decreased it and this decrease was stronger at SA concentration (Torabian, 2011).

Erdal *et al.* (2011) investigated the effects of foliar-application of SA on salt sensitivity of *T. aestivum*. They observed that salt-induced deleterious effect in wheat seedlings were significantly alleviated by the SA treatment. SA can be used as a signal molecule to investigate plant defense to abiotic stress. After the application of SA, increasing tolerance of wheat seedlings to salt stress may be related to increases in antioxidative

enzyme activity. Exogenous SA treatment significantly increased the fresh and dry weights in both root and shoots of wheat plants under salt stress. In parallel to increasing antioxidant activity, SA treatment decreased H_2O_2 content when compared to plants growing under salt stress without SA. In *Brassica juncea*, Yusuf *et al.* (2012) reported that SA enhanced the level of antioxidant system (SOD, CAT and POX) both under stress and stress-free conditions. However, the influence of SA on antioxidant system was more pronounced under stressful condition, therefore, suggesting that the elevated level of antioxidant system might be responsible for increased tolerance of *B. juncea* plants to NaCl stress.

However, some studies demonstrate that application of SA (0.5 mM) may promote the formation of ROS in the photosynthetic tissues and increase oxidative damage during salt and osmotic stresses. For instance, Barba-Espín *et al.* (2011) studied the effect of SA treatment on the response of *P. sativum* plants to salinity. NaCl-induced damage to leaves was increased by SA, which was correlated with a reduction in plant growth. The content of AsA and GSH in leaves of salt-treated plants increased in response to SA, although accumulation of the respective DHA and GSSG occurred. An increase in H_2O_2 also occurred in leaves of salt-exposed plants treated with SA. Negative effect of SA in the *P. sativum* plants exposed to NaCl was also correlated with an imbalance in antioxidant metabolism. Generally, deficiency of SA or a very high level of SA increases plant susceptibility to abiotic stresses. The optimal concentration (0.1–0.5 mM for most plants) enhances abiotic stress tolerance. In mungbean plants SA alleviates salt-induced decrease in photosynthesis and minimizes the leaf Na^+, Cl^-, and H_2O_2 content (Nazar *et al.*, 2011). This was accompanied by increased N and S assimilation through inducing the activity of NR and ATPs.

Cornelia and Bandici (2008) reported that Salicylic acid (SA) plays an important role in response to biotic and abiotic stress. Pretreatment of barley seeds with SA may cause a low level of oxidative stress, improving the antioxidative capacity of the plants. SA can increase their tolerance to salt stress induced by 200 mM NaCl treatments. In tomato plants grown under saline (NaCl) conditions, foliar application of SA significantly

reduced NaCl toxicity effects by decreasing Na^+ and increasing K^+ and Mg^{2+} in the roots and shoots (He and Zhu, 2008).

However, the actual role of SA in abiotic stresses remains unresolved. Several methods of application (soaking the seeds prior to sowing, adding to the hydroponic solution, irrigating, or spraying with SA solution) have been shown to protect various plant species against abiotic stress by inducing a wide range of processes involved in stress tolerance mechanisms (Horvath *et al.* 2007).

Zea mays treated with SA exhibited increased growth, decreased lipid peroxidation and membrane permeability, which were increased by salt stress (Gunes *et al.*, 2007). Exogenous SA also improves grain yield under salt stress in *T. aestivum* (Arfan *et al.*, 2007). The application of SA via root drenching protected *Lens esculentum* against NaCl stress and increased photosynthetic rates under salt stress (Stevens *et al.*, 2006; Poór *et al.* 2011).

El Tayeb (2005) found that SA application to barley induced a pre-adaptive response to salt stress, enhanced the synthesis of Chl *a*, Chl *b* and Car, and maintained membrane integrity, leading to improvement of plant growth. SA-pretreated plants exhibited less Ca^{2+} and more accumulation of K^+, and soluble sugars in roots under saline condition (El Tayeb, 2005). It was found that SA treatment caused accumulation of both ABA and IAA in *T. aestivum* seedlings under salinity. However, the SA treatment did not influence on cytokinin content. Thus, protective SA action includes the development of antistress programs and acceleration of normalization of growth processes after removal of stress factors (Sakhabutdinova *et al.*, 2003).

2.8 Effects of salicylic acid on wheat under salt stressed condition

Howladar and Dennett (2014) conducted an experiment with two wheat variety (Yecora Rojo and Paragon) under salt stressed condition where seeds of wheat were treated with salicylic acid. They showed that, exogenous application of SA through foliar spraying or seed soaking showed a slight increases or decreases with the application method or

between cultivars. SA foliar spraying exhibited a slight improvement over SA seed soaking in most parameters, particularly in Paragon. Although, seed soaking was less effective than foliar spraying, it was a slightly better with Yecora Rojo in some parameters.

Morad *et al.* (2013) reported that maximum height was achieved in control × SA none application treatment and minimum height was achieved in NaCl 8 dsm^{-1} × SA none application treatment. Also SA application increased number of grain in spike. SA application alleviated destructive effect of salt stress. When they worked with three levels (control, salt stress with NaCl 4 and 8 dsm^{-1}) and acid salicylic (application and none application) on traits of wheat.

Maleki *et al.* (2013) conducted an experiment with four bread wheat cultivars (Ghods, Shiraz as salt sensitive and Roshan, Kharchia as salt tolerant) to salinity (0 or 2g NaCl in 1kg of soil) and seed priming with 60 and 120 µM salicylic acid (SA). Primed seeds had significant differences in sodium acumulation in shoot in comparison to control. Application of 60 µM SA reduced the content of Na^+ in Ghods under salt condition. Salinity and priming caused no significant changes in shoot K^+ concentration of all genotypes. In the absence of SA, salinity increased the level of Ca^{2+} in shoot of all genotypes.

In order to investigate the effects of salicylic acid on growth parameters (shoot and root fresh weight), proline, soluble and insoluble sugar, protein and levels malondialdehyde (MDA) in the wheat (*Triticum aestivum* cv. Zarrin) seedlings under salt (NaCl) stress, experiment was conducted by Ghafiyehsanj *et al.* (2013). Eight days after culturing and in complete bifoliolate stage for exertion salinity stress, plants received NaCl treatment (0, 75 and 150 mM). Seven days after exerting the last salt stress, plants received SA as foliar spray (0, 200 and 400 mg L^{-1}). The results showed that with increasing of salinity the amount of protein, insoluble sugar, shoot and root fresh weight were reduced but soluble sugar, proline and malondialdehyde amounts were increased. Exogenous SA

application increased protein, insoluble sugar, shoot and root fresh weight contents, but reduced proline, soluble sugar and MDA in presence of salinity.

Cornelia *et al.* (2013) conducted a pot experiment to examine the influence of the exogenous applied SA solution on some physiological and biochemical parameters of plants, like plant height, leaf area, photosynthetic rate, stomatal conductance, antioxidative enzymes activity, assimilatory pigment contents and proline content in wheat (*Triticum aestivum* cv. Crisana) plantlets under salt stress, in pot experience in milk stage, in comparison with the same parameters of the control lots which were treated with water. They showed that exogenous SA solution, administrated to the wheat seedlings ameliorated the negative effect of salt stress. Positive effects were more pronounced in the case of 0.1 mM SA solution.

Mohamed and Morsy (2013) conducted an experiment during 2011 and 2012 seasons to examine the effects of seeds soaking in salicylic acid (SA) at 0.5 mM on seed emergence %, growth, plant pigments K^+/ Na^+ as well as percentages and uptakes of N, P, K, Mg and Ca in wheat cv. Giza 164 grown under different soil salinity levels (40 & 80 & 160 mM NaCl). Salinity caused by NaCl at 40 to 160 mM measurably reduced emergence %, all growth characters, plant pigments, K^+/ Na^+ as well as percentages and uptake of N, P, K, Mg and Ca in relative to non- salinization. Soaking seeds of wheat cv. Giza164 on salicylic acid solution at 0.5 mM was beneficial to improve growth and nutritional status under soil salinity conditions.

Turkyilmaz (2012) reported that germination of wheat seeds was inhibited by 100 mM NaCl (6%) and by 200 mM NaCl (10%). However, SA and GA_3 treatments increased germination rate of the plants under salinity condition. Radicle elongation decreased in all treatments compared with that of control. Although plumule elongation decreased with 100 mM NaCl and 200 mM NaCl, SA and GA_3 treatments ameliorate this adverse effect. Salinity caused a dramatic decrease especially in 200 mM NaCl in root and shoot lengths and fresh-dry weights in 4 weeks old seedlings, but PGRs treatments ameliorated this adverse effect.

Erdal *et al.* (2011) conducted an experiment to investigate the effects of foliar-applied SA on salt sensitivity, hydrogen peroxide (H_2O_2) generation and activities of antioxidant enzymes like peroxidase (POX) and catalase (CAT) in plant tissues under salt stress was performed. SA treatment significantly increased the fresh and dry weights in both root and shoots of wheat plants under salt stress. Similarly, POX and CAT activities were also augmented by SA treatment. While the highest POX activity was recorded at SA+120 mM NaCl, CAT activity also exhibited an increase compared to salt treatment without SA. In parallel to increasing antioxidative activity, SA treatment decreased H_2O_2 content when compared to plants growing under salt stress without SA. The results revealed that salt-induced deleterious effect in wheat seedlings were significantly alleviated by the SA treatment.

Barakat (2011) reported that the antioxidant enzymes such as catalase, peroxidase and ascorbate peroxidase, photosynthetic pigments, reducing sugar, proteins, amino acids, and proline contents in spike, shoot and root of salinity stressed plants were the most affected parameters specially at high salinity levels (150-200 mM NaCl).Treatments with 0.1 mM of salicylic acid as shoot spraying on NaCl wheat stressed plant organs mitigated the harmful effect of NaCl.

Four glasshouse experiments were conducted by Howladar (2010) for examining salinity stress and tolerance in wheat. The first experiment examined the responses of three wheat cultivars from Saudi Arabia (Local wheat, West bread and Yecora Rojo) and two UK wheat cultivars (Paragon and Belvoir) to different levels of salinity (Tap water, 25, 50, 100, 150 and 200 mM NaCl). In the second experiment, Yecora Rojo and Paragon were selected to test whether improved wheat tolerance to salinity could be obtained by applying exogenous Salicylic acid (0, 0.5, 1 and 2 mM SA) via priming seeds for 6 hours. The third experiment further tested the effect of SA on tolerance to salinity with SA applied through seed soaking for 6h and 24h. The fourth experiment compared the effect of SA applied by seed soaking (6h) or by foliar spray. In all experiments, saline conditions gave significant declines in wheat growth parameters, gas exchange, yield and

yield components with increases in salinity concentration, whereas protein and chlorophyll content increased. Cultivar Paragon grew significantly better than cultivar Yecora Rojo in non-saline conditions but not under salinity stress. Treating wheat with SA produced only a minor improvement in growth parameters, yield and yield components under salinity stress. They showed that the influence of SA depended on genotype, plant stage and SA concentration more than soaking time and application method with 0.5 and 1 mM SA concentrations being the most effective. SA mitigates but does not prevent salinity impacts and has a dual function which can give positive or negative effects under salinity stress.

The interactive effect of salicylic acid and sodium chloride (NaCl) salinity on wheat (*Triticum aestivum* L.) cv. 'Inqlab' (salt-sensitive) and cv. 'S-24' (salt-tolerant) was studied in a sand-culture pot experiment in a net house (Hamid *et al.* 2010). They soaked wheat seeds in water and 100 ppm salicylic acid solution for 6 h were sown in sand salinized with 0, 50, and 100 mM NaCl and pots were irrigated with quarter-strength Hoagland's nutrient solution. Sodium chloride salinity significantly reduced growth parameters. Salicylic acid treatment alleviated the adverse salinity effect on growth. Salinity decreased the chlorophyll *a* and *b* content and chlorophyll *a/b* ratio was less in salt-tolerant wheat variety ('S-24').

Dolatabadian *et al.* (2009) reported that seeds were soaked in salicylic acid solution for 24 h, dried with sterile paper, transferred to sterile Petri dishes, and treated with 10 ml NaCl solution at different concentrations increased germination in stressed and control seeds after 1 week. Salicylic acid increased the level of cell division of seedlings and roots, which increased plant growth. Salt stress significantly increased the activity of the antioxidative enzymes catalase, superoxide dismutase, peroxidase, and polyphenol oxidase in wheat seedlings, and salicylic acid reduced the activity of antioxidant enzymes as stress signal molecules.

Mutlu *et al.* (2009) conducted an experiment with two wheat (*Triticum aestivam* L.) cultivars: salt-tolerant (Gerek-79) and salt-sensitive (Bezostaya) to assess the effects of

salicylic acid (SA) and salinity on the activity of apoplastic antioxidant enzyme. The leaves of 10-d-old seedlings grown at nutrient solution with 0 (control), 250 or 500 mM NaCl were sprayed with 0.01 or 0.1 mM SA. Then, the activities of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) were determined in the fresh leaves obtained from 15-d-old seedlings. The NaCl applications increased CAT and SOD activities in both cultivars, compared to those of untreated control plants. In control plants of the both cultivars, 0.1 mM SA increased CAT activity, while 0.01 mM SA slightly decreased it. SA treatments also stimulated SOD and POX activity in the salt-tolerant cultivar but significantly decreased POX activity and had no effect on SOD activity in the salt-sensitive cultivar. Under salinity, the SA treatments significantly inhibited CAT activity, whereas increased POX activity.

Arfan *et al.* (2007) reported that exogenous application of salicylic acid (SA) through the rooting medium can modulate the photosynthetic capacity of two wheat cultivars differing in salinity tolerance. So, they germinated seeds of a salt tolerant (S-24) and a moderately salt sensitive (MH 97) cultivar at 0 or 150 mM NaCl in Hoagland's nutrient solution containing different levels of salicylic acid (SA) (0, 0.25, 0.50, 0.75 and 1.00mM) for 7d. Seven-day old wheat seedlings were transferred to hydroponics and grown at 0, or 150 mM NaCl for further 30d. Different levels of salicylic acid (SA) were also maintained in the solution culture. After 30d, four plants out of six were harvested. Exogenous application of SA promoted growth and yield, and counteracted the salt stress-induced growth inhibition of salt tolerant S-24, whereas for MH-97 there was no improvement in growth or grain yield with SA application.

Kaydan *et al.* (2007) conducted an experiment to determine the effects of seed soaking in salicylic acid (10^{-2} mol L^{-1}, 10^{-4} mol L^{-1}, 10^{-6} mol L^{-1} and control) on the growth and some physiological characters in wheat (*Triticum aestivum* L.) under salinity (8 dSm^{-1}) and non-salinity conditions. NaCl reduced the emergence percentage, the growth parameters (shoot and root dry weight), K^+/Na^+ ratio, osmotic potential and photosynthetic pigments (Chl *a*, *b* and carotenoids) contents in wheat seedlings. Seed soaking in SA increased the emergence percentage, osmotic potential, shoot and root dry weight, K^+/Na^+ ratio, photosynthetic pigments (Chl *a*, *b* and carotenoids) contents in the salinity stressed wheat seedlings.

Deef (2007) reported that Pre-treatment of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) with Salicylic acid (SA) can be enhanced their tolerance to saline stress during germination. The alleviation of oxidative damage and increased resistance to salt stress induced by 150 mM NaCl treatments often correlate with a more efficient antioxidative defense systems and detoxification mechanisms. Pre-treatment of wheat and barley plants with salicylic acid (SA) enhanced antioxidant activities in concentration dependent manner and increased the stress tolerance of seedlings. Improved acclimation of SA-pre-treated plants to salt stress depended on the activation of the antioxidative and accumulation of ionic and non-ionic osmolytes.

As per the report of Afzal *et al.* (2006), Seeds primed with 50 ppm ascorbic acid and 50 ppm SA not only improved final germination count but also reduced the germination time under saline (15 $dScm^{-1}$) conditions. Seedling raised from primed seeds with 50 ppm SA followed by 50 ppm ascorbic acid had significantly higher lengths and fresh and dry weight of shoot than other treated or non-primed seeds under non-saline and saline conditions. But all hormonal priming treatments decreased the electrolyte leakage of steep water as compared to that of non-primed seeds even after 12 h of soaking. Hormonal priming with 50 ppm SA induced maximum decrease in electrolyte leakage, while an increase in electrolyte leakage was observed by 10 ppm ABA and 100 ppm ascorbic acid.

Hayat *et al.* (2005) conducted an experiment with Grains of wheat (*Triticum aestivum* L. cv. Raj-3077) which were soaked in 0, 10^{-5}, 10^{-4} or 10^{-3} M aqueous solutions of salicylic acid (SA) for 3, 6 or 9 h. The seedlings raised from grains pre-treated with 10^{-5} M SA possessed significantly higher leaf number, fresh and dry mass per plant, and nitrate reductase and carbonic anhydrase activities 30 and 40 days after sowing. However, 10^{-3} M SA reduced all the above-mentioned parameters.

Shakirova *et al.* (2003) reported that, wheat seedlings accumulated large amounts of proline under salinity stress which was further increased when salicylic acid was applied

exogenously, thereby alleviating the deleterious effects of salinity. Further, the treatment also lowered the level of active oxygen species and therefore the activities of SOD and Peroxidase (POX) were also lowered in the roots of young wheat seedlings. SA also increased the resistance of wheat seedlings to salinity. The soaking of wheat seeds in 0.05 mM SA reduced the damaging effects of salinity on seedlings growth and accelerated the growth processes.

Chapter 3

MATERIALS AND METHODS

This chapter presents a brief description about experimental period, site description, climatic condition, crop or planting materials, treatments, experimental design and layout, crop growing procedure, fertilizer application, uprooting of seedlings, intercultural operations, data collection and statistical analysis.

3.1 Location

The experiment was conducted at the experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka ($90^0 77'$ E longitude and $23^0 77'$ N latitude) during the period from November 2013 to March 2014. The location of the experimental site has been shown in Appendix I.

3.2 Soil

The soil of the experimental area belonged to the Modhupur tract (AEZ No. 28). It was a medium high land with non-calcarious dark grey soil. The pH value of the soil was 5.6. The physical and chemical properties of the experimental soil have been shown in Appendix II.

3.3 Climate

The experimental area was under the subtropical climate and was characterized by high temperature, high humidity and heavy precipitation with occasional gusty winds during the period from April to September, but scanty rainfall associated with moderately low temperature prevailed during the period from October to March. The detailed meteorological data in respect of air temperature, relative humidity, rainfall and sunshine hour recorded by the meteorology center, Dhaka for the period of experimentation have been presented in Appendix III.

3.4 Materials

3.4.1 Plant materials

Two wheat varieties BARI Gom 21 (Satabdi) and BARI Gom 25 were used in the experiment. The features of two varieties are presented below:

BARI Gom 21: BARI Gom 21 variety is grown in rabi season. It is a line crossed variety of wheat released by BARI in 2000. Grain colour its white and large in size. The cultivar matures at 105- 112 days of planting. It attains a plant height 90-100 cm. The cultivar gives an average yield of 3.60-5.0 t ha^{-1}

BARI Gom 25: The grains are of large in size and white colour. The cultivar matures at 102-110 days after planting. It attains a plant height 95-100 cm. The cultivar is moderately saline tolerant (6-8 mmohs cm^{-1}).The cultivar gives an average yield of 3.6-4.6 t ha^{-1}.

3.4.2 Earthen pot

Empty earthen pots with 18 inch depth were used for the experiment. Twelve kilogram sun-dried soils were put in each pot. After that, pots were prepared for seed sowing.

3.5 Salinity treatment

The salinity treatments were applied on 28, 35, 42, 49, 56 and 63 DAS. There were five salinity levels including control where developed by adding respected amount commercial NaCl salt to the soil/pot as water dissolved solution. The salinity levels were C (control), S50 (50 mM), S100 (100 mM), S150 (150 mM) and S200 (200 mM). When water added as irrigation without salt then it termed as control (C) while 204.75g salts in S50, 409.5g salts in S100, 614.25g salts in S150 and 819.0 g salts in S200 added in each pot. In order to spread homogenously in each pot the salts were dissolved in 70 liter water and were added to pots for proper salinity imposition.

3.6 Protectant treatments

Salicylic acid (SA) was used as a protectants. The concentration of SA was 1mM and applied as spray solution. 0.0518g powder form of SA was mixed with firstly in 70% alcohol and then in 1500 ml water to prepare solution.

3.7 Treatments

The experiment consisted of two factors as mentioned below:

a) Factor A: varieties

i. BARI Gom 21

ii. BARI Gom 25

b) Factor B: Salinity level

i. Control (C)
ii. 1 mM SA (SA)
iii. 50 mM NaCl (S50)
iv. 50 mM NaCl+1 mM SA (S50+SA)
v. 100 mM NaCl (S100)
vi. 100 mM NaCl+1 mM SA (S100+SA)
vii. 150 mM NaCl (S150)
viii. 150 mM NaCl+1 mM SA (S150+SA)
ix. 200 mM NaCl (S200)
x. 200 mM NaCl+1 mM SA (S200+SA)

3.8 Design and layout of the experiment

The experiment was laid out in a Randomized Completely Block Design (RCBD) with three replications. There were 60 pots all together replication with the given factors.

R_1		R_2		R_3	
BARI Gom 21	BARI Gom 25	BARI Gom 21	BARI Gom 25	BARI Gom 21	BARI Gom 25
C	SA	S50	S50+SA	S100	S100+SA
SA	S50	S50+SA	S100	S100+SA	S150
S50	S50+SA	S100	S100+SA	S150	S150+SA
S50+SA	S100	S100+SA	S150	S150+SA	S200
S100	S100+SA	S150	S150+SA	S200	S200+SA
S100+SA	S150	S150+SA	S200	S200+SA	C
S150	S150+SA	S200	S200+SA	C	SA
S150+SA	S200	S200+SA	C	SA	S50
S200	S200+SA	C	SA	S50	S50+SA
S200+SA	C	SA	S50	S50+SA	S100

3.9 Seed collection

Seeds of BARI Gom 21 and BARI Gom 25 were collected from Bangladesh Agriculture Research Institute, Joydebpur, Gazipur.

3.10 Pot preparation

The collected soil was sun dried, crushed and sieved. The soil and fertilizers were mixed well before placing the soils in the pots. Soils of the pots were poured in polythene bag. Each pot was filled up with 12 kg soil. Pots were placed at the net house of Sher-e Bangla Agricultural University. The pots were pre-labeled for each variety and treatment. Finally, water was added to bring soil water level to field capacity.

3.11 Fertilizer application

Fertilizers used in the experimental pots were urea, triple super phosphate, muriate of potash and gypsum at the rate given value in a tabulated form one-third of urea and the whole amount of other fertilizers were incorporated with soil at final pot preparation

before sowing. Rest of the nitrogen were applied in two equal splits one at 30 days after sowing (DAS) and the other at 60 DAS.

Fertilizer doses are as follows:

Fertilizers	@kg/ha	Actual amount/pot (g)
Urea	220	4.6
Triple super phosphate	180	4.1
Muriate of potash	120	2.7
Gypsum	50	1.2

3.12 Seed sowing technique

Fifteen healthy seeds of each variety were sown in each pot. After germination 9-10 plants were allowed to grow in each pot.

3.13 Intercultural operations

3.13.1 Gap filling and thinning

After sowing seeds continuous observation was kept. It was observed that no single seed failed to germinate. So, there was need of gap filling. Keen observation was made for thinning to maintain 9-10seedlings. Thinning was done to maintain spacing of the plants.

3.13.2 Weeding and irrigation

Sometimes there were some weeds observed in pots which were uprooted manually. Irrigation was given after salt treatment at 35 DAS to maintain field capacity moisture level.

3.13.3 Plant protection measure

There was no insect pests appeared. Moreover, the pots were protected by netting to prevent birds.

3.14 General observation of the experimental pots

Observations were made regularly and the plants looked normal green. No Lodging was observed at any stage. The maximum tillering, panicle initiation, and flowering stages were not uniform.

3.15 Germination test

Germination test was performed before sowing the seeds in the pot. For laboratory test, petridishes were used. Filter paper was placed on petridishes. Firstly seeds were soaked in 10ml of 70% alcohol for 10 minutes. Then half amounts of seeds were soaked in SA solution for 1 hr. The filter paper soaked with 10ml water for Control and 10ml of 50 mM, 100 mM, 150 mM and 200 mM NaCl solution. Seeds were placed in petridishes randomly. Data were collected five days after placement of seed. For each variety data was taken three times after placing the seeds in petridish for three times after and after.

3.16 Collection of data

Data were recorded on the following parameters:

1. Germination parameters:

- Germination (%)
- Normal seedling (%)
- Abnormal seedling (%)
- Shoot and root length (cm)
- Fresh weight of shoot and root (g) seedling^{-1}
- Dry weight (g) seedling^{-1}

2. Crop growth parameters:

- Plant height (cm) at 15 days interval up to harvest
- Tiller no.plant^{-1} at 15 days interval up to harvest
- Above ground fresh weight plant^{-1}(g) at 15 days interval up to harvest
- Above ground dry matter weight plant^{-1} (g) at 15 days interval up to harvest

3. Physiological parameters:

- Chlorophyll (SPAD) value of leaf (mg cm^{-2})
- Relative water content (RWC)

4. Biochemical parameters

- Lipid peroxidation
- Reactive oxygen species generation
- H_2O_2 content
- Ascorbic acid content
- Glutathione content
- Activities of antioxidant enzymes (APX, MDHAR, DHAR, GR, GST, POD and CAT)

5. Yield contributing parameter:

- Spikelets $Spike^{-1}$(no.)
- Spike length(cm)
- 1000-seeds weight (g)
- Effective tiller (no.)
- Non-effective tiller (no.)

4. Yields:

- Grain yield $plant^{-1}$
- Straw yield $plant^{-1}$
- Biological yield $plant^{-1}$
- Harvest index (%)

3.17 Procedure of sampling germination parameter

3.17.1 Germination (%)

Germination (%) was measured by the following formula-

$$\text{Germination (\%)} = \frac{\text{Number of germinated seed}}{\text{Number of seed placed}} \times 100$$

3.17.2 Normal and abnormal seedlings (%)

The normal seedlings and abnormal seedlings were classified according to the prescribed rules given by ISTA (1999).

3.17.3 Shoot and root length (cm)

Shoot and root length was measured from five seedlings randomly.

3.17.4 Fresh weight of shoot and root (g) seedling^{-1}

Five sample seedlings were given for taking fresh weight. Then seedlings shoot and root were weighed in balance and averaged them to take fresh weight seedling^{-1}

3.17.5 Dry weight (g) seedling^{-1}

After weighing the fresh weight, seedlings were them in an electric oven maintaining 60^0c for 24 hours. Then it was weighed in balance to take dry weight and then averaged them.

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

3.18.1 Plant height (cm)

The height of the wheat plants was recorded from 30 days after sowing (DAS) at 15 days interval up to 60 DAS, beginning from the ground level up to tip of the leaf was counted as height of the plant. The average height of five plants was considered as the height of the plant for each pot.

3.18.2 Tiller hill^{-1}

Total tiller number was taken from 30 DAS at 15 days interval up to 60 DAS. The average number of tillers of five plants was considered as the total tiller no plant^{-1}.

3.18.3 Fresh weight plant^{-1} (g)

Three sample plants uprooted from each pot unbiasly and wash them in water. Then the plants were weighed in a balance and averaged them to have fresh weight plant^{-1} and taken from 30 DAS at 15 days interval up to 60 DAS.

3.18.4 Dry weight plant^{-1} (g)

Three sample plants after weighing for fresh weight was dried them in an electric oven maintaining 60^{0}c for 48 hours. Then the plans were weighed in an electric balance and averaged them to have dry weight plant^{-1}. The data were collected from 30 to 60 DAS at an interval of 15 days.

3.19 Procedure of sampling physiological parameters

3.19.1 Chlorophyll content (mg cm^{-2})

Three leaflets were randomly selected from each pot. The top and bottom of each leaflet were measured with atLEAF as atLEAF value. Then it was averaged and total chlorophyll content was measured by the conversion of atLEAF value into SPAD units and then total chl content was measured.

3.19.2 Relative water content (%)

Three leaflets were randomly selected from each pot and cut with scissors. Relative water content (RWC) was measured according to Barrs and Weatherley (1962). Leaf laminas were weighed (fresh wt., FW) and then immediately floated on distilled water in a petridish for 4 h in the dark. Turgid weights (TW) were obtained after drying excess surface water with paper towels. Dry weights (DW) were measured after drying at 80∘C for 48 h. Then calculation was done using the following formula:

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

3.20 Procedure of sampling oxidative stress markers

3.20.1 Lipid peroxidation

The level of lipid peroxidation was measured by estimating MDA, a decomposition product of the peroxidized polyunsaturated fatty acid component of the membrane lipid, using thiobarbituric acid (TBA) as the reactive material following the method of Heath and Packer (1968) with slight modifications. The leaf samples (0.5 g) were homogenized in 3 ml 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 11,500g for 15 min. One ml supernatant was mixed with 4 ml of 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 at 11,500g for 15 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol of MDA g^{-1} fresh weight.

3.20.2 Measurement of H_2O_2

H_2O_2 was assayed according to the method described by Yu *et al.* (2003). H_2O_2 was extracted by homogenizing 0.5 g of leaf samples with 3 ml of 50 mM K-phosphate buffer pH (6.5) at 4^0C. The homogenate was centrifuged at 11,500g for 15 min. 3ml of supernatant was mixed with 1 ml of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v), and the mixture was then centrifuged at 11,500g for 12 min at room temperature. The optical absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content ($\varepsilon = 0.28$ lM^{-1} cm^{-1}) and expressed as l mol g^{-1} fresh weight.

3.21 Extraction and Measurement of Ascorbate and Glutathione

Wheat leaves (0.5 g fresh weight) were homogenized in 3 mL ice-cold acidic extraction buffer (5% metaphosphoric acid containing 1 mM EDTA) using a mortar and pestle. Homogenates were centrifuged at 11,500 ×g for 15 min at 4°C and the supernatant was collected for analysis of ascorbate and glutathione.

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). The AsA was assayed spectrophotometrically at 265nm in 100 mM KP buffer (pH 7.0) with 0.5 unit of ascorbate oxidase (AO). A specific standard curve with AsA was used for quantification.

The glutathione pool was assayed according to previously described methods Murphy *et al.* (2003), Paradiso *et al.* (2008) with modifications utilizing 200 span style of aliquots of supernatant neutralized with 300 span style 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 NADPH in the presence of GR, and glutathione content is evaluated by the rate of absorption changes at 412 nm of 2-nitro-5-thiobenzoic acid (NTB) generated from the reduction of DTNB. GSSG was determined after removal of 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.

3.22 Determination of protein

The protein concentration of each sample was determined following the method of Bradford (1976) using BSA as a protein standard.

3.23 Enzyme extraction and assays

Using a pre-cooled mortar and pestle, 0.5 g of leaf tissue was homogenized in 1 ml of 50 mM ice-cold K-phosphate buffer (pH 7.0) containing 100 mM KCl, 1 mM ascorbate, 5 mM

b-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 a temperature 0–4^{0}C.

Ascorbate peroxidase (EC: 1.11.1.11) activity was assayed following the method of Nakano and Asada (1981). The reaction buffer solution contained 50 mM K-phosphate

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 ll. 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}.

Monodehydroascorbate reductase (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, and 0.5 unit of AO and enzyme solution in a final volume of 700 ll. The reaction was started by the addition of AO. The activity was calculated from the change in ascorbate at 340 nm for 1 min using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

Dehydroascorbate reductase (EC: 1.8.5.1) activity was determined by the procedure of Nakano and Asada (1981). The reaction buffer contained 50 mM K-phosphate 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 an extinction coefficient of 14 mM^{-1} cm^{-1}.

Glutathione reductase (EC: 1.6.4.2) activity was measured by the method of Hossain *et al.* (2010). The reaction mixture contained 0.1 M K-phosphate buffer (pH 7.8), 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 due to NADPH oxidation was recorded for 1 min. The activity was calculated using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

Glutathione S-transferase (EC: 2.5.1.18) activity was determined spectrophotometrically by the method of Hossain *et al.* (2006) with some modifications. The reaction mixture contained 100 mM Tris–HCl buffer (pH 6.5), 1.5 mM GSH, 1mM 1-chloro-2, 4-dinitrobenzene (CDNB) and enzyme solution in a final volume of 700 ll. The enzyme reaction was initiated by the addition of CDNB and the increase in absorbance was

measured at 340 nm for 1 min. The activity was calculated using the extinction coefficient of 9.6 mM^{-1} cm^{-1}.

POD activity was determined by the method of Shannon *et al.* (1966). The reaction mixture contained 2.9 cm^3 of 0.1 M phosphate buffer (pH 7.0), 0.04 cm^3 of 0.1 M H_2O_2, 0.04 cm^3 of 0.2 % O-dianisidine and 0.02 cm^3 of enzyme extract. The change in absorbance was read at 470 nm for 4 min. One enzyme unit is defined as change in 1 unit of absorbance min^{-1}.

Catalase (EC: 1.11.1.6) activity was measured according to the method of Hossain *et al.* (2010) by monitoring the decrease of absorbance at 240 nm for 1 min caused by the decomposition of H_2O_2. The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0), 15 mM H_2O_2 and enzyme solution in a final volume of 700 ll. The reaction was initiated with enzyme extract and the activity was calculated using the extinction coefficient of 39.4 M^{-1} cm^{-1}.

3.24 Procedure of measuring yield and yield contributing parameter

3.24.1 Plant height (cm)

Plant height was measured from the soil level to the apex of the leaf or spike in randomly 5 plants of each pot.

3.24.2 Total number of tillers $hill^{-1}$

The total number of tillers $hill^{-1}$ was counted from selected samples and were grouped in effective and non-effective tillers $plant^{-1}$.

3.24.3 Spike length (cm)

Spike length was recorded from the basal nodes of the rachis to apex of each spike.

3.24.4 Spikelet spike^{-1}

Grains of 5 randomly selected spike of each replication were counted and then the average number of grains for each spike was determined.

3.24.5 1000-grain weight (g)

One hundred clean sun dried grains were counted from the seed stock obtained from the sample plants and weighed by using an electronic balance. Then it was converted into thousand grain weight.

3.24.6 Grain yield (g) plant^{-1}

The grains were separated by threshing per plant and then sun dried and weighed.

3.24.7 Straw yield (g) plant^{-1}

The straw were separated by threshing per plant and weighed.

3.24.8 Biological yield (g) plant^{-1}

Biological yield was calculated by using the following formula:

Biological yield= Grain yield + straw yield

3.24.9 Harvest index (%)

It denotes the ratio of economic yield to biological yield and was calculated following the formula of Gardner *et al.* (1985). It was calculated by using the following formula:

$$\text{Harvest index (HI)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

3.25 Statistical analysis

The data obtained for different parameters were statistically analyzed following computer based software XLSTAT 2014 (AddinSoft, 2014) and mean separation was done by LSD at 5% level of significance.

Chapter 4

RESULTS AND DISSCUSSIONS

4.1 Germination parameters

4.1.1 Germination percentage

4.1.1.1 Effect of variety

Percentage of germination showed significant variation among the different varieties (Fig. 1(A)). BARI Gom 25 (68.88%) had highest germination percentage, where BARI Gom 21 (62.98%) had lowest germination percentage.

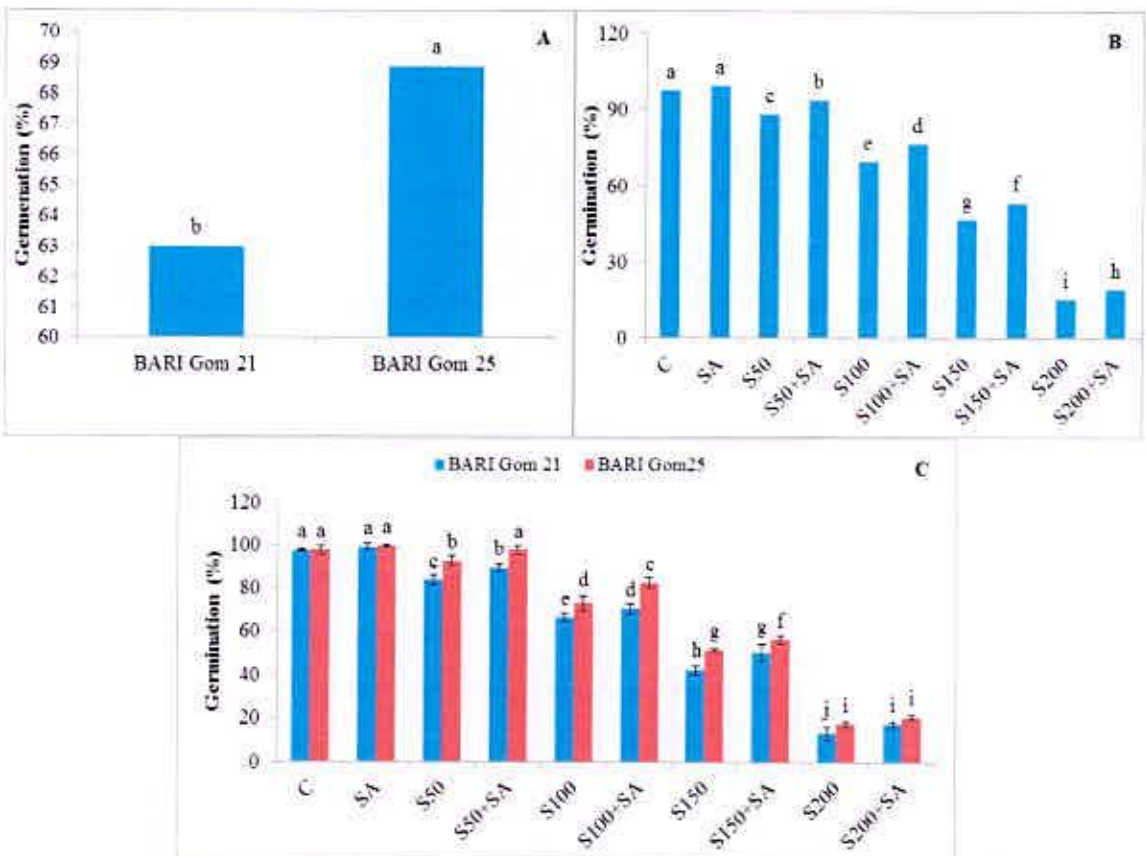

Fig. 1 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on germination percentage of wheat.

Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.1.2 Effect of salinity treatments

The data (Fig. 1B) showed that salinity also reduced the percentage of germination. On the other hand, the magnitude of decrease was less in SA treated salt stressed condition as compared to without treated salt stressed condition. However, germination percentage was higher in control and only SA treated plant (97.67 and 99.33%, respectively).

4.1.1.3 Interaction effect of variety and salinity treatments

Germination percentage decreased with the increase in salinity level. Germination percentage fell to 67 and 73% from 97 and 99% when exposed to 100 mM salinity; SA treatment increased the germination percentage up to 71 and 83% under 100 mM salinity stress for BARI Gom 21 and BARI Gom 25, respectively (Fig. 1C). Under 200 mM salinity stress in case both of both varieties, Germination percentage significantly dropped at 14 and 18% and germination percentage could not be increased significantly even though treated with SA in BARI Gom 25 but in BARI Gom 21 it was sharply increased. In any case, germination percentage was always higher in BARI Gom 25 than BARI Gom 21.

4.1.2 Normal seedling

4.1.2.1 Effect of variety

The number of normal seedling varied significantly due to variety shown in Fig. 2A. It was observed that BARI Gom 25 produced significantly the highest number of normal seedling (57.42%), where BARI Gom 21 produced lower number of normal seedling (48.92%).

4.1.2.2 Effect of salinity treatments

Salinity caused a significant reduction of normal seedling compared to control (Fig. 2B). The highest normal seedling was found in only SA and SA treated 50 mM stressed plant. On the contrary, SA increased normal seedling number compared to its respective control but not similar with only SA and SA treated 50 mM stressed plant (8, 48, 88 and 93% at 50, 100, 150 and 200 mM stressed condition, respectively).

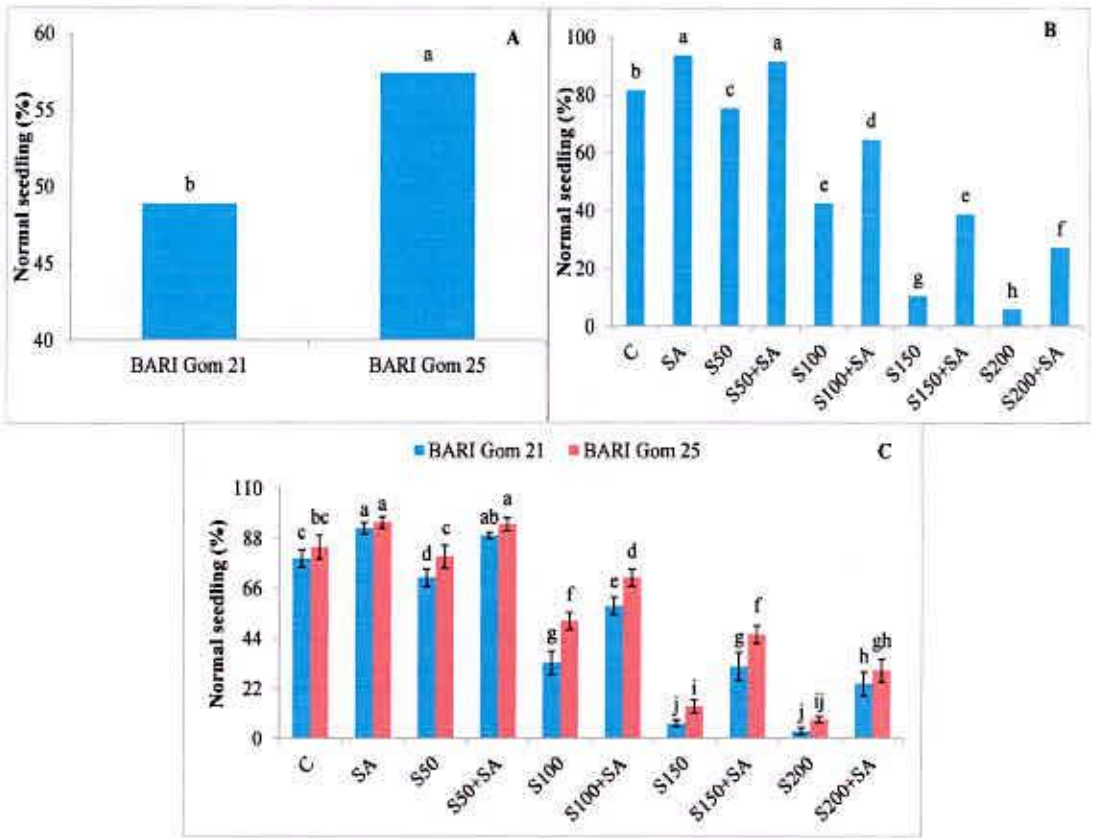

Fig. 2 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on normal seedling of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.2.3 Interaction effect of variety and salinity treatments

Like germination percentage number of normal seedling depends on the condition where it is grown. Seedling growth hampered under salt stress and no. of normal seedling decreased with the increase in salinity level. In case of BARI Gom 21, at 100 and 150 mM salinity stress the number of normal seedling were 33.33 and 6.67% respectively

whereas BARI Gom 21 seedling treated with SA under same level of stresses the number of seedling were 58.33 and 31.67% (Fig. 2C). However, In case of BARI Gom 25, at 100 and 150 mM salinity stress the number of normal seeding were 51.67 and 14.17% respectively whereas BARI Gom 25 seedling treated with SA under same level of stresses the number of seedling were 70.83 and 45.83%. The highest number of Normal seedling (92.5% in case of BARI Gom 21 and 95 and 94.17% in case of BARI Gom 25, respectively) was observed under normal condition and SA treated 50 mM stressed condition (Fig. 2(C)).

4.1.3 Abnormal seedling

4.1.3.1 Effect of variety

Significant variation was observed in number of abnormal seedling due to the effect of variety shown in Fig. 3A. BARI Gom 21 produced higher abnormal seedling (39.14%) compared to BARI Gom 25.

4.1.3.2 Effect of salinity treatments

Upon exposure to salt stress, abnormal seedling increased significantly compared to control (Fig. 3B). The highest abnormal seedling was found at 150 mM salt stressed plant (54.32%). Moreover, the lowest abnormal seedling was found in only SA treated plant (4.61%).

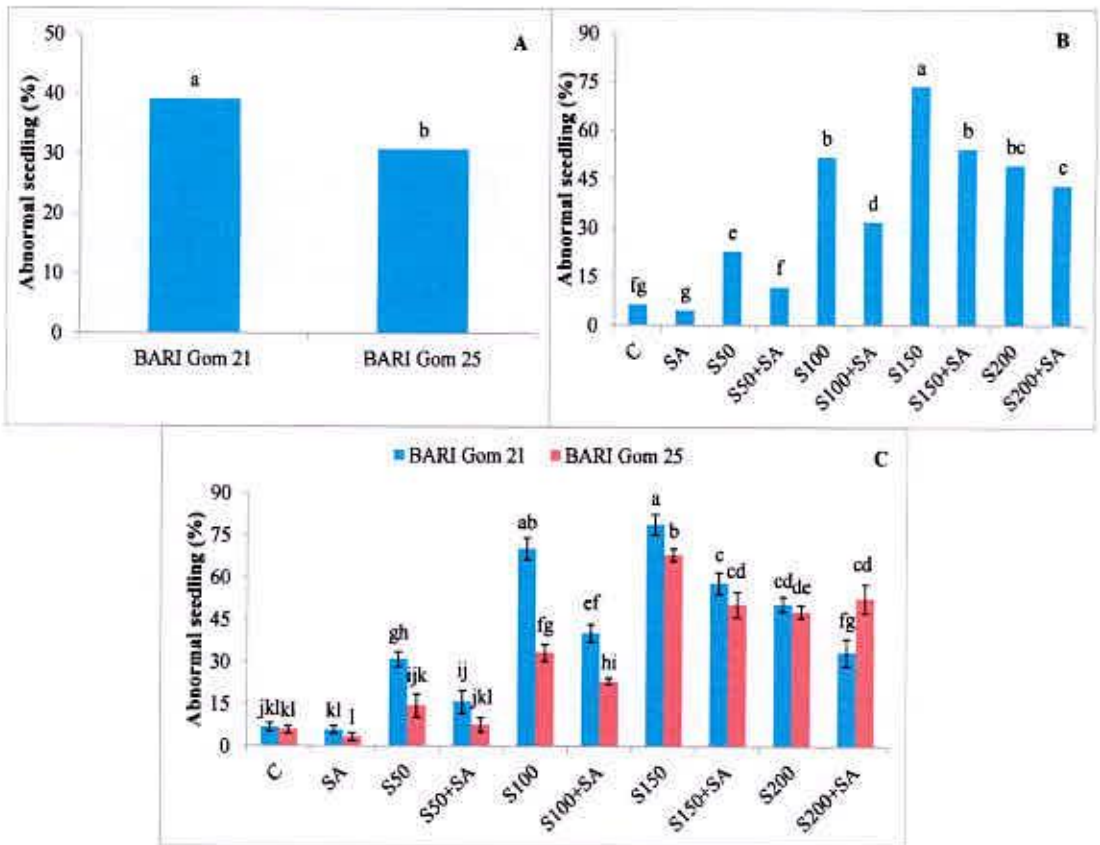

Fig. 3 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on abnormal seedling of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.3.3 Interaction effect of variety and salinity treatments

SA treatment reduced the number of abnormal seedling under salt stress condition. In case of BARI Gom 21 the no. of abnormal seedling was significantly higher than that of BARI Gom 25. At 150 mM salinity level caused the highest no. of abnormal seedling (78.97%) in case of BARI Gom 21 whereas the lowest no. of abnormal seedling (3.35%) found when seedling treated only with SA in case of BARI Gom 25 (Fig. 3C). At any treatment the no. of abnormal seedling was higher in BARI Gom 21 than that of BARI Gom 25.

4.1.4 Length of shoot (cm)

4.1.4.1 Effect of variety

Varietal variation had significant effect on length of shoot over time (Table 1). The highest length of shoot was found in BARI Gom 25 compared to BARI Gom 21 (4.52 cm).

4.1.4.2 Effect of salinity treatments

Different salinity treatments affected shoot length significantly. Salinity treatment reduced length of shoot compared to control (Table 1). On the contrary, SA with saline treatments increased shoot length (11, 49 and 69% at 50, 100 and 150 mM, respectively) where higher level of salinity treatment did not affect by SA spraying (200 Mm salinity stress).

Table 1 Effect of variety and salinity treatments on length of shoot and root of wheat seedling

Variety	Length of shoot (cm)	Length of root (cm)
BARI Gom 21	4.52b	4.35b
BARI Gom 25	5.09a	5.20a
LSD (0.05)	**0.101**	**0.148**
CV (%)	**4.02**	**5.93**
Treatment		
C	7.93a	8.09a
SA	8.01a	8.13a
S50	7.10b	6.53b
S50+SA	7.12b	6.53b
S100	4.10d	4.45d
S100+SA	5.33c	5.31c
S150	2.50f	2.53f
S150+SA	3.30e	3.30e
S200	1.35g	1.47g
S200+SA	1.28g	1.44g

LSD (0.05)	**0.226**	**0.331**
CV (%)	**4.02**	**5.93**

4.1.4.3 Interaction effect of variety and salinity treatments

The data (Fig. 4) showed that salt stress significantly reduced the shoot length as compared to control conditions in BARI Gom 21 (salt sensitive) and BARI Gom 25 (salt tolerant) wheat cultivars. Extent of reduction was higher in BARI Gom 21 than BARI Gom 25. On the contrary, exogenous application of SA increased the shoot length in both the cultivars under saline and non-saline conditions. Higher (8.02 and 8.12g) shoot length was found in control and only SA treated plant of BARI Gom 25 and (7.9g) only SA treated seedlings of BARI Gom 21. On the other hand, control (7.84g) of BARI Gom 21 gave statistically similar result like control and only SA treated seedlings of both varieties (Fig. 4).

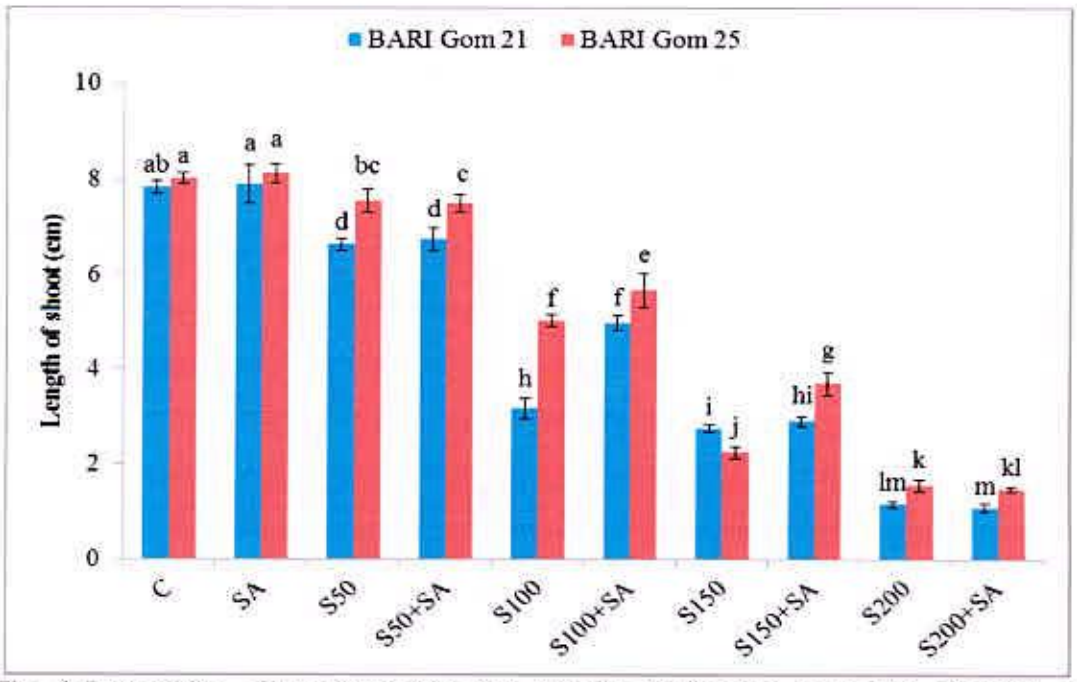

Fig. 4 Interaction effect of variety and salinity treatments on length of shoot of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.5 Length of root

4.1.5.1 Effect of variety

The length of root varied significantly due to variety shown in Table 1. It was observed that BARI Gom 25 produced significantly the highest root length (5.20 cm), where BARI Gom 21 produced lower root length (4.35 cm).

4.1.5.2 Effect of salinity treatments

Salinity caused a significant reduction of root length compared to control (Table 1). The highest root length was found in control and only SA treated plant. On the contrary, SA increased root length compared to its respective control but not similar with control and only SA treated plant (20, 35 and 60% at 50, 100 and 150 mM stressed condition, respectively). 200 mM salt stressed condition was also not affected by SA treatment (Table 1).

4.1.5.3 Interaction effect of variety and salinity treatments

Salinity caused (Fig. 5) a significant reduction ($p \leq 0.05$) in the root length of wheat plants of both cultivars compared to those in non-saline solution and magnitude of decrease was less in BARI Gom 25 as compared to BARI Gom 21. Sharp increases in root length were observed in the seedlings which were treated with SA under salt stressed condition (27, 45 and 63% for BARI Gom 21 and 8, 7 and 4% for BARI Gom 25 at SA treated 50, 100 and 150mM, respectively) than the respective controls (Fig. 5). Moreover, both of variety did not get significant result at 200 mM and SA treated 200 mM salt stressed condition.

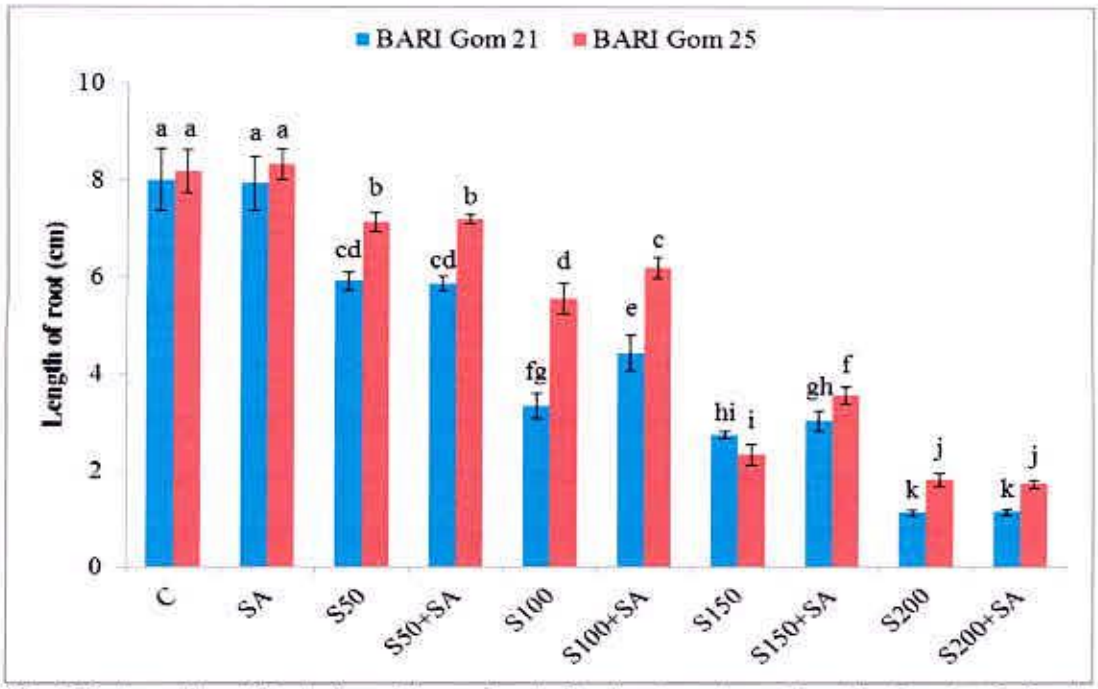

Fig. 5 Interaction effect of variety and salinity treatments on length of root of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.6 Fresh weight of shoot seedling^{-1}

4.1.6.1 Effect of variety

The fresh weight of shoot varied significantly due to variety shown in Table 2. It was observed that BARI Gom 25 produced significantly the highest fresh weight of shoot (0.008 g) seedling^{-1}, where BARI Gom 21 produced lower fresh weight of shoot (0.007 g) seedling^{-1}.

Table 2 Effect of variety and salinity treatments on fresh weight of shoot and root and dry weight seedling^{-1} of wheat seedling

Variety	Fresh weight of shoot (g) seedling^{-1}	Fresh weight of root (g) seedling^{-1}	Dry weight (g) seedling^{-1}
BARI Gom 21	0.007b	0.004b	0.001b
BARI Gom 25	0.008a	0.006a	0.002a
LSD (0.05)	**0.002**	**0.002**	**0.007**
CV (%)	**6.09**	**6.13**	**6.33**
Treatment			
C	0.013a	0.009a	0.0033a
SA	0.013a	0.009a	0.0033a
S50	0.011b	0.007b	0.0029b
S50+SA	0.011b	0.007b	0.003b
S100	0.007d	0.005d	0.0016d
S100+SA	0.008c	0.006c	0.0024c
S150	0.004f	0.003f	0.001f
S150+SA	0.006e	0.005e	0.0013e
S200	0.002g	0.002g	0.001f
S200+SA	0.002g	0.002g	0.001f
LSD (0.05)	**0.005**	**0.004**	**0.002**
CV (%)	**6.09**	**6.13**	**6.33**

4.1.6.2 Effect of salinity treatments

Salinity caused a significant reduction of fresh weight of shoot compared to control (Table 2). The highest (0.013 and 0.013 g) seedling^{-1} fresh weight of shoot was found in control and only SA treated plant, respectively. On the contrary, SA increased effective tiller number compared to its respective control but not similar with control and only SA treated plant. 200 mM salt stressed condition was also not affected by SA treatment (Table 2).

4.1.6.3 Interaction effect of variety and salinity treatments

Fresh weight of shoot was higher in unstressed control of both varieties than salt stressed plants. As shown in Fig. 32, salinity stress treatment decreased fresh weight of shoot by 17, 51, 65 and 93% for BARI Gom 21 and 10, 41, 77 and 86% for BARI Gom 25 at 50, 100, 150 and 200 mM salinity stressed condition (Fig. 6). However, SA supplementation in salt stressed plants caused increases fresh weight of shoot for both of variety. In different, 200 mM and SA treated 200 mM plant gave statistically similar result for both of variety (Fig. 6). Control and only SA treated plant of BARI Gom 25 produced higher fresh weight of shoot which was statistically similar with control and only SA treated plant of BARI Gom 21 fresh weight result.

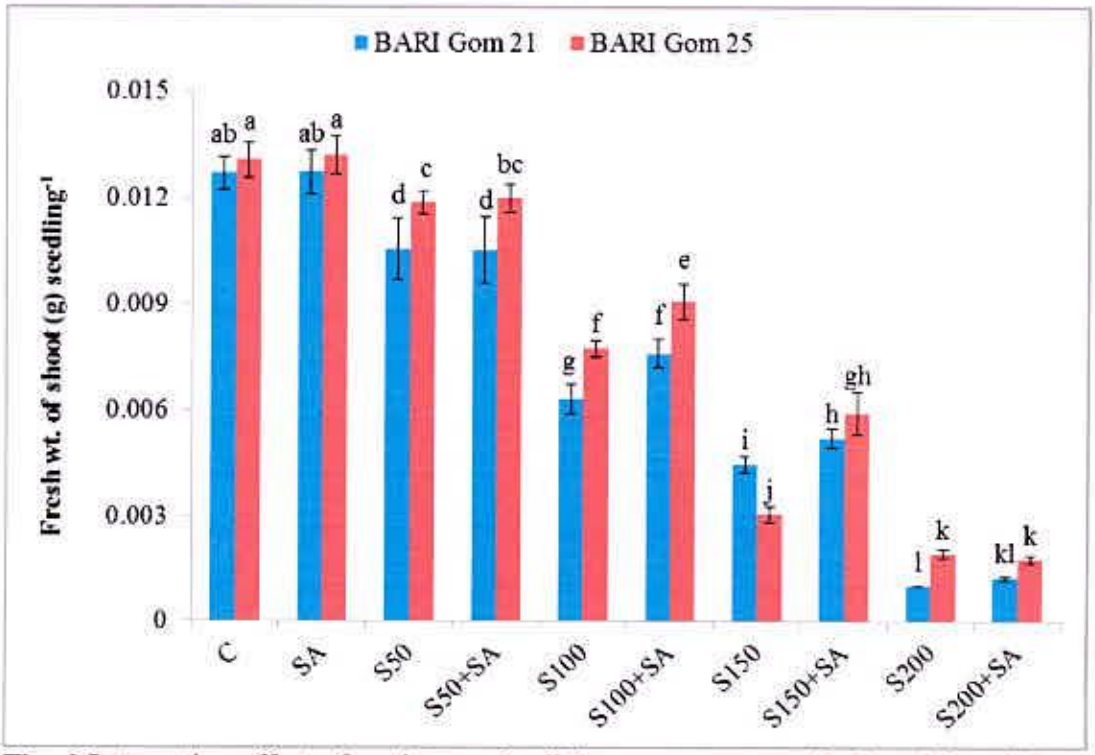

Fig. 6 Interaction effect of variety and salinity treatments on fresh weight of shoot seedling^{-1} of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.7 Fresh weight of root seedling^{-1}

4.1.7.1 Effect of variety

There was significant effect of varieties, salinity and SA treatments on fresh weight of root on wheat varieties (Table 2). Additionally BARI Gom 25 gave highest fresh weight of root (0.006 g) seedling^{-1} compared to BARI Gom 21.

4.1.7.2 Effect of salinity treatments

Exposure to salt stress resulted in significant decreases in fresh weight of root (23, 45, 67 and 78% at 50, 100, 150 and 200 mM stress, respectively). However, SA with saline treatment increased fresh weight up to 200 mM salt stress (Table 2). At 200 mM salinity stress SA produce statistically similar result with respective control.

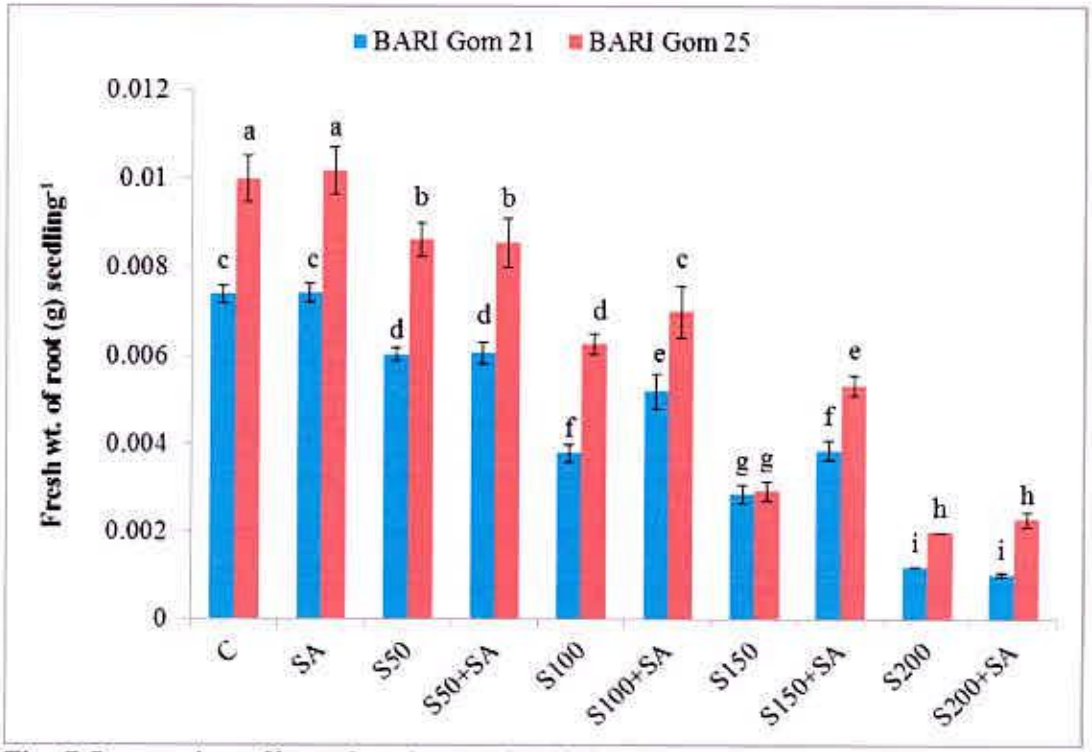

Fig. 7 Interaction effect of variety and salinity treatments on fresh weight of root seedling^{-1} of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.7.3 Interaction effect of variety and salinity treatments

Fresh weight of root was also decreased in the same way which was 19%, 49%, 62%, 84% for BARI Gom 21 and 14%, 38% 71%, 80% for BARI Gom 25 at 50, 100, 150 and 200 mM stressed condition, respectively (Fig. 11). Importantly, SA supplementation in salt treatment significantly increased the fresh weight of root in salt stressed seedlings. The higher result was found in BARI Gom 25 control and only SA treated seedlings (0.01 and 0.0102 g, respectively) than BARI Gom 21. But, the increment result was found up to 150 mM salt treatment. Furthermore it gave no significant result.

4.1.8 Dry weight seedling^{-1}

4.1.8.1 Effect of variety

Significant variation was observed for dry matter weight due to varietal variation shown in Table 2. The highest dry matter found in BARI Gom 25 which was 0.002 g seedling^{-1}. On the other hand, BARI Gom 21 gave lowest dry matter weight which was 0.001 g seedling^{-1}.

4.1.8.2 Effect of salinity treatments

For different salinity treatments with or without SA spraying, significant variation was observed for dry matter weight (Table 2). Control (0.0033 g) seedling^{-1} and only SA treated plant (0.0033 g) seedling^{-1} gave similar highest dry weight compared to other saline treatment and with or without SA treatment. On the contrary, spraying with SA gave higher dry matter weight than saline treatment without SA treatment. But 200 mM saline condition gave similar dry weight with SA treated 200 mM saline condition.

4.1.8.3 Interaction effect of variety and salinity treatments

Fig. 8 shows that seedling dry weight was decreased by adverse effect of salinity treatment when compared with control. Exogenously applied SA into saline treatment ameliorated the salinity stress as indicated by a significant increase in seedling dry matter. Higher seedling dry weight was recorded in only SA treated seedlings (0.0035g) of BARI Gom 25 which was statistically similar with control (0.0034 g) of BARI Gom

25 (Fig. 8). On the other hand, 200 mM salt stressed and SA treated seedlings did not give any significant result. Among the cultivars, BARI Gom 25 (salt tolerant) showed a better performance and produced more dry weight under salt stress when compared with BARI Gom 21; however, the reverse was true under non-saline conditions.

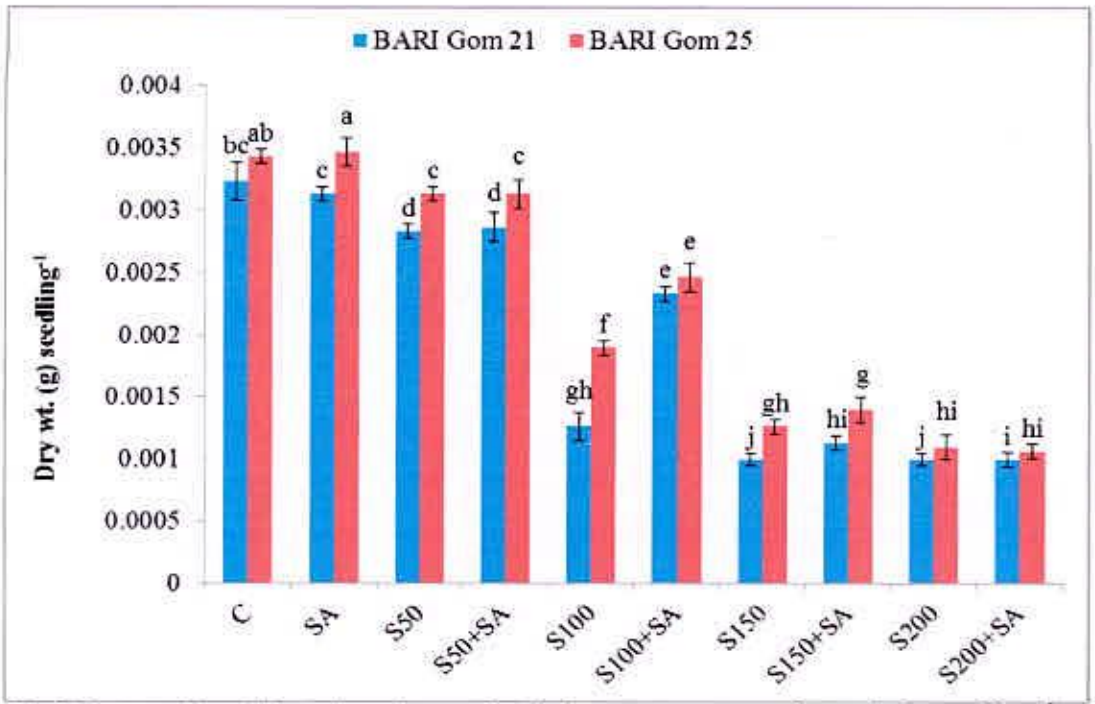

Fig. 8 Interaction effect of variety and salinity treatments on dry weight seedling^{-1} of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a with different letters are significantly different at $p \leq 0.05$ applying LSD test

Germination percentage drastically reduced at 200 mM salinity. Osmotic stresses reduce germination percentage (Cornelia *et al.*, 2008; Maghsoudi *et al.*, 2010). The seeds pre-treated with SA solutions exhibited higher germination percentage. These results are consistent with those of Rajesekaran *et al.* (2002) in carrots, Maghsoudi *et al.* (2010) in wheat and El-Tayeb, (2005) in barley who observed promotion in seed germination with SA. This may indicate that, SA pre-treated wheat seeds exhibited an increase in salt tolerance (Fig. 1).

The growth parameters (fresh and dry mass of roots and shoots, their lengths) decreased progressively with the rise of stress level, compared with the control (Fig. 2, 3, 4, 5, 6, 7, 8). These results are in agreement with those of Ghoulam *et al.* (2002), who showed that salinity caused a marked reduction in growth parameters of sugar beet plants. Salinity caused a dramatic decrease especially in 200 mM NaCl in root and shoot lengths and fresh-dry weights in 5 days old seedlings, but PGRs treatments ameliorated this adverse effect. The plants subjected to NaCl and subsequently treated with SA, possessed higher fresh and dry mass compared to those grown without SA treatment (Fig.6, 7, 8). Exogenous application of SA through the rooting medium had an ameliorative effect as well as growth promoting effect under non-saline and saline conditions (Arfan *et al.*, 2007; Afzal *et al.*, 2006; Karlıdag *et al.*, 2009; Azooz, 2009; Erdal *et al.*, 2011 and Turkyilmaz, 2012). These results were similiar to earlier studies which showed that exogenous application of SA promotes growth and counteracts the stress-induced growth inhibition in some crop species (Tari *et al.*, 2002; Singh and Usha, 2003). While working with wheat, Singh and Usha (2003) reported that foliar spray with SA counteracted growth inhibition caused by water stress, one of the major factors caused by salinity stress in plants. This result was consistent with the report of Kaydan *et al.* (2007), who found that a pre-sowing soaking treatment of the seeds that were treated with SA positively affected the shoot and root dry mass in wheat seedlings under both saline and nonsaline conditions.

4.2 Crop growth parameters

4.2.1 Plant height

4.2.1.1 Effect of variety

Plant height of the cultivars was measured at different growing period (Table 3). The highest plant height was found in BARI Gom 25 at all growth duration (25.95 at 30 DAS, 38.09 at 45 DAS, 61.96 at 60 DAS and 81.33cm at harvest) compared to BARI Gom 21.

4.2.1.2 Effect of salinity treatments

Significant variation was observed in plant height due to different salinity treatments. Salinity reduced the plant height compared to its respective control in all growth duration. However, SA increased plant height up to 200 mM salt stress for all stage (Table 3). But in case of SA treated 150 mM salinity stress, plant height became statistically significant at 30 DAS and at harvest. Furthermore, at 30 and 60 DAS SA treated 200 mM salt stressed condition gave lowest result compared to 200mM stress condition. The highest result found in control and only SA treated plant (30.83 and 30.38cm at 30 DAS, 45.95 and 46.63cm at 45 DAS, 70.85 and 71.08 at 60 DAS and 88.37 and 89.37cm, respectively).

Table 3 Effect of variety and salinity treatments on plant height of wheat at different days after sowing

Variety	Plant height (cm)			
	30 DAS	**45 DAS**	**60 DAS**	**At harvest**
BARI Gom 21	23.32b	33.30b	53.80b	70.54b
BARI Gom 25	25.95a	38.09a	61.96a	81.33a
LSD (0.05)	**0.429**	**0.65**	**0.62**	**0.83**
CV (%)	**3.33**	**3.46**	**2.06**	**2.10**
Treatment				
C	30.83a	45.95a	70.85a	88.37a
SA	30.38ab	46.63a	71.08a	89.37a
S50	25.93c	38.90c	65.68c	80.65c
S50+SA	29.45b	42.53b	67.25b	86.28b
S100	23.38d	35.30d	58.02e	75.32d
S100+SA	26.20c	34.83d	61.08d	79.15c
S150	21.48e	30.05f	50.22g	67.80e
S150+SA	21.92e	31.50e	53.12f	69.20e
S200	19.22f	25.62g	41.97h	61.83f
S200+SA	17.55g	25.67g	39.55i	61.40f
LSD (0.05)	**0.47**	**1.45**	**1.40**	**1.87**
CV (%)	**3.33**	**3.46**	**2.06**	**2.10**

4.2.1.3 Interaction effect of variety and salinity treatments

Sharp decreases in plant height was observed in response to salt stress, compared to the untreated control at 30, 45, 60 DAS and at harvest for both of variety (Table 4). However, SA supplementation with salt treatment increased plant height up to 100 mM salt stressed condition for both of variety. But after 100 mM stressed treatment plant height became statistically similar with SA treated salt treatment at 30, 45, 60 DAS and at harvest. Importantly, SA treatment with 200 mM salt stress at 30 DAS gave lower result than respective control but 100 mM at 45 and 60 DAS gave statistically similar result with SA treated salt stressed plant in case of BARI Gom 25 (Table 4). But in case of BARI Gom

21, SA treatment with 200 mM salt stress produced similar plant height with 200 mM stress treatment.

Table 4 Plant height of two wheat varieties at different growth duration induced by saline, SA and their combination

Variety	Treatment	Plant height (cm)			
		30 DAS	45 DAS	60 DAS	At harvest
BARI Gom 21	C	30.20ab	43.07bc	67.77c	85.10bc
	SA	29.73b	44.97b	68.17c	86.47b
	S50	24.67d	36.00e	61.07e	75.97e
	S50+SA	28.13c	40.17d	62.73de	82.00d
	S100	21.83f	33.60f	53.33g	69.00f
	S100+SA	24.53de	32.77f	58.97f	74.50e
	S150	19.67gh	27.43g	46.80h	60.73g
	S150+SA	20.17g	29.07g	47.67h	62.53g
	S200	17.77ij	22.93h	37.20j	54.87h
	S200+SA	16.47j	23.00h	34.30k	54.23h
BARI Gom 25	C	31.47a	48.83a	73.93a	91.63a
	SA	31.03ab	48.30a	74.00a	92.27a
	S50	27.20c	41.80cd	70.30b	85.33bc
	S50+SA	30.77ab	44.90b	71.77b	90.57a
	S100	24.93d	37.00e	62.70de	81.63d
	S100+SA	27.87c	36.90e	63.20d	83.80cd
	S150	23.30e	32.67f	53.63g	74.87e
	S150+SA	23.67de	33.93f	58.567f	75.87e
	S200	20.67fg	28.30g	46.73hi	68.80f

	S200+SA	18.63hi	28.33g	44.80i	68.57f
CV (%)		**3.33**	**3.46**	**2.06**	**2.10**
LSD (0.05)		**1.336**	**2.044**	**1.974**	**2.634**

Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.2 Tiller hill^{-1}

4.2.2.1 Effect of variety

Varietal variation had significant effect on tillers hill^{-1} over time (Table 5). The highest tiller hill^{-1} was found in BARI Gom 25 compared to BARI Gom 21 (1.45 at 30 DAS, 1.73 at 45 DAS and 1.84 at 60 DAS) throughout the growing period.

4.2.2.2 Effect of salinity treatments

Different salinity treatments affected tiller production significantly throughout the growing period. Salinity treatment reduced tiller number compared to control (Table 5). On the contrary, SA with saline treatments increased tiller number (11, 23 and 32% at 30DAS; 6, 14 and 26% at 45 DAS and 4, 16 and 26% at 60 DAS at 50, 100 and 150 mM, respectively) where higher level of salinity treatment did not affect by SA spraying (200 Mm salinity stress). Sometimes lower level of salinity treatment gave similar result with SA treated salinity treatment.

Table 5 Effect of variety and salinity treatments on total tiller hill^{-1} (no.) of wheat at different days after sowing

Variety	Tiller hill^{-1}		
	30 DAS	**45 DAS**	**60 DAS**
BARI Gom 21	1.45b	1.73b	1.84b
BARI Gom 25	1.76a	1.88a	2.04a
LSD (0.05)	**0.037**	**0.041**	**0.038**
CV (%)	**4.46**	**4.36**	**3.77**
Treatment			
C	2.10a	2.23a	2.42ab
SA	2.05a	2.28a	2.48a
S50	1.78c	2.07b	2.25c
S50+SA	1.88b	2.10b	2.33bc
S100	1.52e	1.67d	1.98d
S100+SA	1.63d	1.92c	2.05d
S150	1.33f	1.50e	1.57f
S150+SA	1.43e	1.67d	1.80e
S200	1.18g	1.30f	1.32g
S200+SA	1.10g	1.33f	1.18h
LSD (0.05)	**0.083**	**0.09**	**0.085**
CV (%)	**4.46**	**4.36**	**3.77**

4.2.2.3 Interaction effect of variety and salinity treatments

The data (Table 6) showed that salinity also reduced the tiller number hill^{-1} in both cultivars of wheat. On the other hand, the magnitude of decrease was less in BARI Gom 25 as compared to BARI Gom 21. The SA treated salt-stressed seedlings had significantly higher tiller number hill^{-1} (9, 31, and 39% at 30 DAS; 7, 16 and 31% at 45 DAS; 6, 19 and 29% at 60 DAS in BARI Gom 21 and 12,15 and 26% at 30 DAS; 6, 14 and 21% at 45 DAS; 2, 13 and 23% at 60 DAS in BARI Gom 25 at SA treated 50, 100 and 150 mM NaCl stresses, respectively), compared to the seedlings subjected to salt stress without SA treatment (Table 6). At 200 mM, SA could not give any higher result

compared to its respective control for both of variety. As a result, tiller number was statistically similar or decreased from its control.

Table 6 Effect of SA on tillers hill^{-1} of wheat cultivars under saline and nonsaline conditions at different age

Variety	Treatment	Tillers hill^{-1}		
		30 DAS	45 DAS	60 DAS
BARI Gom 21	C	1.97b	2.20bcd	2.37bc
	SA	1.93b	2.23abc	2.43ab
	S50	1.63e	2.03ef	2.23de
	S50+SA	1.80cd	2.07ef	2.23de
	S100	1.33g	1.60j	1.83f
	S100+SA	1.37g	1.87gh	1.93f
	S150	1.17ij	1.43l	1.47h
	S150+SA	1.20hi	1.53jkl	1.70g
	S200	1.07jk	1.17m	1.17j
	S200+SA	1.00k	1.20m	1.03k
BARI Gom 25	C	2.23a	2.27ab	2.47ab
	SA	2.17a	2.33a	2.53a
	S50	1.93b	2.10de	2.27cd
	S50+SA	1.97b	2.13cde	2.43ab
	S100	1.70de	1.73i	2.13e
	S100+SA	1.90bc	1.97fg	2.17de
	S150	1.50f	1.57jk	1.67g
	S150+SA	1.67e	1.80hi	1.90f
	S200	1.30gh	1.43l	1.47h

	S200+SA	1.20hi	1.47kl	1.33i
CV (%)		**4.46**	**4.36**	**3.77**
LSD (0.05)		**0.118**	**0.130**	**0.120**

Mean (±SD) was calculated from three replicates for each treatment. Values in a with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.3 Fresh weight plant^{-1}

4.2.3.1 Effect of variety

There was significant effect of varieties on fresh weight of wheat varieties (Table 7). Additionally BARI Gom 25 gave highest fresh weight (3.19g at 30 DAS, 6.72g at 45 DAS and 8.67g at 60 DAS) at all growth duration compared to BARI Gom 21.

4.2.3.2 Effect of salinity treatments

Exposure to salt stress resulted in significant decreases in fresh weight (14, 28, 37 and 53% at 30 DAS; 25, 31, 42 and 48% at 45 DAS and 20, 33, 46 and 59% at 60 DAS at 50, 100, 150 and 200 mM stress, respectively). However, SA with saline treatment increased fresh weight up to 200 mM salt stress (Table 7). At 200 mM salinity stress SA produce statistically similar result with respective control.

Table 7 Effect of variety and salinity treatments on fresh weight of wheat at different days after sowing

Variety	Fresh weight (g) plant^{-1}		
	30 DAS	45 DAS	60 DAS
BARI Gom 21	2.71b	5.57b	6.68b
BARI Gom 25	3.19a	6.72a	8.67a
LSD (0.05)	**0.085**	**0.144**	**0.162**
CV (%)	**5.50**	**4.48**	**4.05**
Treatment			
C	3.87a	8.42a	10.67a
SA	3.86a	8.56a	10.83a
S50	3.33b	6.33c	8.54c
S50+SA	3.78a	7.48b	9.54b
S100	2.82c	5.87d	7.20e
S100+SA	3.22b	6.22c	7.98d
S150	2.46d	4.89f	5.84f
S150+SA	2.47d	5.23e	7.06e
S200	1.84e	4.39g	4.48g
S200+SA	1.82e	4.08g	4.61g
LSD (0.05)	**0.189**	**0.322**	**0.363**
CV (%)	**5.50**	**4.48**	**4.05**

4.2.3.3 Interaction effect of variety and salinity treatments

As shown in Table 8, the fresh weight in wheat plants decreased significantly under salt stress compared to the control. Control and only SA treated plant of BARI Gom 25 gave significantly higher fresh weight (3.96, 4.01g at 30 DAS; 9.22, 9.46g at 45 DAS and 11.89, 12g at 60 DAS, respectively) compared to other salt stressed and SA treated stressed plants of those variety and other variety (BARI Gom 21). On the contrary, supplementation of SA under stressed condition could increase fresh weight of plant compared to its control for both of variety. But, it had limitation. Because it could not affect 200 mM stressed condition, where fresh weight would not increase or decrease.

Table 8 Fresh weight of two wheat varieties grown on normal and saline condition at different growth durations as affected by SA application

Variety	Treatment	Fresh weight (g) plant^{-1}		
		30 DAS	45 DAS	60 DAS
BARI Gom 21	C	3.78abc	7.62b	9.44d
	SA	3.72bc	7.67b	9.67cd
	S50	2.85de	5.67d	7.00h
	S50+SA	3.71bc	6.96c	8.85ef
	S100	2.56f	5.08e	6.67h
	S100+SA	2.78ef	5.67d	6.77h
	S150	2.11g	4.67ef	4.96i
	S150+SA	2.15g	4.78ef	5.44i
	S200	1.74h	4.04g	3.85j
	S200+SA	1.70h	3.56h	4.11j
BARI Gom 25	C	3.96ab	9.22a	11.89a
	SA	4.01a	9.46a	12.00a
	S50	3.81abc	7.00c	10.07bc
	S50+SA	3.85abc	8.00b	10.22b
	S100	3.09d	6.67c	7.72g
	S100+SA	3.67c	6.77c	9.18de
	S150	2.82ef	5.11e	6.72h
	S150+SA	2.79ef	5.68d	8.67f
	S200	1.93gh	4.74ef	5.11i
	S200+SA	1.94gh	4.60f	5.11i
CV (%)		**5.50**	**4.48**	**4.05**
LSD (0.05)		**0.268**	**0.455**	**0.513**

Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.4. Dry weight plant^{-1}

4.2.4.1 Effect of variety

Significant variation was observed for dry matter weight due to varietal variation shown in Table 9. The highest dry matter found in BARI Gom 25 which was 0.54g at 30 DAS, 1.89g at 45 DAS and 2.65g at 60 DAS. On the other hand, BARI Gom 21 gave lowest dry matter weight which was 0.47g at 30 DAS, 1.64g at 45 DAS and 1.99g at 60 DAS.

4.2.4.2 Effect of salinity treatments

For different salinity treatments with or without SA spraying, significant variation was observed for dry matter weight (Table 9). Control (0.67g at 30 DAS, 2.38g at 45 DAS and 3.14g at 60 DAS) and only SA treated plant (0.67g at 30 DAS, 2.37g at 45 DAS and 3.09g at 60 DAS) gave similar highest dry weight compared to other saline treatment and with or without SA treatment. On the contrary, spraying with SA gave higher dry matter weight than saline treatment without SA treatment. But 200 mM saline condition gave similar dry weight with SA treated 200 mM saline condition.

Table 9 Effect of variety and salinity treatments on dry weight of wheat at different days after sowing

Variety	Dry weight (g) plant^{-1}		
	30 DAS	**45 DAS**	**60 DAS**
BARI Gom 21	0.47b	1.64b	1.99b
BARI Gom 25	0.54a	1.89a	2.65a
LSD (0.05)	**0.011**	**0.041**	**0.055**
CV (%)	**4.16**	**4.47**	**4.52**
Treatment			
C	0.67a	2.38a	3.14a
SA	0.67a	2.37a	3.09a
S50	0.64b	2.02c	2.68b
S50+SA	0.64b	2.16b	2.79b
S100	0.47d	1.67d	2.25d
S100+SA	0.53c	1.98c	2.54c
S150	0.39e	1.44e	1.77f
S150+SA	0.41e	1.53e	2.01e
S200	0.32f	1.13f	1.43g
S200+SA	0.33f	0.96g	1.53g
LSD (0.05)	**0.025**	**0.092**	**0.123**
CV (%)	**4.16**	**4.47**	**4.52**

4.2.4.3 Interaction effect of variety and salinity treatments

A significant reduction in dry weight plant^{-1} was observed in both varieties of wheat plants exposed to salt stress as compared to the untreated control (Table 10). However, addition of SA, in combination with salt stress significantly increased dry weight in both varieties, compared to addition of salt only. But, after 200 mM SA treatment could not increase dry weight of wheat plant. In BARI Gom 21, at 30, 45 and 60 DAS and in BARI Gom 25, at 45 DAS, the dry weight of wheat plant became decreased with the treatment of SA at 200 mM salt stressed condition, where in other growth duration for both varieties gave similar result with 200mM salt stressed condition. Besides, when only SA

was applied, the dry weight of wheat plant was similar to that in the untreated control. The highest dry weight was found in BARI Gom 25 at all growth duration (0.71 and 0.70 g at 30 DAS, 2.56 and 2.55 g at 45 DAS and 3.48 and 3.48 g at 60 DAS at control and only SA treated plant, respectively).

Table 10 Effect of SA application on dry weight of two wheat varieties under normal and salt affected condition

Variety	Treatment	Dry weight (g) plant^{-1}		
		30 DAS	45 DAS	60 DAS
BARI Gom 21	C	0.63bc	2.20c	2.80c
	SA	0.64b	2.21c	2.71cd
	S50	0.60cd	1.93ef	2.17fg
	S50+SA	0.59d	1.93e	2.42e
	S100	0.44g	1.53gh	1.96h
	S100+SA	0.49f	1.91ef	2.21f
	S150	0.38ij	1.30i	1.53ij
	S150+SA	0.39i	1.45h	1.55h
	S200	0.30k	1.05j	1.21k
	S200+SA	0.29k	0.91k	1.40j
BARI Gom 25	C	0.71a	2.56a	3.48a
	SA	0.70a	2.55a	3.48a
	S50	0.68a	2.11cd	3.18b
	S50+SA	0.68a	2.39b	3.17b
	S100	0.50f	1.80f	2.55de
	S100+SA	0.56e	2.04de	2.88c
	S150	0.40hi	1.58g	2.01gh
	S150+SA	0.43gh	1.61g	2.47e

	S200	0.35j	1.21i	1.64i
	S200+SA	0.37ij	1.01jk	1.65i
CV (%)		**4.16**	**4.47**	**4.52**
LSD (0.05)		**0.035**	**0.130**	**0.173**

Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Salt stress constrains plant growth by adversely affecting various physiological and biochemical processes, such as photosynthesis, antioxidant phenomena, proline metabolism, and osmolyte accumulation (Borsani *et al.*, 2001; Fariduddin *et al.*, 2003; Misra *et al.*, 2009 and Idrees *et al.*, 2011). Given that SA plays key roles in the regulation of plant growth, development, the interaction with other organisms, and the responses to environmental stresses (Senaratna *et al.*, 2000 and Hayat *et al.*, 2010). Therefore, in the present study, the effect of exogenously treated SA on growth rate of wheat plants growing under different salt stress when compared with their corresponding non-SA applied plants (Table 4, 6, 8, 10). This is not consistent with the reports of Idrees *et al.* (2011) where it was shown that SA treatment ameliorated the adverse effects of salt stress in terms of growth parameters in *Catharanthus roseus*. Similarly, SA treatment enhanced the growth of wheat plants under water stress (Singh and Usha, 2003), maize (Khodary, 2004), mustard (Yusuf *et al.*, 2008) and barley (El Tayeb, 2005) under NaCl stress. Increase in growth of wheat under non-saline or saline conditions from which SA treatment resulted, can be attributed to an increase in photosynthesizing tissue, that is, the leaves (Dhaliwal *et al.*, 1997), which is in agreement with the results obtained also. Moreover, it can be suggested that foliar spray with SA might have affected certain metabolic factors in carbon uptake of fixation including Rubisco enzyme concentration and activity and or photosynthetic carbon reduction (PCR) cycle (Arfan *et al.*, 2007). These results are in agreement with those obtained by Ali and Mahmoud (2013); Kaydan *et al.* (2007); Hussein *et al.* (2007); Karlidag *et al.* (2009) and Erdal *et al.* (2011).

4.3 Physiological parameters

4.3.1 Relative water content

4.3.1.1 Effect of variety

There was significant variation observed for relative water content due to varietal variation (Fig. 9(A)). BARI Gom 25 (84.35%) recorded the highest relative water content compared to BARI Gom 21 (77.3%).

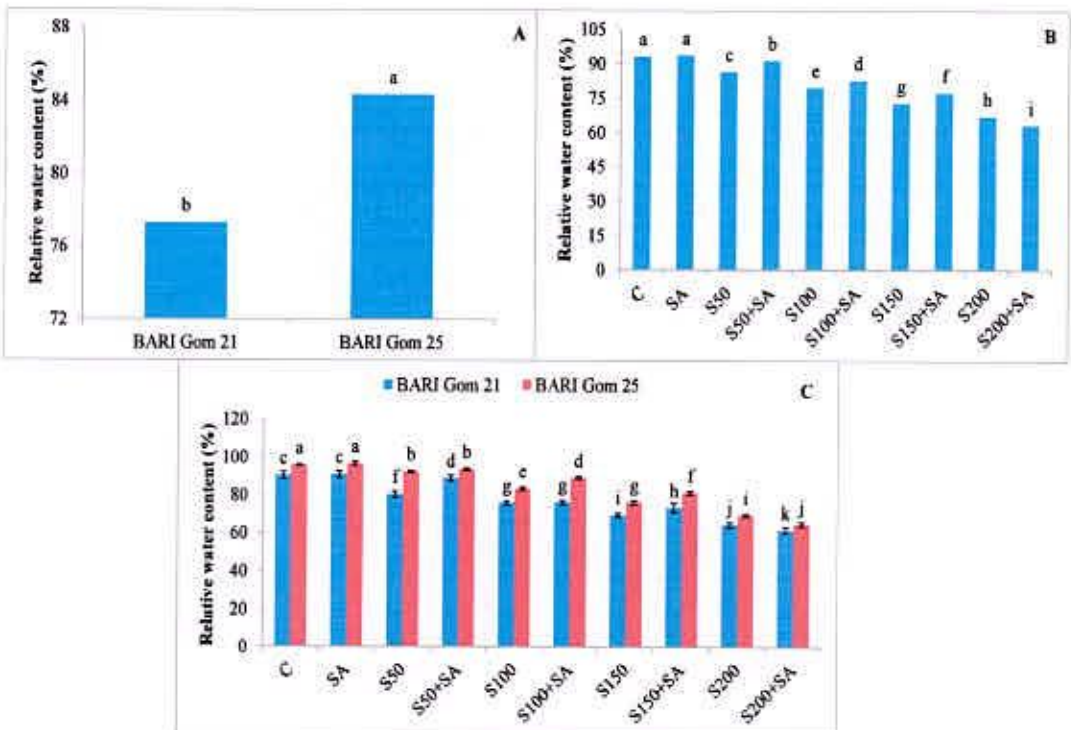

Fig. 9 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on relative water content of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.3.1.2 Effect of salinity treatments

Sharp decreases in relative water content (8, 15, 22 and 28% at 50, 100, 150 and 200 mM salt stressed condition) were observed in response to salt stress, compared to untreated

control (Fig. 9(B)). Moreover, SA could increase relative water content under salt stressed condition up to 150 mM stressed condition. At 200 mM stressed condition, applying of SA reduced the relative water content percentage.

4.3.1.3 Interaction effect of variety and salinity treatments

Upon exposure to salt stress, leaf relative water content decreased significantly in both wheat varieties when compared to their controls (Fig. 9(C)). However, decline in RWC was lower in BARI Gom 25 as compared to BARI Gom 21. At 100 mM of NaCl it was decreased by 16 and 12% in BARI Gom 21 and BARI Gom 25, respectively over control, while at 200 mM NaCl the RWC decreased by 29 and 27% (Fig. 9(C)). The application of SA effectively maintained the RWC in salt stressed seedlings. In BARI Gom 21, SA could increase RWC by 16 and 32% in seedlings exposed to 100 and 200 mM NaCl, respectively. In case of BARI Gom 25, the increases were 7 and 32% at 100 mM NaCl and 6 and 34% at 200 mM NaCl (Fig. 9(C)).

4.3.2 Chlorophyll content

4.3.2.1 Effect of variety

Variety showed significant variation in chlorophyll content (Fig. 10(A)). BARI Gom 25 showed the highest chlorophyll content (0.049mg cm^{-2}) compared to BARI Gom 21 (0.043mg cm^{-2}).

4.3.2.2 Effect of salinity treatments

Different salinity treatments affected chlorophyll production significantly throughout the growing period. Salinity treatment reduced total chlorophyll content compared to its respective control (Fig. 10(B)). On the contrary, SA with saline treatments increased chlorophyll content (4, 17 and 25% at 50, 100 and 150 mM, respectively) where higher level of salinity treatment did not affect by SA spraying (200 Mm salinity stress).

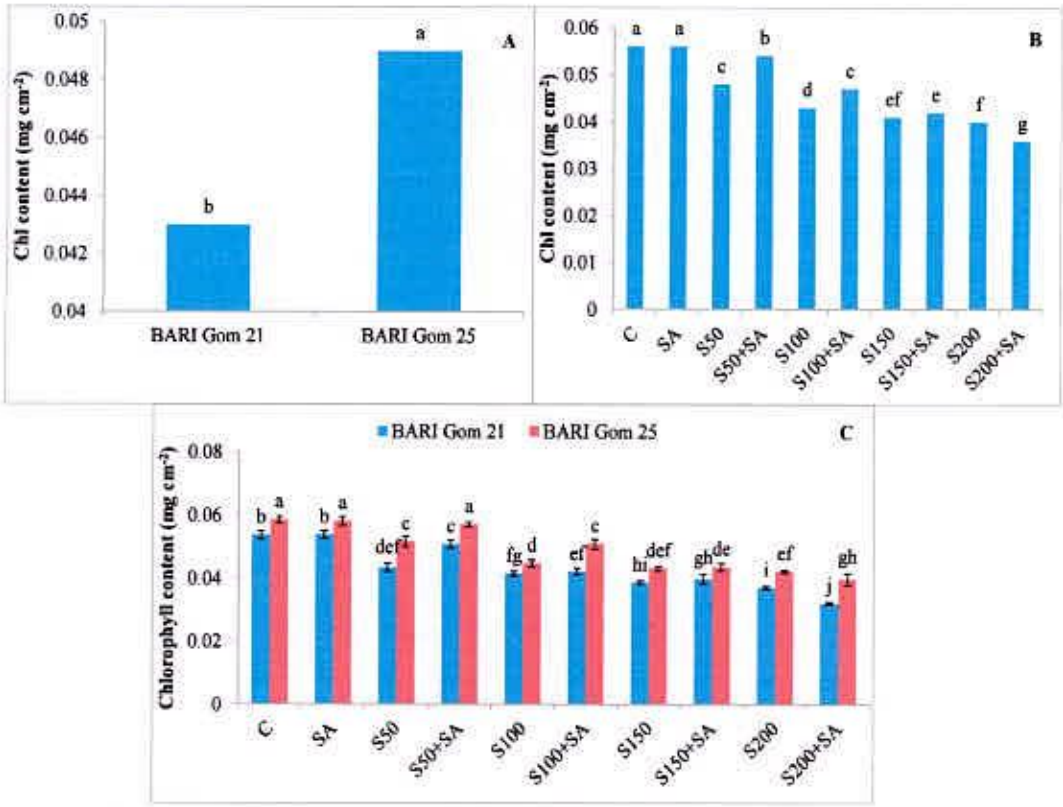

Fig. 10 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on chlorophyll content of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.3.2.3 Interaction effect of variety and salinity treatments

Chlorophyll content also affected by salinity stress, according to Fig. 10(C). In case of BARI Gom 21, chlorophyll content decreased 19, 23, 28, and 31% at 50, 100, 150 and 200 mM salinity stress, respectively (Fig. 10(C)). On the contrary, reductions in chl content were 12, 23, 26 and 28% at 50, 100, 150 and 200 mM salinity stress, respectively. Though SA treatment significantly increased the chl content under stress condition but it failed to increase chl content under 200 mM salinity stress. The highest amount chl (0.05863 mg cm^{-2}) was found in BARI Gom 25 under control and the lowest amount chl was 0.0321 mg cm^{-2} in BARI Gom 21 under 200 mM salinity treated with SA. However, when plant treated with SA only, chl content was not affected significantly in relation to control.

Since salt stress causes osmotic stress, the decline in RWC is a common phenomenon in plants growth under salinity and hence RWC is considered as a potent indicator for evaluating plants for tolerance to salt stress. In our study, salt stress led to a significant decrease of RWC in wheat leaves irrespective to NaCl concentration and the wheat cultivars (Fig. 9). Similar decrease in RWC due to salt stress was reported earlier (Vysotskaya *et al.*, 2010; Chaparzadeh and Mehrnejad, 2013). Decrease in RWC was due to loss of turgor that results in limited water availability for cell extension processes (Katerji *et al.*, 1997). However, when salt treated seedlings were supplemented with SA they showed enhanced RWC which was due to the retention in water in their tissue (Fig. 9). The enhanced water content in plants due to exogenous application of SA was also observed by other researchers (Alam *et al.*, 2013 and Li *et al.*, 2014). In BARI Gom 21 the RWC was slightly higher than BARI Gom 21 which was due to its better tolerance.

In our experiment salt caused reduction in chl content, in both wheat varieties. However, the reduction was higher in salt sensitive BARI Gom 21 (Fig. 10). Salt stress often causes alteration in photosynthetic pigment biosynthesis (Maxwel and Jhonson, 2000). Similar decrease in chl content was observed by Amirjani *et al.* (2011) in rice. However, exogenous application of SA in salt treated seedlings could elevate the chl content which might be due to the higher biosynthesis of the pigment. These results are in agreement with Hasanuzzaman *et al.* (2014) and Alam *et al.* (2013).

4.4 Oxidative stress markers

4.4.1 MDA content

4.4.1.1 Effect of variety

As shown in Fig. 11(A) highest MDA content also found in BARI Gom 21 compared to BARI Gom 25. 35.94 nmol g^{-1} FW MDA content was found in BARI Gom 21 where 32.61 nmol g^{-1} FW found in BARI Gom 25.

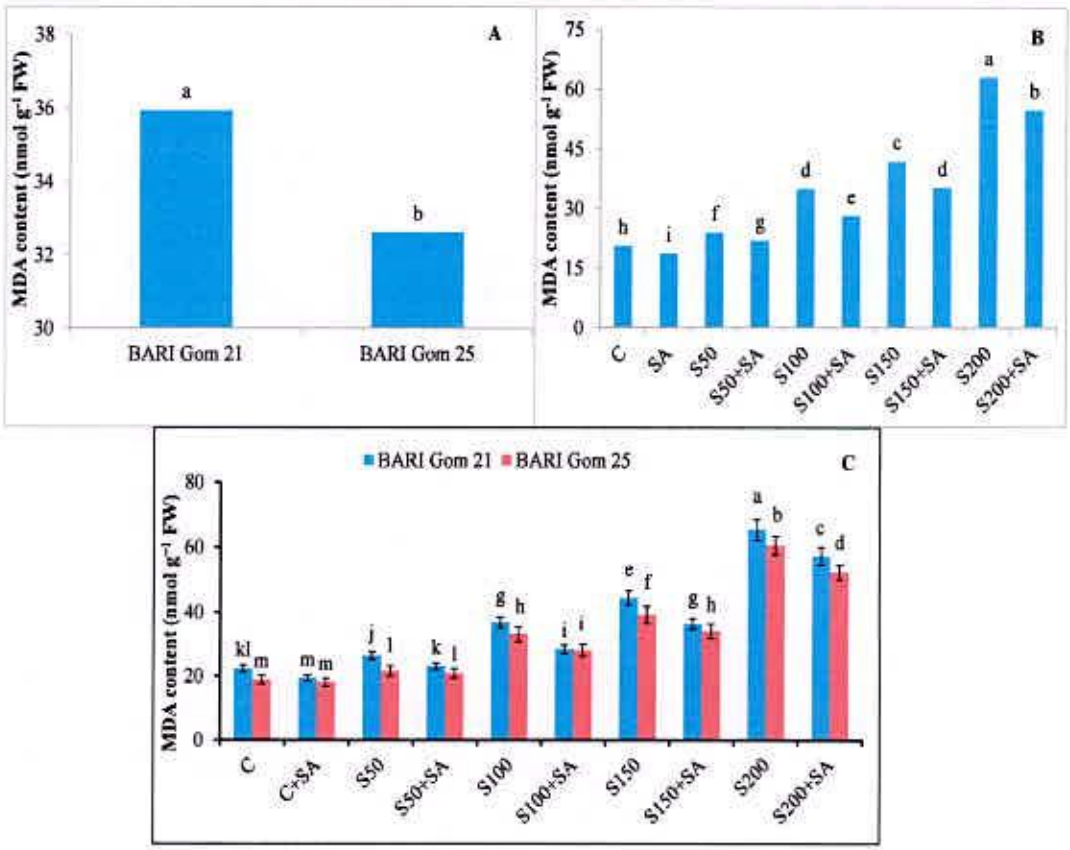

Fig. 11 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on MDA content of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.4.1.2 Effect of salinity treatments

MDA content was also affected by salinity stress, according to Fig. 11(B). Saline treatment increased the MDA content compared to control and SA treated plant. 17, 70, 107 and 204% reduced due to 50, 100, 150 and 200 mM salinity stress, respectively. Furthermore, the increment was less in SA treated stressed plant compared to respective control (Fig. 11(B)).

4.4.1.3 Interaction effect of variety and salinity treatments

The malondealdehyde (MDA) content (indicator of lipid peroxidation) sharply increased at any level of salt stress in both wheat varieties. The highest amount of MDA content was 65 and 60 nmol g^{-1} FW salinity level 200 mM whereas the lowest amount of MDA content were 13 and 4 nmol g^{-1} FW found when seedling treated with SA alone in salt sensitive and salt tolerant variety respectively. However, the rate on increment was higher in salt sensitive BARI Gom 21. In BARI Gom 21, 100 and 200 mM NaCl caused 65 and 194% increase in MDA content while in BARI Gom 25 it was 75 and 221%, respectively, compared to control (Fig. 11(C)). The seedlings supplemented with SA could maintain the level of MDA significantly lower compared to the seedlings exposed to salt stress without supplementation (Fig. 11(C)). MDA content was always higher in BARI Gom 21 than that of BARI Gom 25.

Lipid peroxidation is considered as important index as it determines the degree of oxidative stress because it is found that MDA content increases with the extent of oxidative stress caused by abiotic stress including salt stress (Hasanuzzaman *et al.*, 2011). Reactive oxygen species such as $OH^{\cdot}$ and $^1O_2$ are highly reactive and attack PUFA thus MDA formed as oxidation product (Gill, 2010). In this study, MDA content increase with salinity level (Fig. 11). More severe stress generates more ROS that causes more damage to the membrane which is reflected by higher MDA content. MDA content increase under different stress condition (Hasanuzzaman *et al.*, 2011; Alam *et al.*, 2014). Application of salicylic acid reduced the MDA content significantly under drought stress in Brassica (Alam *et al.*, 2013). Our results suggested the same findings.

4.4.2 H_2O_2 content

4.4.2.1 Effect of variety

Significant variation was observed for H_2O_2 content due to varietal variation in Fig. 12(A). BARI Gom 21 produced higher H_2O_2 content (13.32 nmol g^{-1} FW) compared to BARI Gom 25.

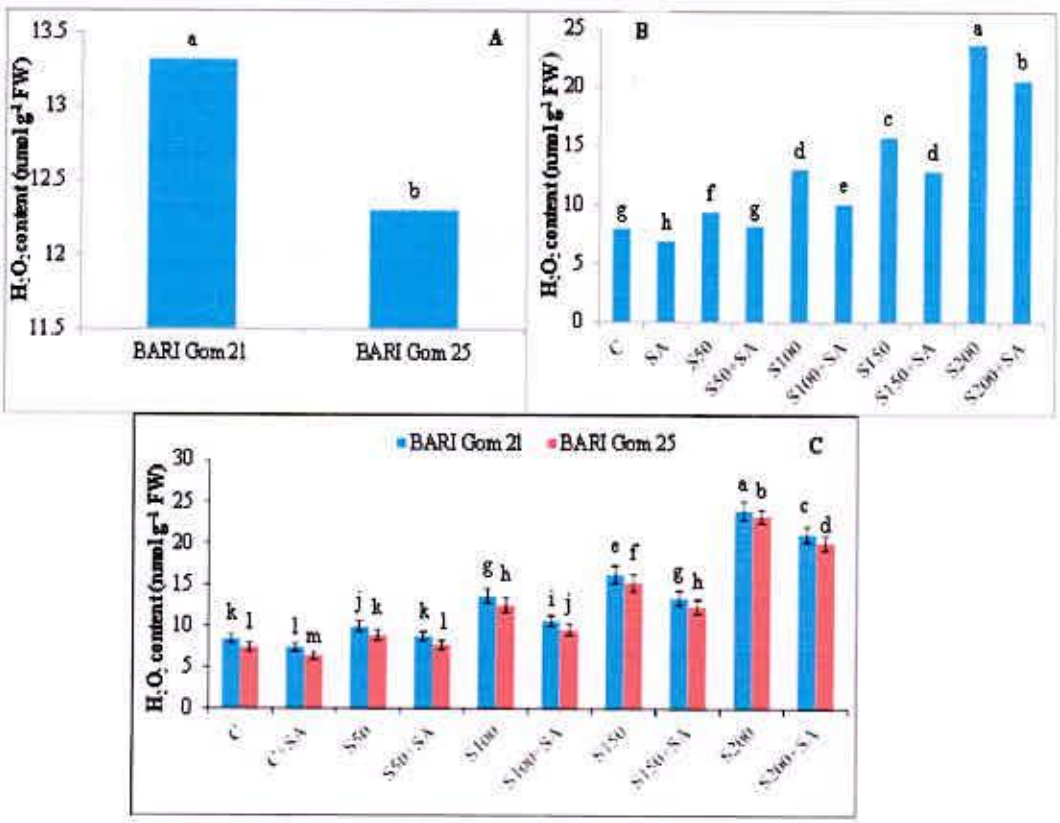

Fig. 12 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on H_2O_2 content of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.4.2.2 Effect of salinity treatments

For different salinity stress treatments, significant variation was observed for H_2O_2 content (Fig. 12(B)). The highest H_2O_2 content was found in 200 mM salt stressed plant. H_2O_2 content decreased sharply in case of SA treated stressed plant compared to salt

stressed treatment. However, SA treatment decreased H_2O_2 content 4, 27, 63 and 161% at 50, 100, 150 and 200 mM stressed condition.

4.4.2.3 Interaction effect of variety and salinity treatments

The levels of H_2O_2 also increased noticeably upon exposure to NaCl. At salinity level 100 mM , The amount of H_2O_2 were 14 and 12 nmol g^{-1} FW and then this amount fell to 11 and 10 nmol g^{-1} FW when treated with SA. In BARI Gom 21, the H_2O_2 content was increased by 93 and 184% at 150 and 200 mM NaCl, while in BARI Gom 25 it was increased by 106 and 215%, respectively, compared to control (Fig. 12(C)). SA could maintain the H_2O_2 content lower in salt-stressed seedlings compared to the seedlings grown without SA supplementation (Fig. 12(C)). In all cases there was significant difference between BARI Gom 21 and BARI Gom 25 in respect of H_2O_2 content.

Higher accumulation of H_2O_2 causes oxidative stress in plant. In present study, H_2O_2 content significantly increased under salinity stress (Fig. 12). With the increase in salinity level, H_2O_2 content also increased. Increased amount of H_2O_2 found under different stress (Hasanuzzaman *et al.*, 2014; Alam *et al.*, 2014; Nahar *et al.*, 2015). Rao *et al.*, (2013) reported salt tolerant wheat varieties (Sehar-06, Lu-26) accumulate lower H_2O_2 than sensitive varieties. 1mM and 0.5mM SA spray reduced the H_2O_2 content in mungbean under 50 mM salt stress (Khan *et al.*, 2010).

4.5 Antioxidant defense system

4.5.1 AsA content

4.5.1.1 Effect of variety

AsA content showed significant variation among the different varieties (Fig. 13(A)). BARI Gom 25 produced highest AsA content (3620.4 nmol g^{-1} FW). The lowest AsA content (3035.4 nmol g^{-1} FW) was obtained from BARI Gom 21.

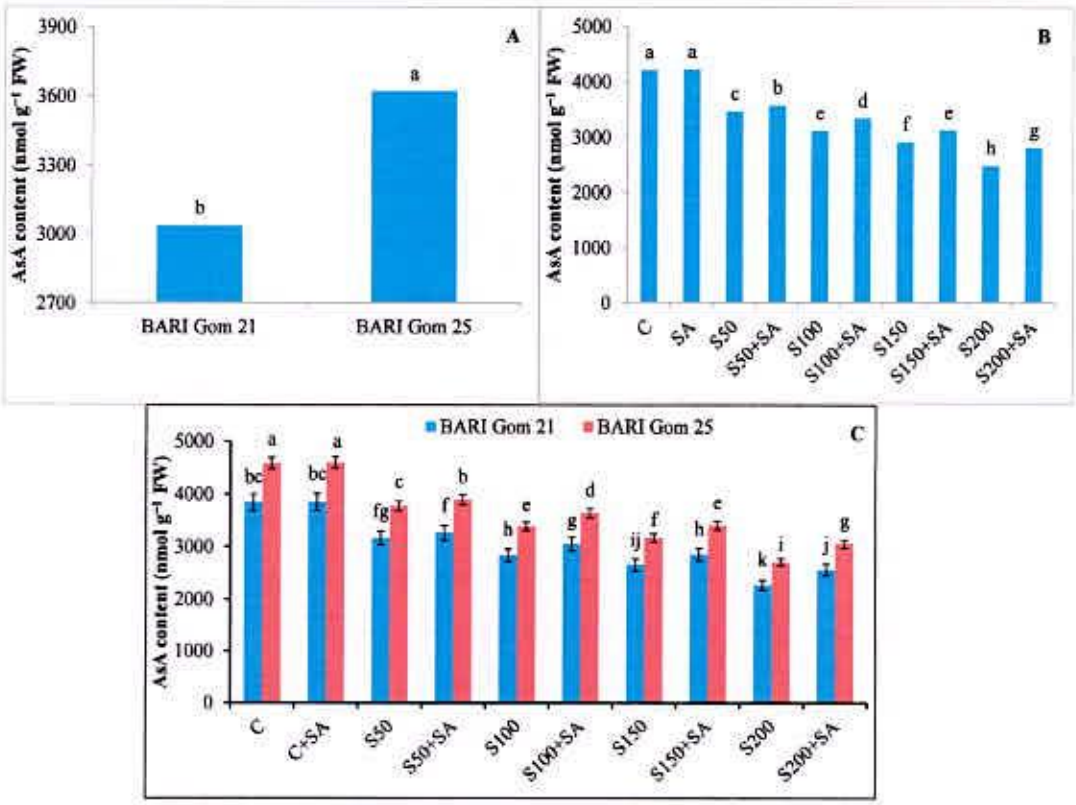

Fig. 13 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on AsA content of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.1.2 Effect of salinity treatments

Salinity reduced AsA content compared to control (Fig. 13(B)). SA treatment increased AsA content under stressed condition (16, 21, 26 and 34% at 50, 100, 150 and 200 mM).

4.5.1.2 Interaction effect of variety and salinity treatments

According to (Fig. 13(C)) Gradual decrease in AsA content over control was observed, for both BARI Gom 21 and BARI Gom 25, as the plant exposed to salt stress. Compared to control AsA content decrease 18, 26, 31 and 41% in case of BARI Gom 21 and BARI Gom 25 due to 50, 100, 150 and 200 mM salinity respectively. When seedling treated with SA, AsA content increases significantly for all cases. AsA content of BARI Gom 25 was always higher than that of BARI Gom 21. The highest amount of AsA content (3841 and 4582 nmol g^{-1} FW) for both genotypes was observed in untreated control and the lowest amount of AsA content (2271 and 2709 nmol g^{-1} FW) for both genotypes was observed in seedling exposed to 200 mM salinity. There was no significant difference between control and SA treated alone seedling AsA content for both varieties.

4.5.2 GSH content

4.5.2.1 Effect of variety

GSH content varied significantly for different varieties shown in Fig. 14 (A). The highest GSH content (347.51 nmol g^{-1} FW) was recorded by BARI Gom 25 compared to BARI Gom 21 (316.34 nmol g^{-1} FW).

4.5.2.2 Effect of salinity treatments

Significant variation was observed for GSH content due to different salinity treatments (Fig. 14(B)). GSH content became reduced due to saline treatment. However, SA treated under salt stressed condition increased GSH content up to higher level of salt stressed condition (23, 29, 18 and 29% at 50, 100, 150 and 200 mM stress treatment, respectively) compared to their respective control.

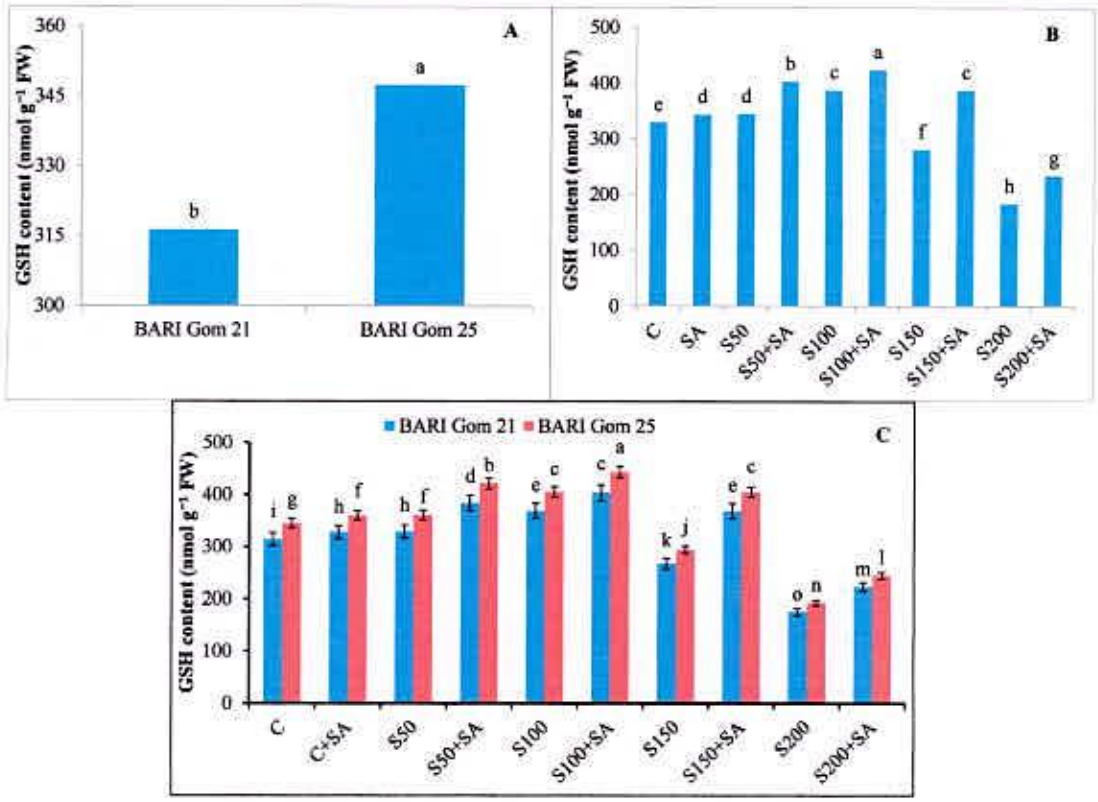

Fig. 14 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on GSH content of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.2.3 Interaction effect of variety and salinity treatments

Significant increase in GSH content were observed (5 and 18 % by the 50 and 100 mM NaCl stresses respectively) in response to salt stress, compared to the untreated control (Fig. 14(C)) .However, sharp decrease was also observed when plant exposed to 150 and 200 mM salinity stress for both genotypes. An increase in GSH content was also observed in SA treated salt-stressed seedlings and, particularly at the 100 mM stress, the SA treated seedlings showed a significant increase (9%) in GSH content compared to seedlings of BARI Gom 21 and BARI Gom 25 subjected to salt stress alone. At any level of stress treatment, GSH content was higher in BARI Gom 25 than that of BARI Gom 21 (Fig. 14(C)).

4.5.3 GSSG content

4.5.3.1 Effect of variety

There was significant variation observed for GSSG content due to varietal variation (Fig. 15(A)). BARI Gom 21 recorded highest GSSG content (35.61 nmol g^{-1} FW) and the lowest GSSG content (32.69 nmol g^{-1} FW) was obtained from the other variety named BARI Gom 25.

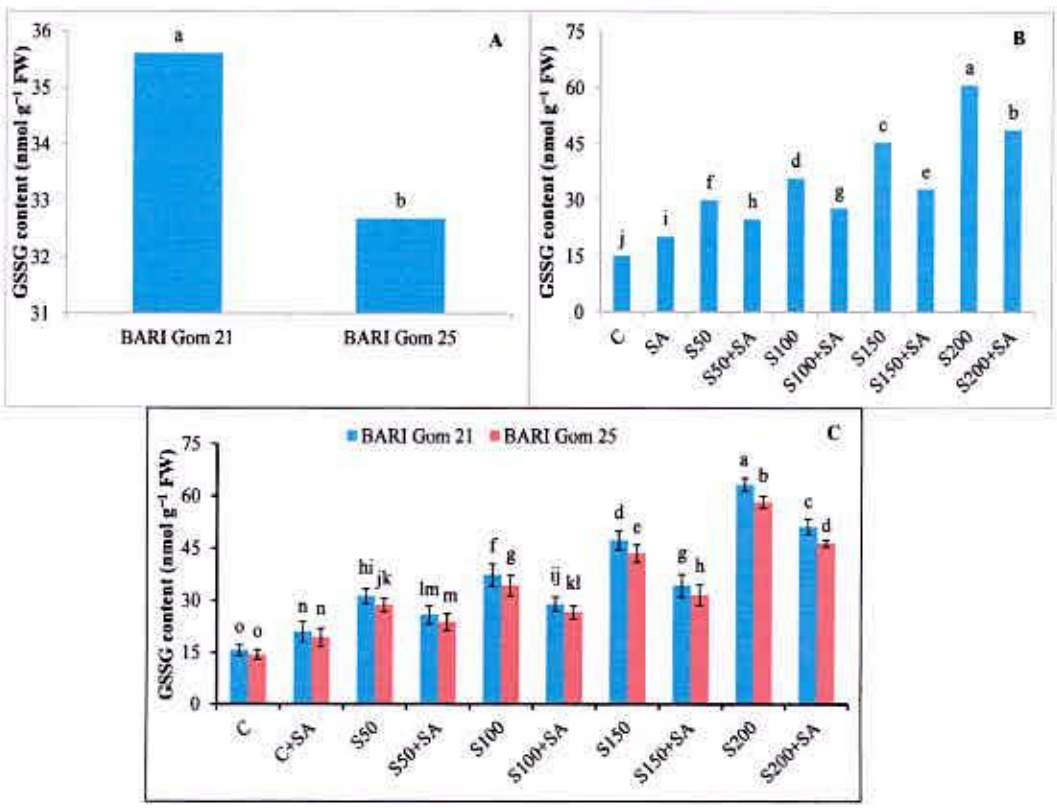

Fig. 15 (A) Effect of variety, (B) Effect of treatment, and (C) Interaction effect of variety and salinity treatments on GSSG content of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.3.2 Effect of salinity treatments

Sharp increases in GSSG content were observed (100, 140, 204 and 306% at 50, 100, 150 and 200 mM stressed condition, respectively) due to NaCl salinity stress treatment (Fig. 15(B)). Furthermore, SA treatment decreased GSSG content under stress condition. The

highest (60.78 nmol g^{-1} FW) GSSG content was found in 200 mM salt stressed condition, where the lowest (14.98 nmol g^{-1} FW) GSSG content was found in control.

4.5.3.3 Interaction effect of variety and salinity treatments

The GSSG content in wheat seedlings of any variety sharply increased at any level of salt stress. The highest amount of GSSG content was 63 and 58 nmol g^{-1} FW salinity level 200 mM whereas the lowest amount of MDA content were 16 and 14 nmol g^{-1} FW found when seedling without stress treatment or SA in salt sensitive BARI Gom 21 and salt tolerant BARI Gom 25 variety respectively (Fig. 15(C)). In salt sensitive BARI Gom 21 the levels were increased by 139 and 305% at 100 and 200 mM NaCl, respectively. Exogenous SA, on the other hand, maintained the GSSG content significantly lower under salt stress compared to the seedlings grown without SA supplementation (Fig. 15(C)). GSSG content was always higher in BARI Gom 21 than that of BARI Gom 25.

4.5.4 GSH/GSSG ratio

4.5.4.1 Effect of variety

Variety showed significant variation in GSH/GSSG ratio (Fig. 16(A)). BARI Gom 25 showed the highest GSH/GSSG ratio (12.71) whereas lowest GSH/GSSG ratio (10.17) in BARI Gom 21.

4.5.4.2 Effect of salinity treatments

For different salinity treatments with or without SA spraying, significant variation was observed for GSH/GSSG ratio (Fig. 16(B)). Control (19.98) produced higher GSH/GSSG ratio compared to other salt stressed condition and SA treated stressed condition. However, SA treated stressed plant produced higher GSH/GSSG ratio (21, 25, 42 and 77% at 50, 100 and 150 mM salt stressed condition, respectively) compared to salt stressed condition.

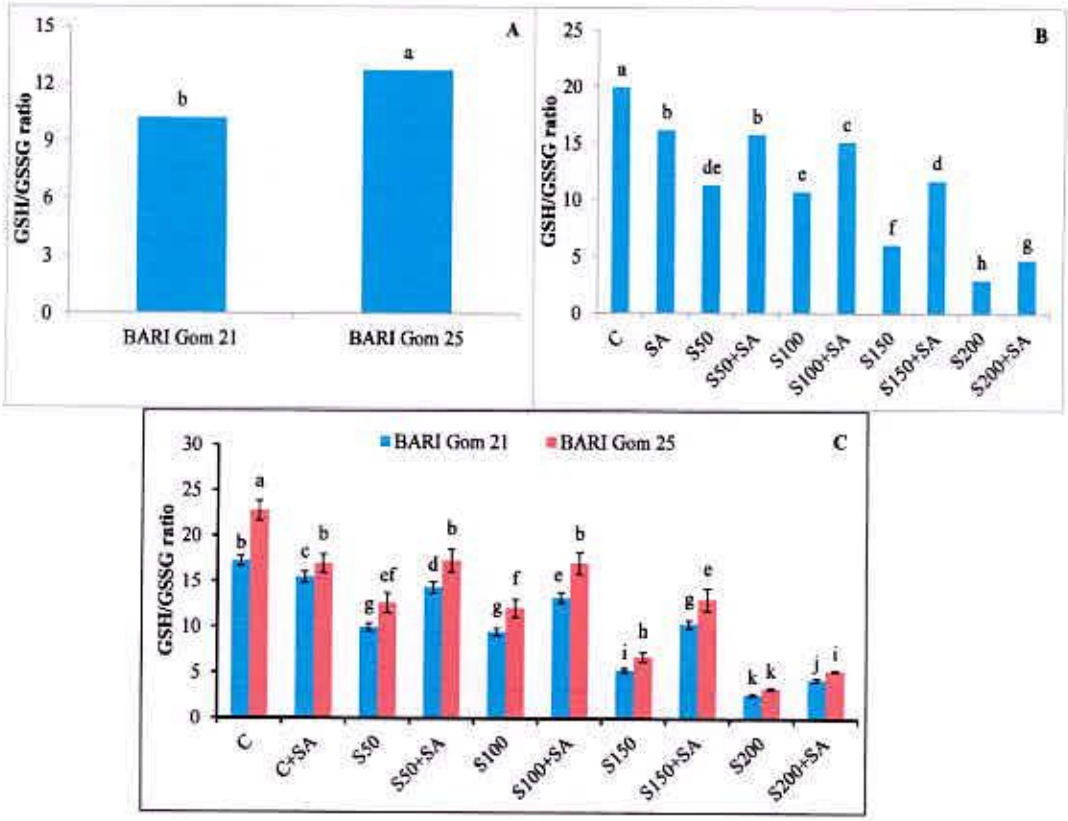

Fig. 16 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on GSH/GSSG ratio of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.4.3 Interaction effect of variety and salinity treatments

The ratio of GSH/GSSG decreased markedly under salt stress in dose dependent manners and it greatly varied with varieties (Fig. 16(C)). In salt sensitive BARI Gom 21, 150 and 200 mM NaCl resulted in 69 and 85% decrease in GSH/GSSG ration, while in salt tolerant BARI Gom 25, it decreased by 70 and 85%, respectively, compared to control (Fig. 16(C)). At salinity level 100 mM, the ratios of GSH/GSSG were 9 and 12 and then it rose up to 13 and 17 FW when treated with SA. In all cases there was significant difference between BARI Gom 21 and BARI Gom 25 in respect of GSH/GSSG except at salinity level 200 mM.

4.5.5 CAT activity

4.5.5.1 Effect of variety

There was significant effect of varieties, salinity and SA treatments on CAT activity of wheat varieties (Fig. 17(A)). Additionally BARI Gom 25 gave highest CAT activity (54.2 μmol m^{-1} mg^{-1} protein) compared to BARI Gom 21.

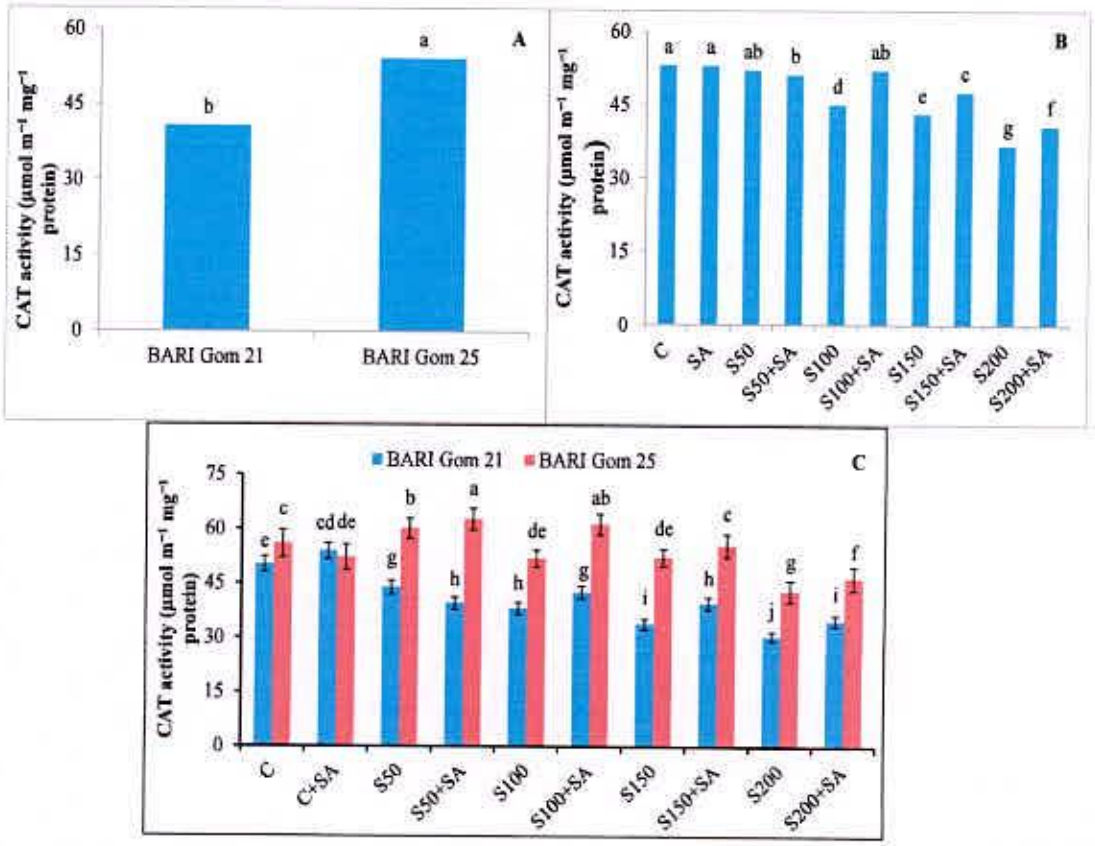

Fig. 17 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on CAT activity of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.5.2 Effect of salinity treatments

Exposure to salt stress resulted in significant decreases in CAT activity (2, 16, 19 and 31% at 50, 100, 150 and 200 mM stress, respectively). However, SA with saline treatment increased CAT activity compared to its respective control (Fig. 17(B)).

4.5.5.3 Interaction effect of variety and salinity treatments

Catalase activity showed differential responses in wheat seedlings with variable salt tolerance levels and also induced by salt levels (Fig. 17(C)). At salinity level 100 mM, the activity of catalase enzyme observed 38 μmol m^{-1} mg^{-1} proteins in BARI Gom 21 whereas 52 μmol m^{-1} mg^{-1} protein activities found in BARI Gom 21. In salt sensitive BARI Gom 21, the activity decreased by any level of salt stress (24 and 39% lower at 100 and 200 mM NaCl, respectively, compared to the control). Salt tolerant BARI Gom 25 showed significant increase in CAT activity under mild stress (50 mM NaCl), whereas a noticeable decrease (23%) was observed at severe stress (200 mM). However, exogenous SA enhanced the CAT activity in salt-treated seedlings (Fig. 17(C)). In most cases BARI Gom 25 showed higher CAT activity than that of BARI Gom 21 except seedling treated with SA.

4.5.6 APX activity

4.5.6.1 Effect of variety

Significant variation was observed for APX activity due to varietal variation shown in Fig. 18(A). The highest APX activity found in BARI Gom 25 which was (1.03 μmol m^{-1} mg^{-1} protein). On the other hand, BARI Gom 21 gave lowest APX activity which was (0.82 μmol m^{-1} mg^{-1} protein).

4.5.6.2 Effect of salinity treatments

For different salinity treatments with or without SA spraying, significant variation was observed for APX activity (Fig. 18(B)). SA treated 100 mM salt stressed condition gave highest (1.10 μmol m^{-1} mg^{-1} protein) APX activity compared to control, other saline treatment and with or without SA treatment. On the contrary, 200 mM saline condition gave lower (0.72 μmol m^{-1} mg^{-1} protein) APX activity.

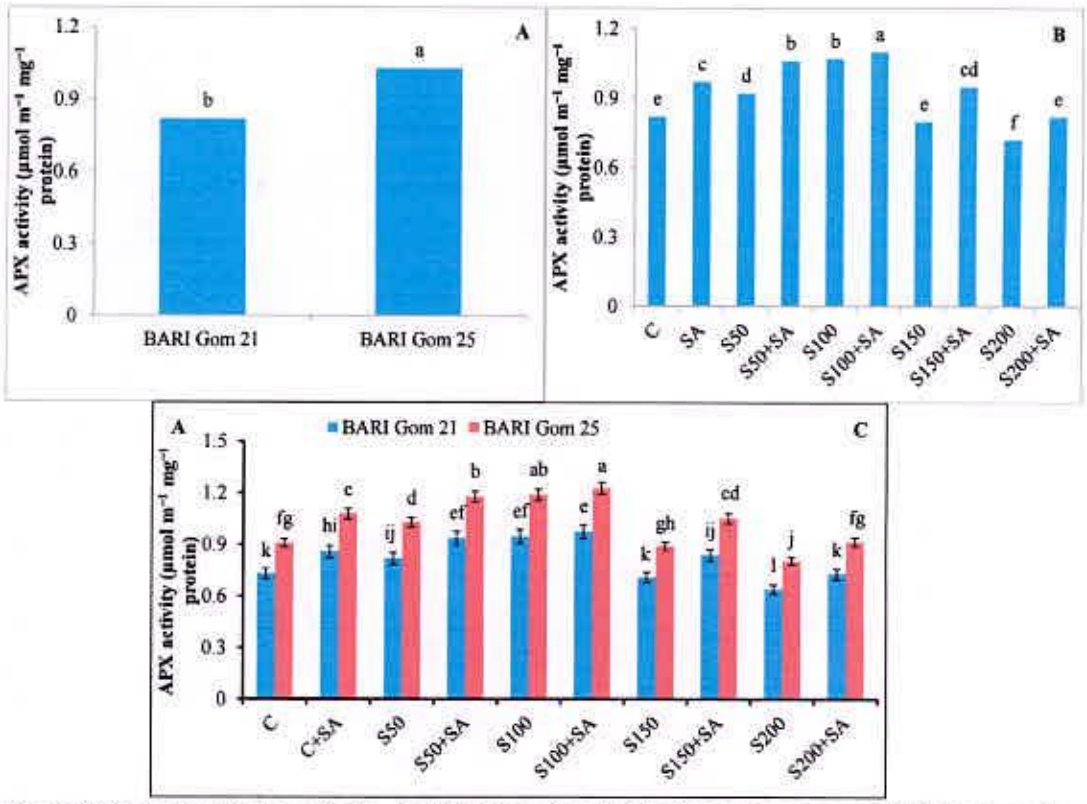

Fig. 18 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on APX activity of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.6.3 Interaction effect of variety and salinity treatments

Imposition of salt stress of 100 mM significantly increased the APX activity by 30% in salt sensitive BARI Gom 21while in salt tolerant BARI Gom 25 it was increased by 31% compared to control. At 100 mM salinity level , the activity of APX enzyme observed 0.94 µmol m^{-1} mg^{-1} protein in BARI Gom 21 whereas 1.19 µmol m^{-1} mg^{-1} protein activity found in BARI Gom 25.Under severe salt stress (200 mM NaCl), APX activity was decreased by 12% in salt sensitive cultivar and11% in salt tolerant cultivar (Fig. 18(C)). Exogenous SA supplementation in salt stressed seedlings maintained higher APX activities, compared to salt stress alone, whereas in salt tolerant BARI Gom 25 the activity was always higher than BARI Gom 21 (Fig. 18(C)).

4.5.7 MDHAR activity

4.5.7.1 Effect of variety

There was significant variation observed for MDHAR activity due to varietal variation (Fig. 19(A)). BARI Gom 25 (43.12 µmol m^{-1} mg^{-1} protein) recorded the highest MDHAR activity compared to BARI Gom 21 (36.15 µmol m^{-1} mg^{-1} protein).

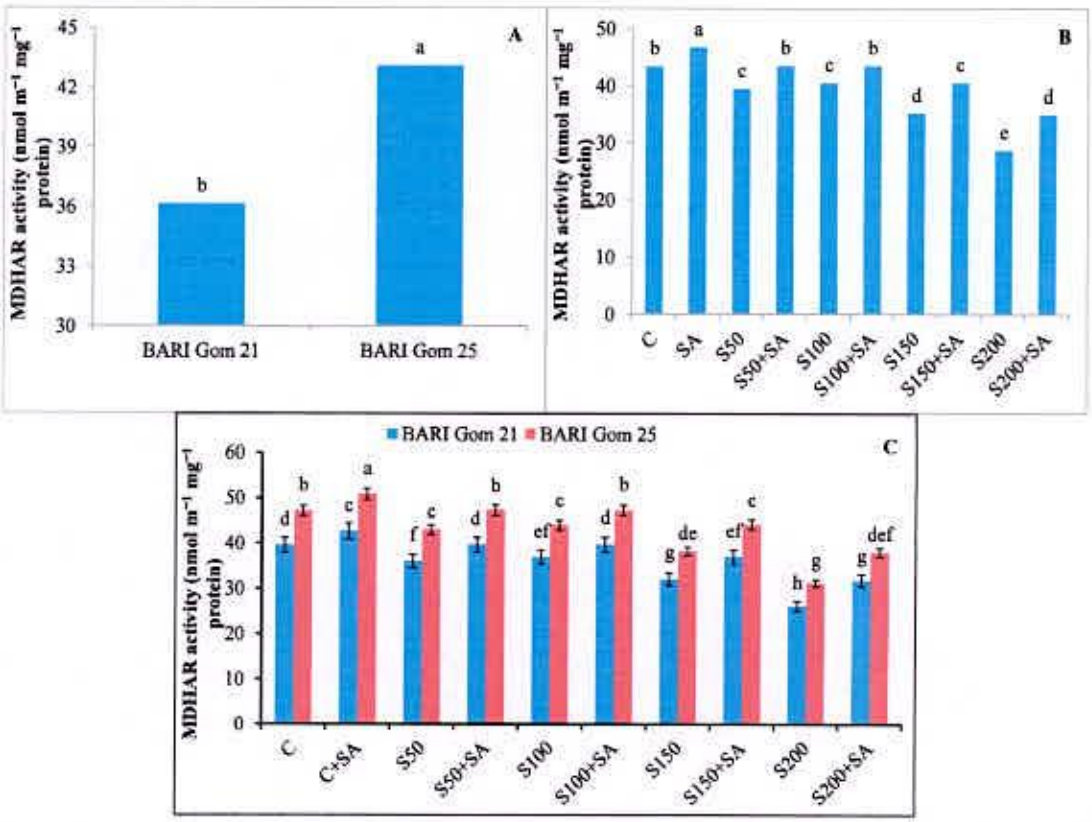

Fig. 19 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on MDHAR activity of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.7.2 Effect of salinity treatments

Sharp decreases in MDHAR activity (9, 7, 19 and 34% at 50, 100, 150 and 200 mM salt stressed condition) were observed in response to salt stress, compared to untreated

control (Fig. 19(B)). Moreover, SA could increase MDHAR activity under salt stressed condition. At 200 mM stressed condition, MDHAR activity was lower (Fig. 19(B)).

4.5.7.3 Interaction effect of variety and salinity treatments

Salt stress at any level decreased the MDHAR activity in salt sensitive BARI Gom 21 by which were 19 and 34% lower at 150 and 200 mM NaCl, respectively, compared to control (Fig. 19(C)). The highest activity was 43 nmol m^{-1} mg^{-1} protein found BARI Gom 25 treated with SA alone while the lowest MDHAR activity was 26 µmol m^{-1} mg^{-1} protein found in BARI Gom 21 exposed to 200 mM salinity stress. Exogenous SA addition under any levels of salt stress significantly increased MDHAR activities irrespective of cultivars (Fig. 19(C)).

4.5.8 DHAR activity

4.5.8.1 Effect of variety

Variety showed significant variation in DHAR activity (Fig. 20(A)). BARI Gom 25 showed the highest DHAR activity (221.34 µmol m^{-1} mg^{-1} protein) compared to BARI Gom 21 (180.94 µmol m^{-1} mg^{-1} protein).

4.5.8.2 Effect of salinity treatments

Different salinity treatments affected DHAR activity significantly throughout the growing period. Salinity treatment reduced DHAR activity compared to its respective control (Fig. 20(B)). On the contrary, SA with saline treatments increased DHAR activity (4, 9, 10 and 21% at 50, 100, 150 and 200 mM, respectively).

4.5.8.3 Interaction effect of variety and salinity treatments

Salt stress caused a marked decrease in DHAR activity at any level of stress except when seedling exposed to 200 mM stress irrespective of genotypes. At 100 mM Salt stress, In case of BARI Gom 25, DHAR activity was 206 nmol m^{-1} mg^{-1} protein and rose up to 267 nmol m^{-1} mg^{-1} protein when treated with SA under stress condition (Fig. 20(C)). In BARI Gom 21, due to exogenous SA application DHAR activities were increased by 10 and

21% at 150 and 200 mM NaCl, respectively. In BARI Gom 25, exogenous SA supplemented seedlings showed increased DHAR activities by 25% at 150 mM NaCl, however no significant change in DHAR activity observed due to SA supplementation at 200 mM NaCl, compared to salt stress alone (Fig. 20(C)).

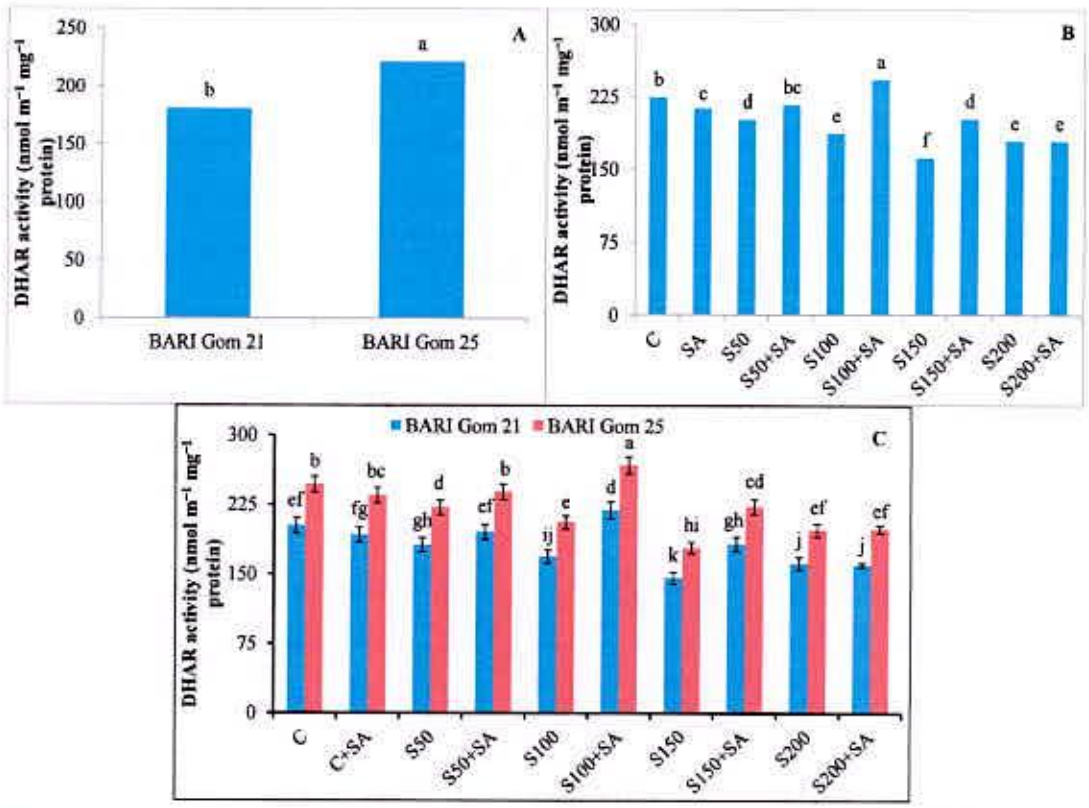

Fig. 20 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on DHAR activity of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.9 GR activity

4.5.9.1 Effect of variety

GR activity showed significant variation among the different varieties. BARI Gom 25 (25.8 μmol m^{-1} mg^{-1} protein) had highest GR activity, where BARI Gom 21 (23.79 μmol m^{-1} mg^{-1} protein) had lowest GR activity (Fig. 21(A)).

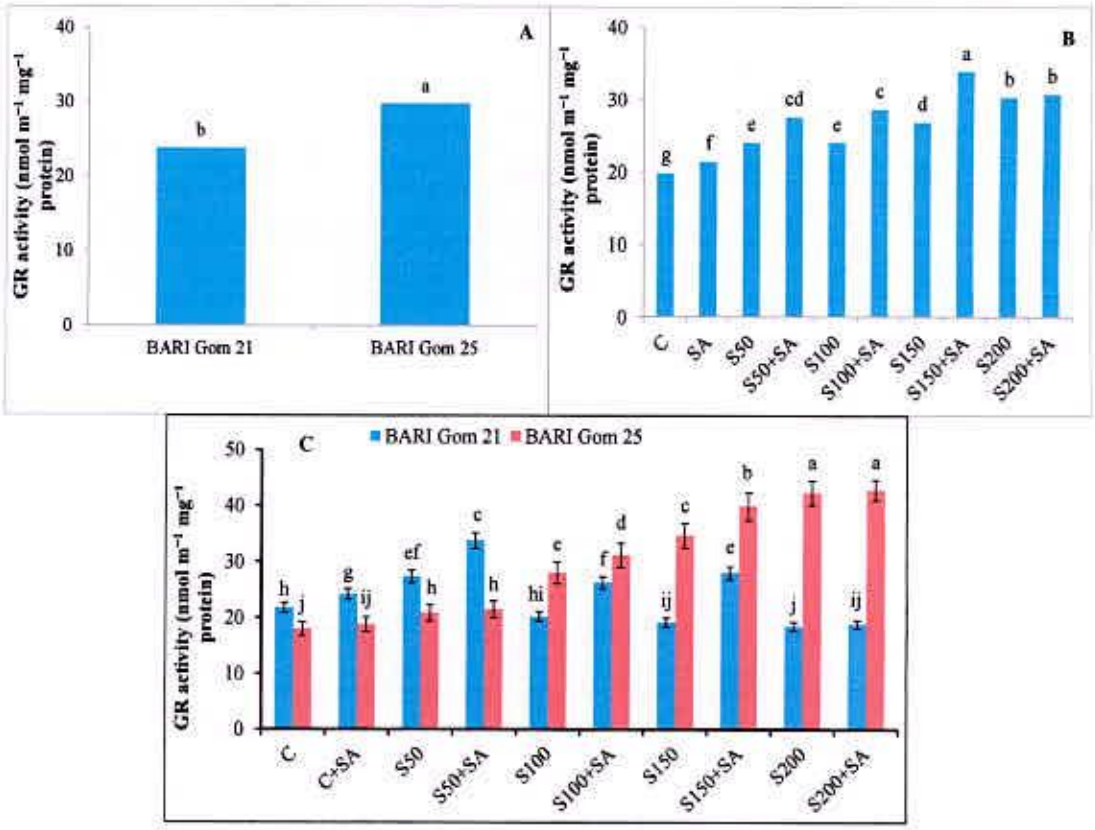

Fig. 21 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on GR activity of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.9.2 Effect of salinity treatments

The data (Fig. 21(B)) showed that salinity also reduced GR activity. On the other hand, the magnitude of decrease was less in SA treated salt stressed condition as compared to without treated salt stressed condition. As a result, GR activity was statistically similar (72.08% at 200 mM stressed condition, 74.25% at SA treated 200 mM stressed condition) from its control.

4.5.9.3 Interaction effect of variety and salinity treatments

The GR activity showed different responses in two wheat varieties in salt stress. Compared to control, the salt sensitive BARI Gom 21 had decreased GR activities of 12% and 15% in exposure to 150 and 200 mM NaCl, respectively (Fig. 21(C)). In

opposition, salt tolerant BARI Gom 25 had significantly higher GR activities of 35% and 42% with 150 and 200 mM NaCl, respectively. Nonetheless, exogenous SA enhanced its activity further in both sensitive and tolerant varieties irrespective of salt doses, compared to the activity in the seedlings exposed to salt stress alone (Fig. 21(C)).

4.5.10 POD activity (Unit mg^{-1} protein)

4.5.10.1 Effect of variety

The POD activity varied significantly due to variety shown in Fig. 22(A). It was observed that BARI Gom 25 produced significantly the highest POD activity (64.72 Unit mg^{-1} protein), where BARI Gom 21 produced lower POD activity (51.96 Unit mg^{-1} protein).

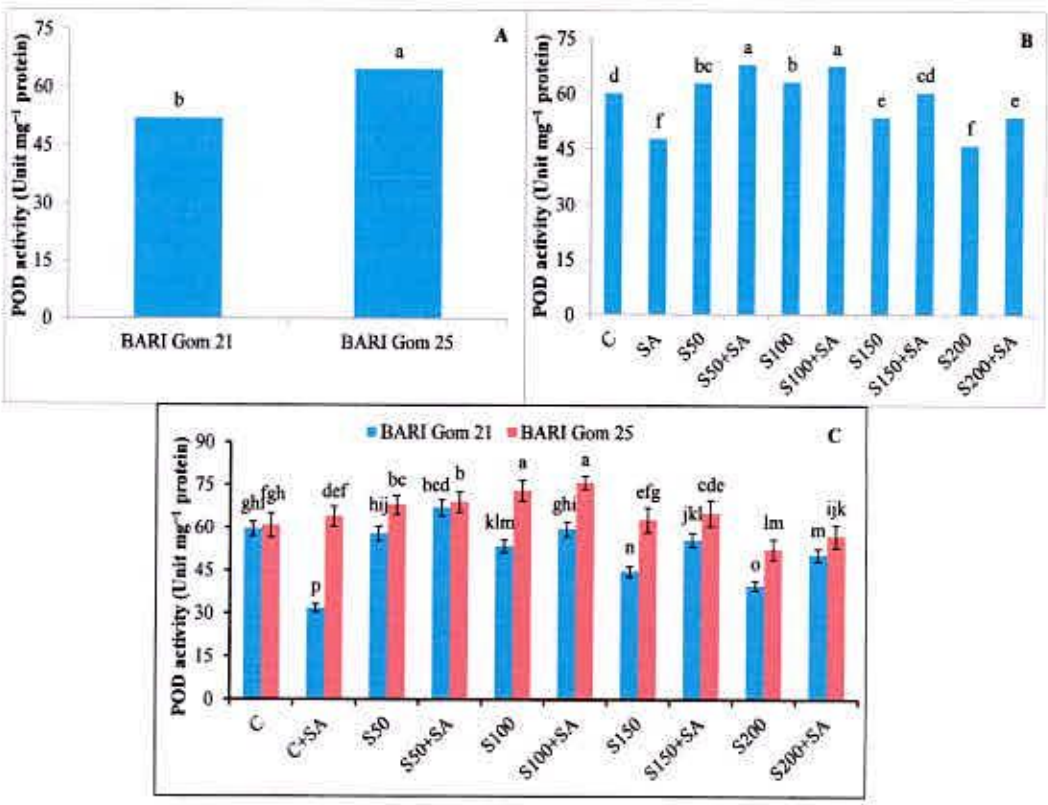

Fig. 22 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on POD activity of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.10.2 Effect of salinity treatments

Salinity caused a significant reduction of normal seedling compared to control (Fig. 22B). The highest (67.92 Unit mg^{-1} protein) POD activity was found in SA treated 50 mM stressed plant. On the contrary, 200 mM salt stressed condition produced lower POD activity (46.05 Unit mg^{-1} protein).

4.5.10.3 Interaction effect of variety and salinity treatments

Salt stress caused significant decerase in POD activities 25% and 33% in BARI Gom 21 whereas BARI Gom 25 showed 3% increase and 14% decrease in POD activity at 150 and 200 mM Saline stress respectively (Fig. 22(C)). For BARI Gom 25 POD activities ranges from 63 to 76 Unit mg^{-1} protein and in many case showed insignificant difference among different treatment. However in all case, POD activity was higher in Salt tolerant BARI Gom 25 than that of BARI Gom 21.

4.5.11 GST activity

4.5.11.1 Effect of variety

Significant variation was observed in GST activity due to the effect of variety shown in Fig. 23(A). BARI Gom 25 produced higher GST activity (175.66 nmol m^{-1} mg^{-1} protein) compared to BARI Gom 21.

4.5.11.2 Effect of salinity treatments

Upon exposure to salt stress, GST activity increased significantly compared to control (Fig. 23(B)). The highest (213.90 and 211.99 nmol m^{-1} mg^{-1} protein, respectively) GST activity was found at SA treated 150 and 200 mM salt stressed plant. Moreover, the lowest (91.58 and 94.26 nmol m^{-1} mg^{-1} protein) was found in control and only SA treated plant.

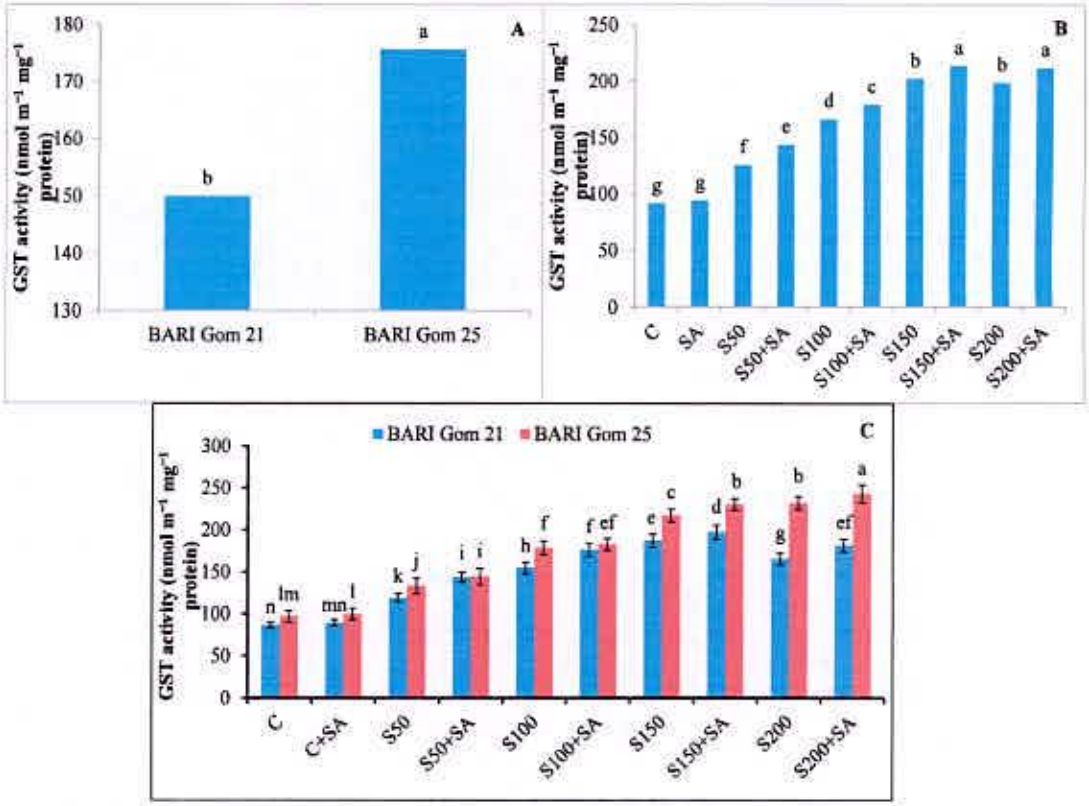

Fig. 23 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on GST activity of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.11.3 Interaction effect of variety and salinity treatments

The activity of GST sharply increased in all wheat seedlings induced by all levels of salt stress although its activity was slightly higher in salt tolerant BARI Gom 25 (Fig. 23(C)). In BARI Gom 21, 150 and 200 mM NaCl resulted in 116% and 91% increases in GST activities, compared to control, while in BARI Gom 25 its activity increased 125% and 140% over control under 150 and 200 mM NaCl (Fig. 23(C)). SA increased GST activity significantly. GST activity was higher in all cases except when seedling treated with SA under 50 mM salt stress.

AsA is able to doneate electron to many enzymatic and non enzymatic reactions what makes it a important ROS scavenging molecule. It can protect membrane by scavenging $OH^{\cdot}$ and $O_2^{\cdot-}$ directly regenerating α-tocopherol from tocopheroxyl radical (Gill, 2010).

GSH is another important substance especially for photosynthetic organelles such as chloroplast. AsA and GSH play vital role in the AsA-GSH cycle to enhance stress tolerance under stress condition (Pastori *et al*, 2003). AsA- GSH cycle composed of APX, MDHAR, DHAR and GR and these enzymes works coordinately to remove ROS such as H_2O_2 (Kadioglu *et al*., 2010). AsA content strongly related to oxidative stress tolerance and higher AsA content in plants showed better tolerance to oxidative stress (Nahar *et al*., 2015). Increased AsA or GSH content can effectively reduce ROS produced under stress conditions including salt stress and thus prevents oxidative stress. In the present study, it is examined that the performance of salt tolerance and salt sensitive wheat cultivars against different salinity levels and we also examined how they are protected from salt stress by exogenous SA application. It was observed that under mild salt stress condition AsA level of salt sensitive BARI Gom 21 was reduced whereas the AsA level of BARI Gom 21 was higher (Fig. 13). Severe salt stress also reduced the AsA level of salt sensitive and salt tolerant cultivar. In this study, a slight increase in APX activity was observed in leaves of salt treated seedlings which were supported by Gusman *et al*. (2013), Tari *et al*. (2015). However, SA supplementation could not enhance the activity further under severe salt stress (Fig. 17). This result is correlated to MDHAR and DHAR activities which regulate the recycling of AsA within the cell. From Fig. 19 and 20, it is clear that when the MDHAR or DHAR activity was reduced in salt sensitive BARI Gom 21, then its AsA levels were reduced irrespective of different salt doses. The higher MDHAR and DHAR activities of salt tolerant BARI Gom 21 were also related to its AsA levels (Fig. 13, 19 and 20). In our experiment, the GSH content (Fig. 14) also increased with increased salinity stress, but decrease under severe stress. Similar results reported by Hasanuzzaman *et al*. (2014) and Alam *et al*. (2013). The increased GSH content might be due to the increase in GR activities as well as higher GSH biosynthesis (Mittova *et al*., 2003). Under stressful condition GR helps in maintaining the GSH redox state by recycling of GSSG to GSH. It also plays a vital role in maintenance of sulfhydryl (–SH) group and acts as a substrate for glutathione *S*-transferases (Yousuf *et al*., 2012). However, supplementation with SA under salinity stress showed significant increase of both AsA and GSH (Fig. 13 and 14) which indicated a clear role of SA in producing non-enzymatic antioxidant. SA might took part in the regeneration of AsA by

up-regulating the related enzymes i.e. MDHAR and DHAR; SA also accelerated efficient recycling of GSH is also ensured by GR activity. In this experiment salt stress could increase the GR activity to a small extent. However, when SA treated seedlings were subjected to salt stress the activity markedly increased which rendered rapid recycling of GSH in line with better synthesis of GSH under salt stress conditions (Fig. 14). The role of SA in enhancing the activity of GR was reported in many plant studies (He and Zhu, 2008). Earlier, it was reported the correlation between enhanced GR activity and better GSH levels as well as abiotic stress tolerance including salinity (Hasanuzzaman *et al.*, 2011 a, b; Hasanuzzaman and Fujita, 2011;Hasanuzzaman and Fujita, 2013). In this study, AsA-GSH cycle actively work in tolerant varieties than that of susceptible varieties supported by Hasanuzzaman *et al.* (2014); Sekmen *et al.* (2007); Aghaei *et al.* (2009).

In our experiment, the GSSG content at severe salinity stress was astonishingly higher (Fig. 15) than control ones. This increase might be partly attributed to a decrease in the rate of GSH recycling or to an increase in the rate of degradation of GSH (Noctor and Foyer, 1998). However, SA treated salinity-stressed seedlings showed significantly lower GSSG. The GSH/GSSG ratio also markedly enhanced by SA application under salt stress condition (Fig. 16). It has been suggested that the GSH/GSSG ratio, indicative of the cellular redox balance, may be involved in ROS perception (Shao *et al.*, 2005). Similar observations were reported by several researchers (Kadioglu *et al.*, 2011; Hasanuzzaman and Fujita, 2011; Nahar *et al.*, 2014).

Catalase is one of the vital enzymes in scavenging H_2O_2 in plant cells exposed to various abiotic stresses due its higher turnover rate of reaction (Garg and Manchanda, 2009). The role of CAT in scavenging H_2O_2 was observed in several studies (Hasanuzzaman *et al.*, 2011a, b; Hasanuzzaman and Fujita, 2013). In this study, CAT activity was significantly decreased upon exposure to salt stress in susceptible variety BARI Gom 21 and this decrease in CAT activity in BARI Gom 21 under salt stress might be due to its inactivation by the accumulated H_2O_2 induced by water shortage or ineffective enzyme synthesis or change in assembly of enzyme sub-units (Gupta *et al.*, 2009). On the other

hand, CAT activity was significantly increased at mild salt stress and decreased under severe salt stress in BARI Gom 21 (Fig. 18) (Gupta *et al.*, 2009; Khan *et al.*, 2009).This trend was supported by earlier reports (Lin *et al.*, 2010; Azooz *et al.*, 2009, Hasanuzzaman *et al.*, 2014). In contrary, SA-supplemented salt-stressed seedlings showed enhanced activity CAT than those under salt treatment without SA which suggests an unambiguous role of SA in scavenging H_2O_2 under salt stress. Similar increases in CAT activity after SA supplementation was observed under salt stress by other researchers (Yusuf *et al.*, 2008; Noriega *et al.*, 2012).

POD activity increased under salinity stress (Rohman *et al.*, 2015, Li *et al.*, 2014). In this study, POD activity increased at mild stress but decreased at severe stress. In higher plants, H_2O_2 is scavenged by the ascorbate-glutathione pathway and/or by CAT and non-specific PODs (Scandalios, 2005; Miller *et al.*, 2010). CAT, POD and APX are reported to scavenge H_2O_2 to water in plant species (Gill & Tujeta, 2010; Miller *et al.*, 2010).The increased activities of POD and GPX under salt stress played important role in H_2O_2 scavenging (Rohman *et al.*, 2015). SA in salt treatments increased the activities of POD (Fig. 20) which reduced the H_2O_2 level and MDA production as well. Li *et al.* (2014) found upregulation of SOD, POD, CAT and APX by application of SA in salt stressed *T. grandis* seedlings. Plant GSTs are also associated with responses to various forms of abiotic stress (Hossain *et al.*, 2006 and Dixon *et al.*, 2010) and stress tolerance is often correlated with enhanced activity of GST (Hasanuzzaman *et al.*, 2012). In both wheat varieties of our experiment, GST activity markedly increased under salt stress where comparatively higher activity was observed in salt tolerant BARI Gom 21 (Fig. 21). Our results are partially supported by Hoque *et al.* (2008) and Hasanuzzaman *et al.* (2014).

4.6 Yield contributing characters

4.6.1 Effective tiller hill^{-1}

4.6.1.1 Effect of variety

The effective tiller varied significantly due to variety shown in Fig. 24(A). It was observed that BARI Gom 25 produced significantly the highest effective tiller (3.29), where BARI Gom 21 produced lower effective tiller (2.81).

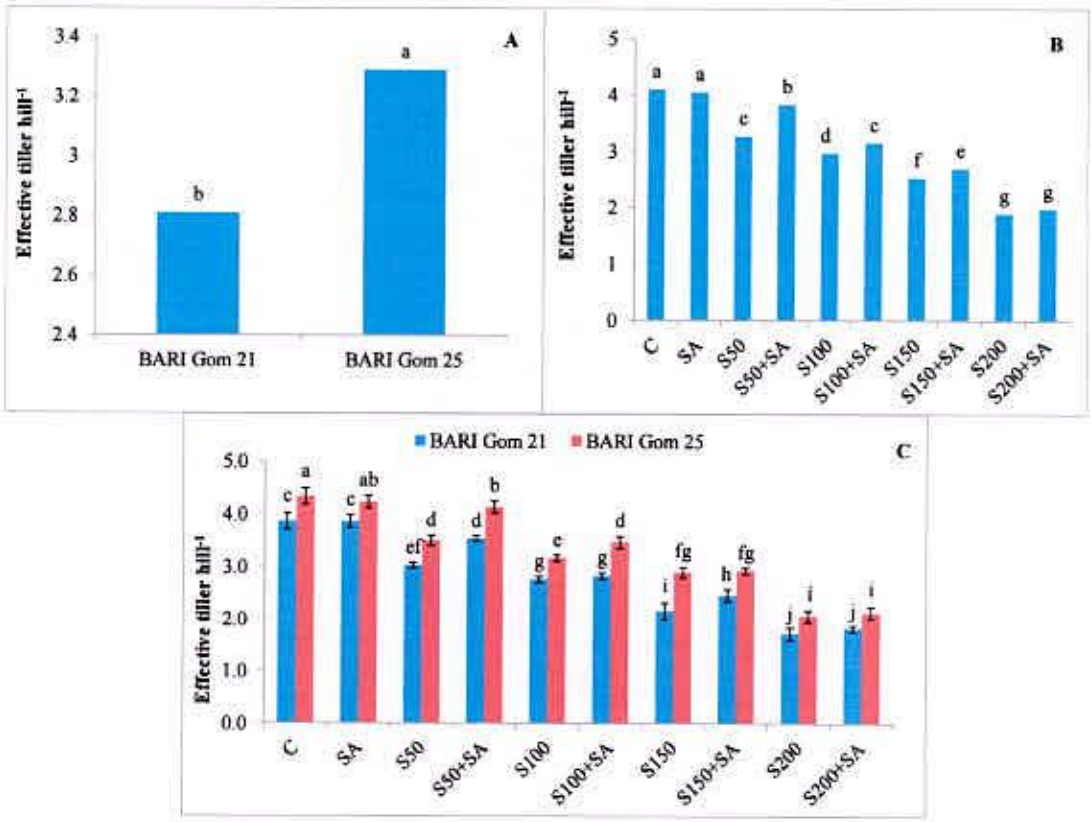

Fig. 24 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on effective tillers hill^{-1} (no.) of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.6.1.2 Effect of salinity treatments

Salinity caused a significant reduction of effective tiller compared to control (Fig. 24(B)). The highest effective tiller was found in control and only SA treated plant. On the contrary, SA increased effective tiller number compared to its respective control but not similar with control and only SA treated plant (7, 24 and 35% at 50, 100 and 150 mM stressed condition, respectively). 200 mM salt stressed condition was also not affected by SA treatment (Fig. 24(B)).

4.6.1.3 Interaction effect of variety and salinity treatments

Exposure to salt stress resulted in significant decreases in effective tiller number $hill^{-1}$: 22, 29, 44 and 56% for BARI Gom 21 and 20, 27, 34 and 53% for BARI Gom 25 at 50, 100, 150 and 200 mM salinity stressed conditions, respectively when compared to unstressed control plant(Fig. 24(C)). Addition of exogenous SA combination with salinity stress significantly increased the effective tiller number up to 150 mM by 9, 27 and 37% for BARI Gom 21 and 5, 21 and 33% for BARI Gom 25 in SA treated 50, 100 and 150 mM stressed plant respectively (Fig. 24(C)), when compared to plants exposed to salt stress alone. Furthermore, in case of 200 mM salt stressed condition there had no statistically difference from each other of both varieties.

4.6.2 Non-effective tiller $hill^{-1}$

4.6.2 1 Effect of variety

Significant variation was observed in non-effective tiller due to the effect of variety shown in Fig. 25(A). BARI Gom 21 produced higher non-effective tiller (1.2) compared to BARI Gom 25.

4.6.2 2 Effect of salinity treatments

Upon exposure to salt stress, non-effective tiller increased significantly compared to their controls (Fig. 25(B)). The highest non-effective tiller number was found in SA treated 200 mM salt stressed plant (1.52). Moreover, the lowest non-effective tiller was found in only SA treated plant (0.88).

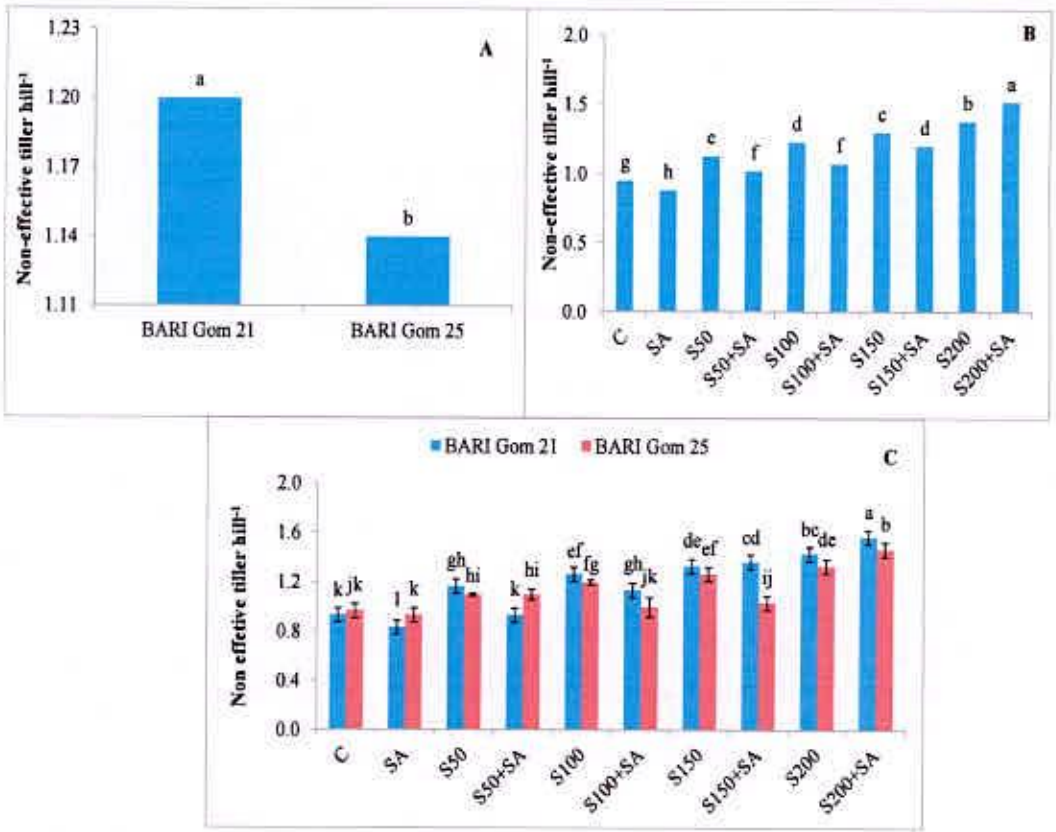

Fig. 25 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments non-effective tillers hill^{-1} (no.) of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.6.2.3 Interaction effect of variety and salinity treatments

Salinity stress caused increased number of non-effective tiller hill^{-1} of both varieties. The highest non-effective tiller was found in 200mM salt stressed condition of BARI Gom 21 which was treated with SA (Fig. 25(C)). Upon salinity stress treatment the number of non-effective tiller was increased by 25, 36, 43 and 54% for BARI Gom 21 and 14, 25, 32 and 38% for BARI Gom 25 at 50, 100, 150 and 200 mM, respectively, as compared to their respective control (Fig. 25(C)). SA supplementation reduced the number of non-effective tiller in the salt stressed condition up to 150 mM for both varieties. On the other

hand, it became increased for both of variety. The increment of non-effective tiller number higher in BARI Gom 21 compared to BARI Gom 25.

4.6.3 Length of spike

4.6.3.1 Effect of variety

As shown in Table 11 highest length of spike also found in BARI Gom 25 compared to BARI Gom 21. 11.69cm spike length was found in BARI Gom 25 where 10.1cm found in BARI Gom 21.

Table 11 Effect of variety and salinity treatments on length of spike and spikelet spike^{-1} of wheat

Variety	Length of spike (cm)	Spikelet spike^{-1}
BARI Gom 21	10.1b	30.51b
BARI Gom 25	11.69a	35.48a
LSD (0.05)	**0.236**	**0.526**
CV (%)	**4.14**	**3.05**
Treatment		
C	14.47a	41.77a
SA	14.27a	42.33a
S50	11.98c	36.73c
S50+SA	13.03b	39.68b
S100	9.8d	31.4d
S100+SA	11.62c	36.77c
S150	8.83e	28.23e
S150+SA	9.58d	28.78e
S200	7.76f	23.3f
S200+SA	7.61f	20.95g
LSD (0.05)	**0.528**	**1.176**
CV (%)	**4.14**	**3.05**

4.6.3.2 Effect of salinity treatments

Length of spike was also affected by salinity stress, according to Table 11. Saline treatment reduced the length of spike compared to control and SA treated plant. 18, 33, 39 and 47% reduced due to 50, 100, 150 and 200 mM salinity stress, respectively. Furthermore, the reduction was less in SA treated stressed plant compared to respective control (Table 11). But SA could not affect 200 mM salt stressed condition where it gave similar result with 200 mM stressed condition.

4.6.3.3 Interaction effect of variety and salinity treatments

Length of spike of BARI Gom 21 and BARI Gom 25 varieties were decreased by 28%, 38%, 39%, 47% and 8%, 28, 40%, 47% in 50, 100, 150 and 200 mM respectively, as compared to their respective control (Fig. 26). In contrary, exogenous SA supplementation caused increased the length of spike of both varieties up to 150 mM. Moreover, it had no statistically significance with each other. Maximum reduction in length of spike due to salinity stress was observed in BARI Gom 21 compared to BARI Gom 25.

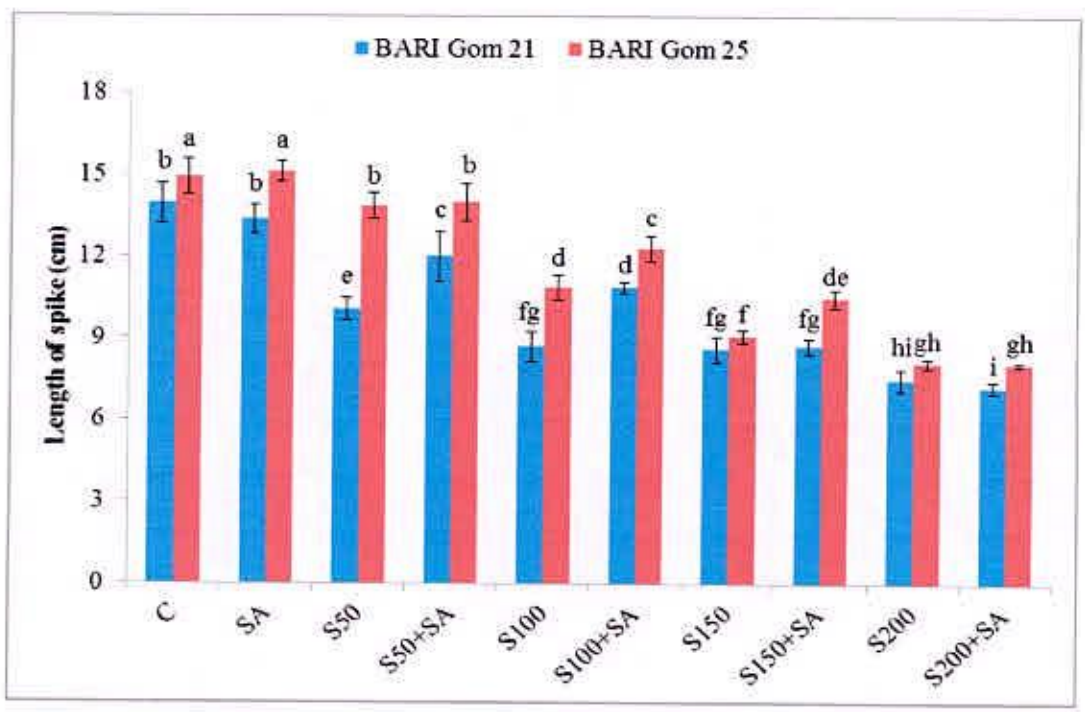

Fig. 26 Interaction effect of variety and salinity treatments on length of spike of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.6.4 Spikelet spike^{-1}

4.6.4.1 Effect of variety

Significant variation was observed for spikelet spike^{-1} due to varietal variation in Table 11. BARI Gom 25 produced higher spikelet spike^{-1} (35.48) compared to BARI Gom 21.

4.6.4.2 Effect of salinity treatments

For different salinity stress treatments, significant variation was observed for spikelet spike^{-1} (Table 11). The highest spikelet spike^{-1} was found in control and only SA treated plant. Spikelet spike^{-1} decreased sharply in case of salt stressed treatment compared to SA treated stressed plant. However, SA treatment increased spikelet number 6, 12 and 32% at 50, 100 and 150 mM stressed condition, but not increased in case of 200 mM stressed condition.

4.6.4.3 Interaction effect of variety and salinity treatments

Number of spikelet per spike was also decreased in the same way which was 9, 26, 34 and 48% for BARI Gom 21 and 15, 24, 31 and 42% for BARI Gom 25 at 50, 100, 150 and 200 mM of salinity stress, respectively (Fig. 27). Extent of reduction was higher in BARI Gom 21 than BARI Gom 25. However, exogenous application of SA increased the shoot length in both the cultivars under saline and non-saline conditions. The SA application increased the spikelet number under control and saline conditions in both cultivars. Significantly higher number of spikelet per spike was recorded in controlled condition, only SA treated and SA treated 50 mM saline condition of BARI Gom 25. After all, both of variety gave same result in case of 200 mM saline condition. SA treated 200 mM saline condition gave significantly lower number of spikelet per spike than 200 mM saline condition without treatment for both of variety (Fig. 27).

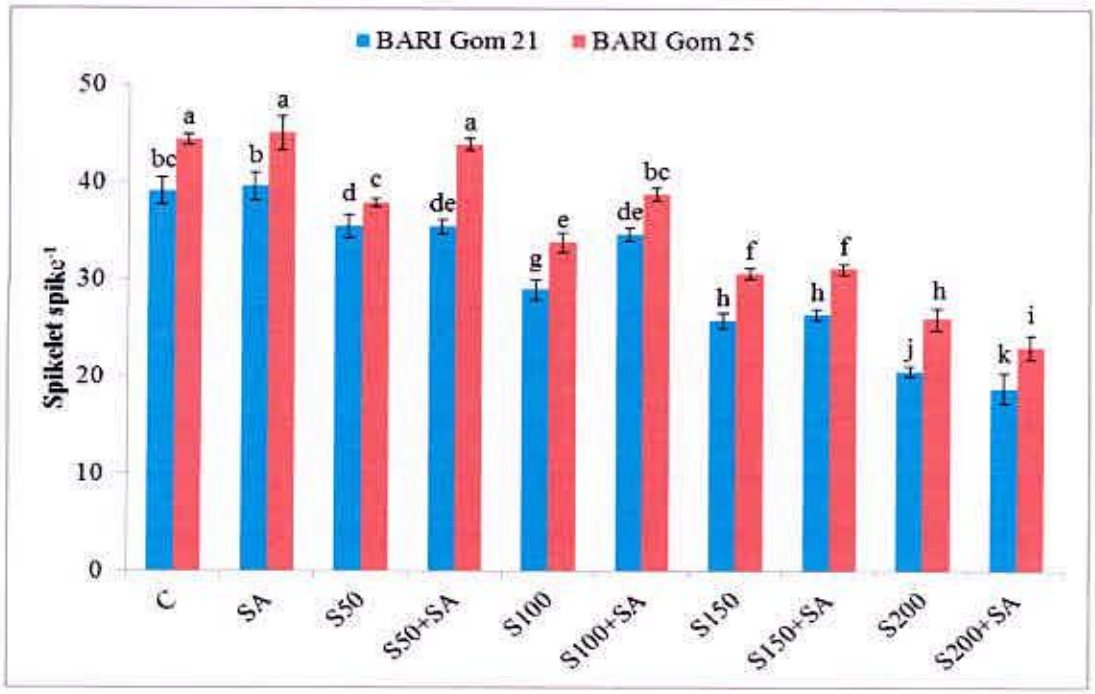

Fig. 27 Interaction effect of variety and salinity treatments on spikelet spike^{-1} of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.6.5 1000-grain weight

4.6.5.1 Effect of variety

Weight of 1000 grains showed significant variation among the different varieties (Fig. 28(A)). BARI Gom 25 produced highest 1000 grain weight (45.99g). The lowest 1000 grain weight (36.99g) was obtained from BARI Gom 21.

4.6.5.2 Effect of salinity treatments

Salinity reduced 1000 grain weight compared to control. SA treatment increased 1000 grain weight under stressed condition (1, 16 and 24% at 50, 100 and 150 mM). But 200 mM stressed condition produced highest 1000 grain weight compared to SA treated salt stressed condition (Fig. 28(B)).

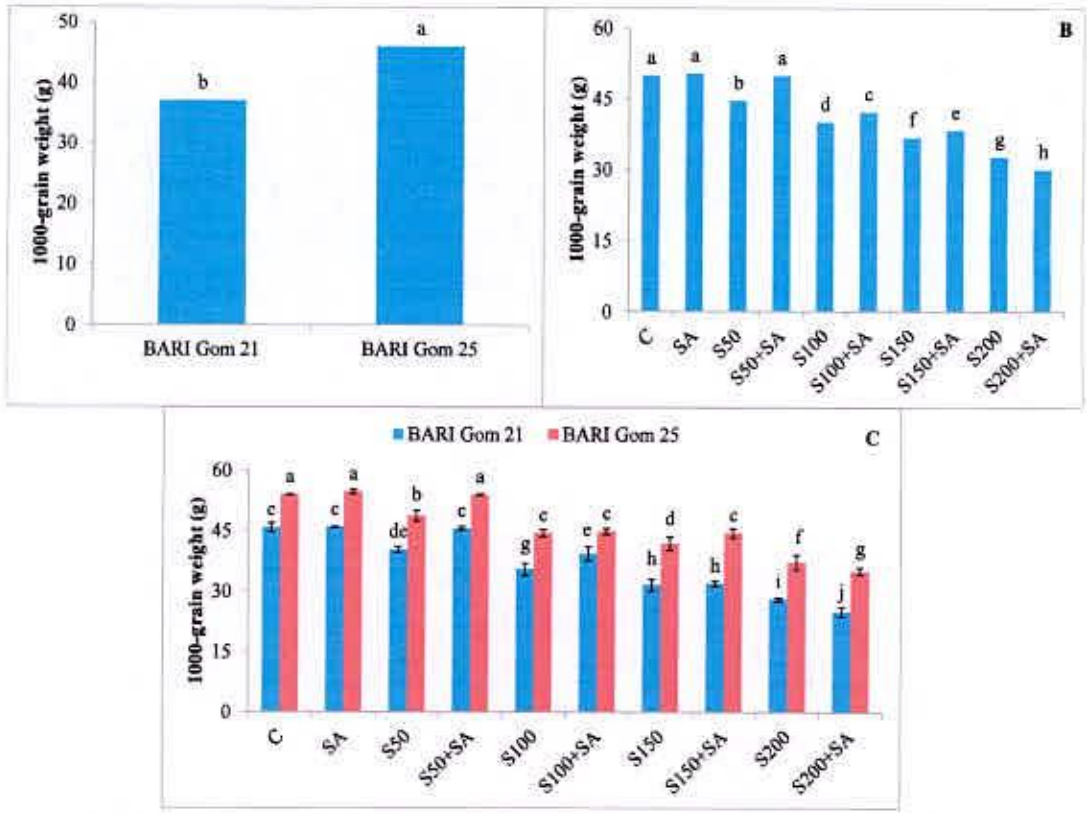

Fig. 28 (A) Effect of variety, (B) Effect of salinity treatment, and (C) Interaction effect of variety and salinity treatments on 1000 grain weight of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.6.5.3 Interaction effect of variety and salinity treatments

As shown in Fig. 28(C) 1000 grain weight of wheat plants decreased under salinity stress. Marked decreases in 1000 grain weight were observed (12, 23, 31 and 39% in BARI Gom 21 and 10, 18, 23 and 31% in BARI Gom 25 at 50, 100, 150 and 200 mM, respectively) in response to salt stress. Anyhow, salt stressed plants treated with SA up to 150 mM had significantly higher 1000 grain weight in both variety compared to plants which were subjected to salt stress without SA. Even so, 1000 grain weight significantly decreased after treating with SA at 200 mM salt stress in both variety of wheat plant compared to plants which were treated with 200 mM without SA treatment (Fig. 28(C)). Highest 1000 grain weight was found in control (54.07g) of BARI Gom 25 which was similar to only SA treated (54.77g) and SA treated 50 mM (48.73g) stressed plant of that variety. Comparing cultivars, under control conditions, BARI Gom 25 produced more

1000 grain weight in comparison to BARI Gom 21. However, both wheat cultivars behaved similarly under saline environment.

4.7 Yields

4.7.1 Grain yield plant^{-1}

4.7.1.1 Effect of variety

Grain yield varied significantly for different varieties shown in Fig. 29(A). The highest grain yield (29.92g) was recorded by BARI Gom 25 compared to BARI Gom 21 (23.47g).

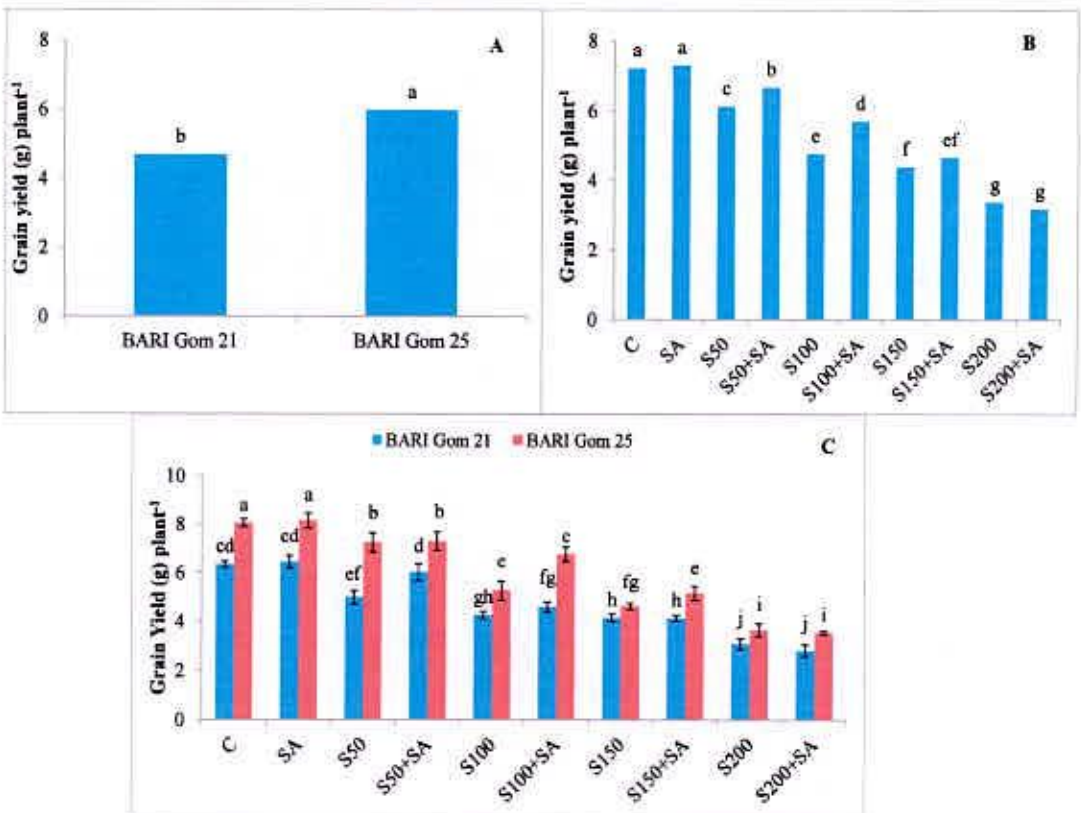

Fig. 29 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on grain yield plant^{-1} of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.7.1.2 Effect of salinity treatments

Significant variation was observed for grain yield due to different salinity treatments (Fig. 29(B)). Grain yield became reduced due to saline treatment. However, SA treated under salt stressed condition increased grain yield up to 100 mM salt stressed condition (8 and 28% at 50 and 100 mM stress treatment, respectively) compared to their respective control. At 150 and 200 mM stress condition could not affected by SA treatment (Fig. 29(B)).

4.7.1.3 Interaction effect of variety and salinity treatments

Salinity caused (Fig. 29(C)) a significant reduction in grain yield of wheat plants of both cultivars compared to those in non-saline solution and magnitude of decrease was less in BARI Gom 25 as compared to BARI Gom 21. 12, 35, 43 and 55% grain yield decreased for BARI Gom 25 and 22, 33, 35 and 52% for BARI Gom 21 at 50, 100, 150 and 200mM, respectively. The highest grain yield was found in control (40.33g) and only SA treated plant (40.77g) of BARI Gom 25 variety (Fig. 29(C)). For all that, the lowest grain yield was found in 200mM saline condition of both varieties which was not increased or decreased after treating with SA.

4.7.2 Straw yield plant^{-1}

4.7.2.1 Effect of variety

There was significant variation observed for straw yield due to varietal variation (Fig. 30(A)). BARI Gom 25 recorded highest straw yield (22.09g) and the lowest straw yield (20.09g) was obtained from the other variety named BARI Gom 21.

4.7.2.2 Effect of salinity treatments

Sharp decreases in straw yield were observed (13, 30, 41 and 51% at 50, 100, 150 and 200 mM stressed condition, respectively) due to NaCl salinity stress (Fig. 30(B)). Furthermore, SA treatment increased straw yield under stress condition. But at 200 mM salt stressed condition SA treatment reduced straw yield compared to 200 mM stressed condition without SA treatment.

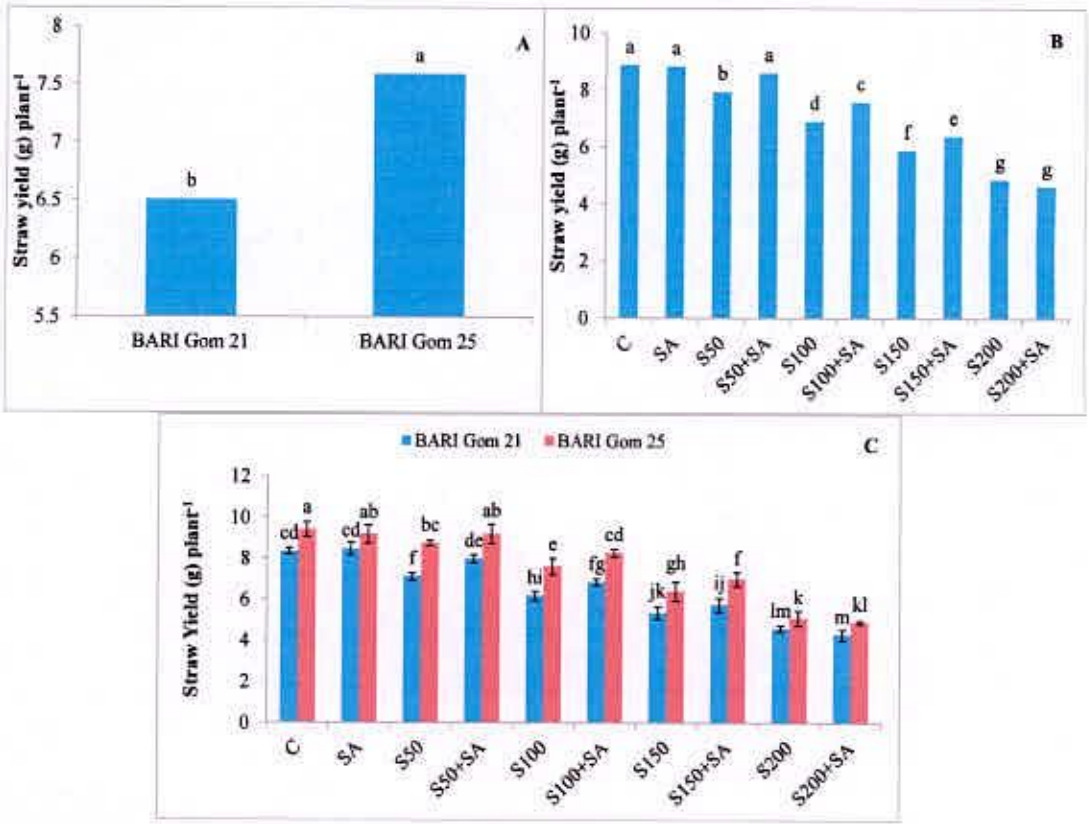

Fig. 30 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on straw yield plant^{-1} of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.7.2.3 Interaction effect of variety and salinity treatments

Straw yield was noticeably decreased in both wheat varieties under salt stressed condition. Straw yield was decreased by 11, 32, 42 and 51% for BARI Gom 21 and 15, 28, 41 and 50% for BARI Gom 25 at 50, 100, 150 and 200mM respectively, compared to respective control (Fig. 30(C)). Highest straw yield was observed in only SA treated plant (30.14g) of BARI Gom 25 variety when grown under non-saline treatment. Control of BARI Gom 25 variety produced 29.53g straw yield pot^{-1} which is statistically similar with the highest result (Fig. 30(C)). However, exogenous application with SA mitigated the salt effect up to 150 mM for both of varieties. After 150 mM treatment it did not give any significant result in BARI Gom 21. But for BARI Gom 25, it became decreased.

4.7.3 Biological yield plant^{-1}

4.7.3.1 Effect of variety

Biological yield varied significantly for different varieties shown in Fig. 31 (A). The highest biological yield (13.58 g) was recorded by BARI Gom 25 compared to BARI Gom 21 (11.2 g).

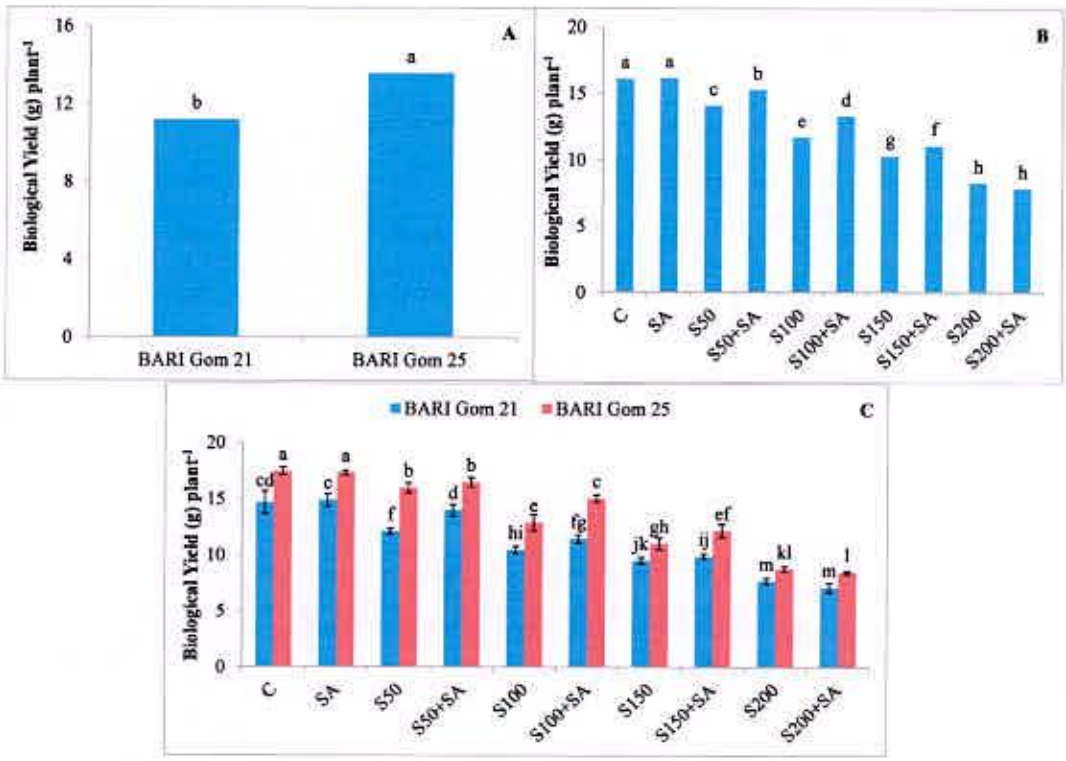

Fig. 31 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on biological yield plant^{-1} of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.7.3.2 Effect of salinity treatments

Exposure to salt stress resulted in significant decreases in biological yield (13, 28 and 37% at 50, 100 and 150 mM stress, respectively). However, SA with saline treatment increased biological yield compared to its respective control up to 200 mM salt stressed condition, where it was given similar result (Fig. 31(B)).

4.7.3.3 Interaction effect of variety and salinity treatments

According to (Fig. 31(C)) Gradual decrease in biological yield over control was observed, for both BARI Gom 21 and BARI Gom 25, as the plant exposed to salt stress. When seedling treated with SA, biological yield increases significantly for all cases. Biological yield of BARI Gom 25 was always higher than that of BARI Gom 21. The highest amount of biological yield (17.48 and 17.34 g) was observed in untreated control and only SA treated plant of BARI Gom 25 and the lowest amount of biological yield (7.70 and 7.15 g) was observed in seedling exposed to 200 mM salinity and SA treated 200 mM salt stressed condition of BARI Gom 21.

4.7.4 Harvest index

4.7.4.1 Effect of variety

Variety showed significant variation in harvest index (Fig. 32 (A)). BARI Gom 25 showed the highest harvest index (54.63%) whereas lowest harvest index (49.44%) in BARI Gom 21.

4.7.4.2 Effect of salinity treatments

For different salinity treatments with or without SA spraying, significant variation was observed for harvest index (Fig. 32 (B)). Control (66.27%) and only SA treated plant (66.74%) produced higher harvest index compared to other salt stressed condition and SA treated stressed condition. However, SA treated stressed plant produced higher harvest index (62.08, 56.03 and 45.9% at 50, 100 and 150 mM salt stressed condition, respectively) compared to salt stressed condition.

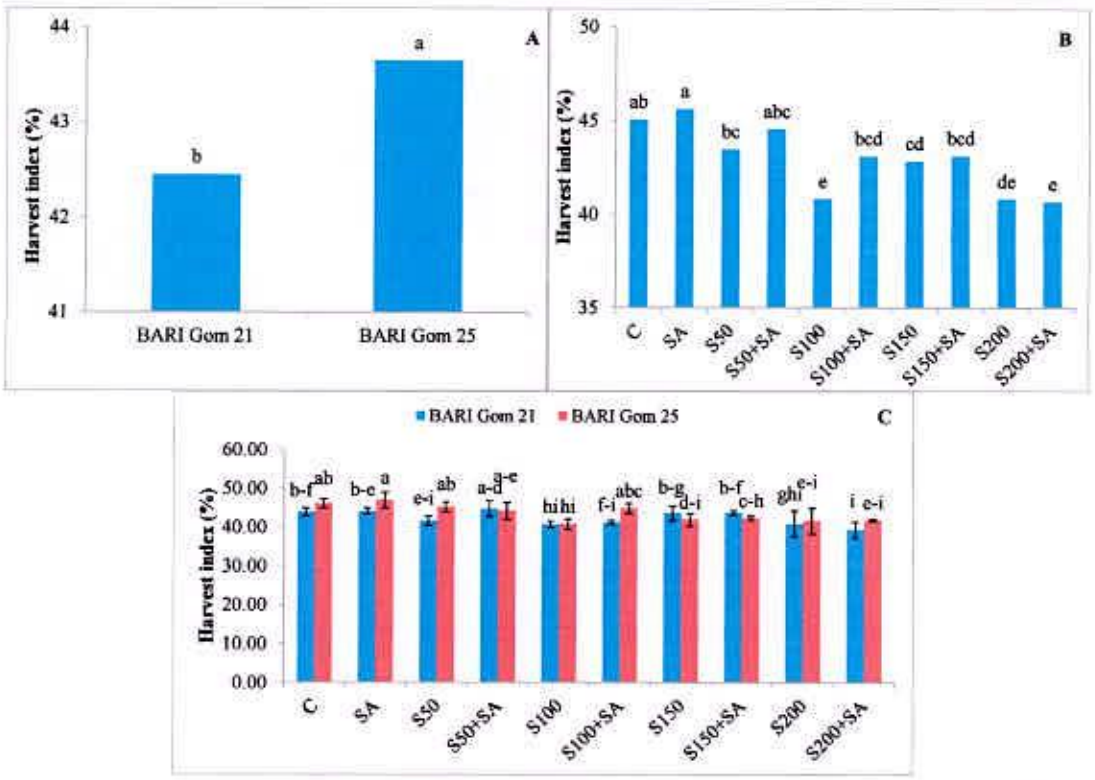

Fig. 32 (A) Effect of variety, (B) Effect of salinity treatments, and (C) Interaction effect of variety and salinity treatments on harvest index of wheat. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.7.4.3 Interaction effect of variety and salinity treatments

Sharp decreases in harvest index were observed (21, 29, 39 and 45% for BARI Gom 21 and 8, 25, 35 and 42% for BARI Gom 25 at 50, 100, 150 and 200 mM NaCl stresses, respectively) in response to salt stress, compared to the respective control and untreated control (Fig. 32 (C)). SA treated salt stressed plants had significantly higher HI, compared to plants subjected to salt stress without SA treatment. However, the level was significantly lower than that of untreated control. The HI level of SA treated control plants was similar to that of the untreated control.

Yield is a result of the integration of metabolic reactions in plants; consequently any factor that influences this metabolic activity at any period of plant growth can affect the yield (Ibrahim and Aldesuquy, 2003). In this investigation, Yield and yield attributes

(spike length, plant height, number of spikelet spike^{-1}, 100 grain weight, grain weight, straw weight, crop yield per plant) are reduced due to salt stress in both wheat cultivars. The reduction in yield of stressed wheat plants can be attributed to the decrease in photosynthetic pigments, carbohydrates accumulation (polysaccharides) and nitrogenous compounds (total nitrogen and protein). The decrease in yield and yield components in different crops under similar conditions has also been reported by many workers (Arfan *et al.*, 2007; Sankar *et al.*, 2008 and Aldesuquy *et al.*, 2012). These workers clearly indicated that salt tolerant genotypes showed less reduction in yield plants in respect of susceptible ones. Therefore, maintenance of better yield of the wheat cultivar, BARI Gom 21 than that of BARI Gom 21 under salt stress (Fig. 24, 25, 26, 27, 28, 29, 30, 31, 32). Salt stress during the early stage of reproductive growth tends to reduce yield by reducing seed number. During seed development stress reduces yield by reducing seed size. Prolonged moisture stress during reproductive growth can severely reduce yield because of reduced seed number and seed size (Dombos *et al.*, 1989). However, treating plants with SA under salinity stress caused increments in all the studied yield criteria. It could be stated that the beneficial effect of SA on improving yield may be due to the translocation of more photo assimilates to the seeds (Fig. 26, 27). These results may be due to the role of salicylic acid in enhancing some physiological and biochemical aspects. These findings are in agreement with those reported by Ali and Mahmoud (2013), Arfan *et al.* (2007) and Singh and Usha (2003) on wheat, Gunes *et al.* (2005) on maize and Elwan and El-Hamahmy (2009) on pepper.

Chapter 5

SUMMARY AND CONCLUSION

The present piece of work was done at the experimental shed of the Department of Agronomy, Sher-e Bangla Agricultural University, Dhaka during the period from November to March, 2013 to find out the influence of SA to mitigate the effect of salt stress on wheat which was applied exogenously.

The experiment was laid out in a Randomized Completely Block Design (RCBD) with three replications. There were 60 pots all together replication with the given factors. Empty earthen pots with 18 inch depth were used for the experiment. There were 20 treatment combinations. The treatments were control (C), control with salicylic acid (C+SA), 50 mM NaCl (S50), 50 mM NaCl with salicylic acid (S50+SA), 100 mM NaCl (S100), 100 mM NaCl with salicylic acid (S100+SA), 150 mM NaCl (S150), 150 mM NaCl with salicylic acid (S150+SA), 200 mM NaCl (S50), 200 mM NaCl with salicylic acid (S200+SA) for two varieties viz. BARI Gom 21 and BARI Gom 21. The salinity treatments were applied on 28, 35, 42, 49, 56 and 63 DAS. Fifteen healthy seeds of each variety were sown in each pot.

Germination test was performed before sowing the seeds in the pot. The data were collected from five days week seedlings for three times with some parameters viz. germination (%), number of normal and abnormal seedling, length of shoot and root, fresh weight of shoot and root and dry weight of seedling. The data on growth parameters viz. plant height, tillers $hill^{-1}$, fresh weight $plant^{-1}$, and dry weight $plant^{-1}$ were recorded during the period from 30 to 60 DAS. Two physiological parameters viz. relative water content and chlorophyll content were also collected. Some biochemical parameters were collected from each variety viz. lipid peroxidation, H_2O_2 content, ascorbic acid content, glutathione content, activities of antioxidant enzymes (APX, MDHAR, DHAR, GR, GST, POD and CAT). At harvest, characters like plant height, effective tillers $hill^{-1}$, non-

effective tillers hill^{-1}, length of spike, spikelet spike^{-1}, 1000 grain weight, grain yield, straw yield and harvest index were recorded.

Germination (%) was also affected by the salt stress. The highest germination (%) was found in control and only SA treated plant of both varieties. Only SA treatment of BARI Gom 25 produced higher number of normal seedling compared to other treatment, where 150 mM of NaCl stress of BARI Gom 21 produced higher number of abnormal seedling. Length of shoot and root was highest in control and only SA of both varieties. Control and only SA treatment of both varieties produced highest fresh weight of shoot. On contrary, control and only SA treated plant of BARI Gom 25 produced higher fresh weight of root and dry weight of seedling.

Different salinity with or without SA treatments had significant effect on crop growth parameters viz. plant height, tillers hill^{-1}, fresh weight plant^{-1} and dry weight plant^{-1} at different DAS. The highest plant height was observed in BARI Gom 25 with control (31.47cm) at 30; control (48.83cm) and only SA (48.3cm) at 45 DAS and control (73.93cm) and only SA (74cm) at 60 DAS. The highest tillers hill^{-1} was observed in BARI Gom 25 with control (2.23) and only SA (2.17) at 30 DAS; only SA (2.33) at 45 DAS and only SA (2.53) at 60 DAS. Fresh weight plant^{-1} was highest in BARI Gom 25 with only SA (4.01g) at 30 DAS; control (9.22g) and only SA (9.46g) at 45 DAS and control (11.89g) and only SA (11.99g) at 60 DAS. Dry weight plant^{-1} was highest in BARI Gom 25 with control (0.71g), only SA (0.70g), S50 (0.68g) and S50+SA (0.68g) at 30 DAS; control (2.56g) and only SA (2.55g) at 45 DAS and control (3.48g) and only SA (3.48g) at 60 DAS.

Salinity treatments had significant effect on the physiological parameters viz. relative water content and chlorophyll content was highest in BARI Gom 21 with control (191.72%), only SA (193.03%) and control (0.059 mg cm^{-2}), only SA (0.058 mg cm^{-2}), S50+SA (0.057 mg cm^{-2}).

The biochemical parameters viz. MDA and H_2O_2 content was highest in BARI Gom 21 with S200 (65.46 and 23.98 nmol g^{-1} FW, respectively). The highest AsA content was found in BARI Gom 25 at control and SA (4581.41 and 4592 nmol g^{-1} FW, respectively).

GSH content was highest in BARI Gom 25 at S100+SA (3637.59 nmol g^{-1} FW). GSSG was highest in BARI Gom 21 at S200 (63.32 nmol g^{-1} FW). GSH/GSSG ratio was highest in BARI Gom 25 at control (22.74). The highest CAT was in BARI Gom 25 at S50+SA (62.77 µmol m^{-1} mg^{-1} protein), APX at S100+SA (1.23 µmol m^{-1} mg^{-1} protein), MDHAR at SA (50.84 nmol m^{-1} mg^{-1} protein) and DHAR at S100+SA (267.53 nmol m^{-1} mg^{-1} protein). The highest GR was in BARI Gom 25 at S200 (42.34 nmol m^{-1} mg^{-1} protein), S200+SA (42.77 nmol m^{-1} mg^{-1} protein); POD at S100 (72.91 Unit mg^{-1} protein), S100+SA (75.68 Unit mg^{-1} protein) and GST at S200+SA (243.34 nmol m^{-1} mg^{-1} protein).

Salinity treatments had significant effect on the yield and yield contributing characters viz. plant height, effective tillers $hill^{-1}$, length of spike, spikelet $spike^{-1}$, 1000 grain weight, grain yield, straw yield and harvest index was highest in BARI Gom 25 at control and SA treatment. Where, non- effective tiller was highest in BARI Gom 21 at S200.

Based on result of the present experiment, together with results found in the available literature, we therefore concluded that exogenous 1 mM SA spray is an effective way to overcome the adverse effects of osmotic stress on growth, physiology and yield components of wheat. It could be partially attributed to the increase in non-enzymatic and enzymatic antioxidants. In all the cases BARI Gom 25 was a better performer under salt stress. All parameters decreased at any level of salt stress. Exceptions were abnormal seedling, non-effective tiller $hill^{-1}$, MDA content, H_2O_2, GSSG content and GST activity which increased in response to salinity.

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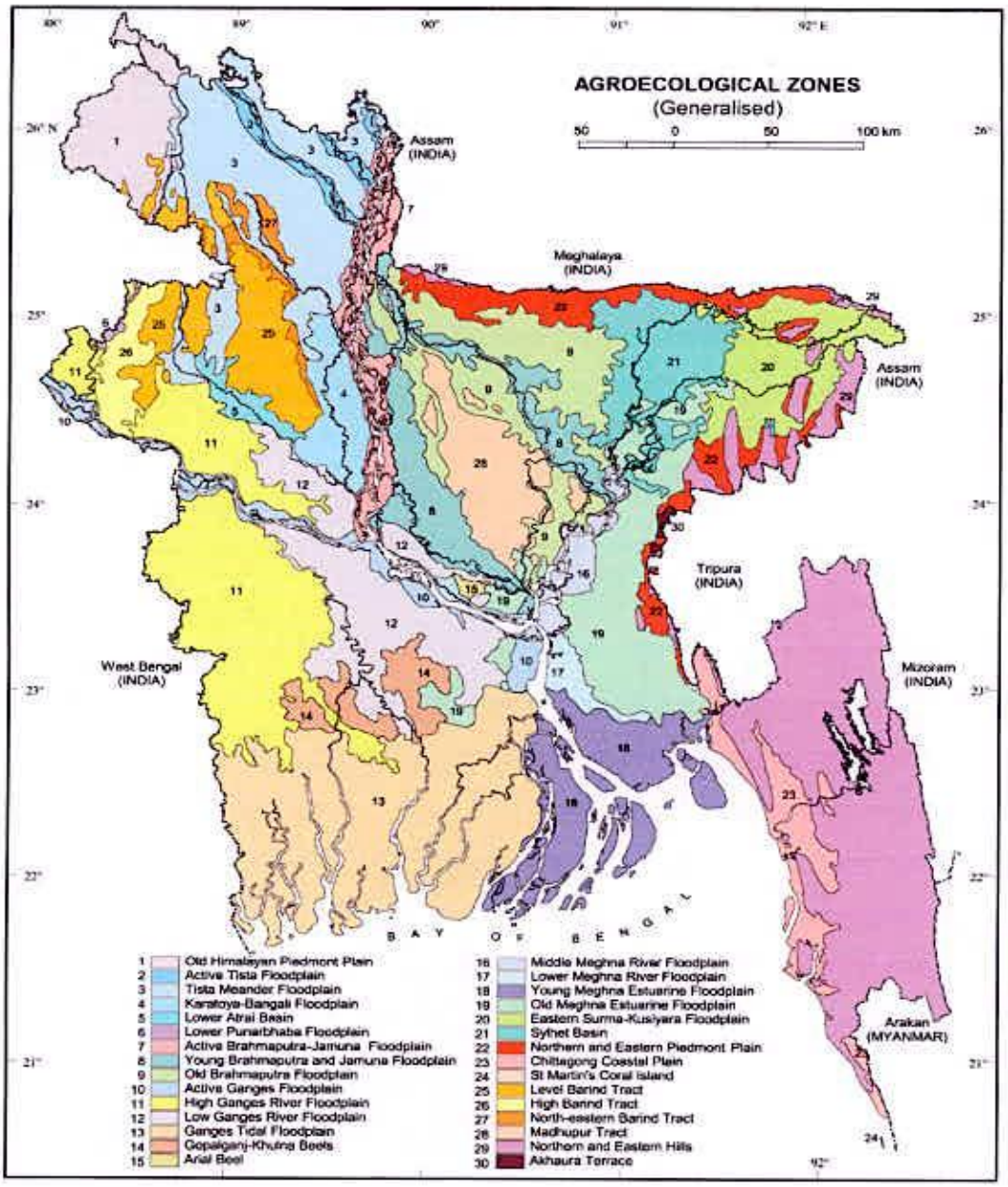
APPENDICES

Appendix I. Experimental location on the map of Agro-ecological Zones of Bangladesh



Appendix II: Physical and chemical properties of experimental soil analyzed at Soil Resources Development Institute (SRDI), Farmgate, Dhaka.

Characteristics	Value
Particle size analysis	
%Sand	27
%Silt	43
%Clay	30
Textural class	Silty-clay
pH	5.6
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total N (%)	0.03
Available P (ppm)	20.00
Exchangeable K (me/100 g soil)	0.10
Available S (ppm)	45

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

Appendix III. Monthly average air temperature, rainfall and relative humidity of the experimental site during the period from November 2013 to March 2014

Months	Air temperature		Relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum		
November, 2013	28.10	6.88	58.18	1.56
December, 2013	25.36	5.21	54.3	0.63
January, 2014	21.17	15.46	64.02	00
February, 2014	24.30	19.12	53.07	2.34
March, 2014	29.78	22.37	48.66	0.12