COMPARATIVE PHYSIOLOGY OF SALINITY AND DROUGHT STRESS TOLERANCE IN INDICA AND JAPONICA RICE SEEDLINGS

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CERTIFICATE

*This is to certify that thesis entitled, "**COMPARATIVE PHYSIOLOGY OF SALINITY AND DROUGHT STRESS TOLERANCE IN INDICA AND JAPONICA RICE SEEDLINGS**" 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 in AGRONOMY**, embodies the result of a piece of bona fide research work carried out by **Md. Shahadat Hossen**, Registration No. **10-03779** 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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DEDICATED TO MY BELOVED PARENTS
AND Prof. Dr. Quazi Abdul Fattah

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The Author

Comparative physiology of salinity and drought stress tolerance in indica and japonica rice seedlings

ABSTRACT

Salinity and drought stress is the major and frequently co-occurring abiotic stresses which disturb the morphophysiological and biochemical attributes within the plants. Therefore, the present study was conducted to investigate the morphophysiological and biochemical responses of two popular cultivars of rice (BRRI dhan29 and BRRI dhan48 from indica and Koshihikari from japonica) under salinity and drought stress either alone or in combination at early vegetative stage. Eighteen-day-old seedlings were subjected to salinity (150 mM NaCl), drought (PEG-6000, 15%) and combined salinity and drought (150mM NaCl + PEG-6000, 15%) *in vitro* for 72h. Salinity and drought alone and in combination increased mortality rate, decreased seedlings height, reduced biomass, abated water status and lowered photosynthetic pigments content in all three cultivars but the worst effects were observed in BRRI dhan29 and Koshihikari compared to BRRI dhan48. Moreover, under stress conditions compared with control a substantial increase was seen in the rate of electrolyte leakage (EL), elevated levels of H_2O_2, lipoxygenase (LOX) activity, malondialdehyde (MDA) and methylglyoxal (MG) content which indicated an enhancement of lipid peroxidation in rice cultivars. The reduction of reduced ascorbate (AsA), lower AsA/DHA and GSH/GSSG ratio under salinity stress and combined stress indicate the disruption of redox balance in the cell. But under stress conditions compared with other varieties BRRI dhan48 showed lower Na^+/K^+ ratio, elevated proline (Pro.) content, higher AsA and reduced glutathione (GSH) activity, higher AsA/DHA and GSH/GSSG ratio and enhanced activities of MDHAR, DHAR, GPX and glyoxalase system. The results suggested that higher tolerant capacity of BRRI dhan48 against salinity, drought and combined stress is related to lower Na^+/K^+ ratio, enhanced Pro content and better performance of glyoxalase system and antioxidant defense for scavenging reactive oxygen species (ROS) and these results may provide insight into possible responses associated with single or combined stress of salinity and drought in rice cultivars.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	ELABORATIONS
ABA	Abscisic acid
APX	Ascorbate peroxidase
AsA	Ascorbic acid
BRRI	Bangladesh Rice Research Institute
CAT	Catalase
Chl	Chlorophyll
Car	Carotenoid
DAE	Department of Agricultural Extension
DAS	Days after sowing
DHAR	Dehydroascorbate reductase
DHA	Dehydroascorbate
DW	Dry weight
EL	Electrolyte leakage
et al.	and others
FAO	Food and Agricultural Organization
FW	Fresh weight
FC	Field capacity
Gly I	Gyoxalase I
Gly II	Gyoxalase II
GR	Glutathione reductase
GSSG	Oxidized glutathione
GPX	Glutathione peroxidase
GSH	Reduced glutathione
GST	Glutathione-S-transferase
LOX	Lipoxygenase
LSD	Least Signicance Difference
MDA	Malondealdehyde

LIST OF ABBREVIATIONS (cont'd)

ABBREVIATIONS	ELABORATIONS
MDHAR	Monodehydroascorbate reductase
MDHA	Monodehydroascorbate
MG	Methylglyoxal
NADPH	Nicotinamide adenine dinucleotide phosphate
PEG	Polyethylene glycol
Pro.	Proline
POD	Peroxidase
ROS	Reactive oxygen species
RWC	Relative water content
SOD	Superoxide dismutase
USDA	United States Department of Agriculture

Chapter I

INTRODUCTION

Climatic change is a serious environmental threat to crop production. Among all environmental stresses, drought and salinity are the important ones that cause large reductions in agricultural productivity worldwide. These two abiotic stresses occur simultaneously and due to drought stress salinity becomes concentrated in the remaining soil solution (Munns, 2002; Ahmed *et al.,* 2013a). Commonly, co-occurrence of several abiotic stresses is worst for crop production than single stress such as the combined effects of drought and salinity on crops are more detrimental than single stress observed in rice (Ma *et al.,* 2016), potato (Levy *et al.,* 2013), barley (Ahmed *et al.,* 2013a) and wheat (Yousfi *et al.,* 2012). However, most of the studies up to date have addressed the effects of single stress on crops (Wu *et al.,* 2013). Hence, it is essential to develop tolerant crop varieties against single and combined stress of salinity and drought for achieving better productivity.

Under drought and salinity stress plants show a common response and both of them cause reduction of plant growth. Additionally, due to saline stress the sodium (Na^+) and chloride (Cl^-) ions disturb the water relations to plants through decreased the water availability in the soil which ultimately reduced the osmotic potential. It was assumed that the low soil moisture in saline soil increased the salt concentration, thus restricting the root growth. For this, plant roots couldn't uptake water from the subsoil, thus plants may be strongly arrested to withstand drought stress (Singh, 2010). Higher concentration of Na^+ in the soil can also prevent plants from absorption of K^+ by roots (Ueda *et al.,* 2013) and cause a decrease in the K^+/Na^+ ratio in the cytosol, thus disturbs cytoplasmic homeostasis, (Kibria *et al.,* 2017). When plants fall into drought stress it induces stomatal closure, which causes CO_2 starvation in leaves, thus preventing CO_2 fixation (Ueda *et al.,* 2013). Due to lack of sufficient CO_2 in the chloroplast, triggers the ROS generation, and leads to damage of photosynthetic machinery that may irreversibly affect photosynthetic activity.

Furthermore, reactive oxygen species (ROS) can act as signaling molecules, which maintain many physiological processes, but high salinity and drought increased the production of ROS such as superoxide ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) (Hasanuzzama *et al.,* 2013a, b). These caused oxidative damage to plant which disrupts physiological and biochemical life processes and even leads to plants death (Hasanuzzama and Fujita, 2011). However, plants have possessed enzymatic and non-enzymatic antioxidant mechanisms to reduce the ROS damage. In plants under stress the activity of enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase (GST) may be increased or decreased and upregulated or downregulated non-enzymatic antioxidants contents such as AsA and GSH, if these enzymatic and non-enzymatic antioxidants increased or upregulated under stress that can be acted as ROS-scavengers (Hasanuzzama *et al.,* 2014a). Moreover, under salinity and drought stress another highly reactive cytotoxic MG compound increase, which damage the proteins, lipids and DNA (Yadav *et al.,* 2005). For detoxifying this MG plants can possess another detoxification system which is known as glyoxalase system consisting of two enzymes viz. glyoxalase I (Gly I) and glyoxalase II (Gly II) (Hasanuzzama *et al.,* 2014a; Hasanuzzama *et al.,* 2017a). Therefore, it is important to understand the antioxidant defense and glyoxalase system under drought and salinity stresses (alone or combine) on plant growth and develop to generate abiotic stress tolerance in plants.

Rice (*Oryza* spp.) is the most important cereal crop in Asia, which belongs to the family Poaceae (formerly known as Gramineae). Globally, rice crop ranks second among cereal crops after maize in terms of grain production. It is a staple food for over 50% of the world's people and the annual production is about 754.6 million metric tons over the world (FAOStat, 2017). Rice provides 20% dietary energy in worldwide and 70% of dietary energy comes from rice in Asia (FAO, 2015). It is also the staple food of 156 million people of Bangladesh (Shelley *et al.,* 2016). Among 114 rice growing countries of the world Bangladesh ranks fourth (FAO, 2015). The total rice growing area is about 11.38 million hectares, leading to the

production of 34.70 million metric tons of rice with an average yield 3.05 t ha^{-1} (DAE, 2015).

Drought and salinity are the major abiotic stresses for reducing the rice production in Bangladesh (Shelley *et al.,* 2016). Due to semiaquatic polygenic origins of rice and the diversity of rice ecosystem and growing conditions, rice production system depend on more water supply than other cropping systems (O'Toole, 2004). For this reasons, rice production in Bangladesh is hampered under drought and salinity stress. The northwestern part of Bangladesh is exposed to severe drought in Kharif season, which decreased the production more than 40% (IRRI, 2016; Dey *et al.,* 2011) and also in rabi season around 1.2 million hectares cultivable land face drought (Dey *et al.*, 2011). On the other hand, at the southern part of Bangladesh, salinity is a serious threat to rice production and almost 1.06 million hectares of arable lands are affected by salinity (SRDI, 2010). However, recently drought and salinity co-occurrence in our country, because of when agricultural land exposed to drought the ground salt affected water come to the surface soil through the capillary pore and disturbs the crop production system. For this, in future rice production in Bangladesh faces a serious threat to feed increasing populations. Therefore, it is important to find out the drought and salinity stress (alone or combine) tolerance cultivars from different geography to increase the production under these stresses. *Oryza sativa* L. subsp. Indica (BRRI dhan29; BRRI dhan48) and subsp. Japonica (Koshihikari) are two different geographical races. A number of studies indicated the differences in stress tolerance between contrasting genotypes, or sub-species (Kavitha *et al.,* 2012; Borah *et al.,* 2017; Frouin *et al.,* 2018). In this regards, find out the tolerant genetic resources and the underlying tolerance mechanisms under drought and salinity stress is important. Although several types of research have been conducted to investigate the factors involved in rice plant's tolerance to salt and drought stress, only a few are focused on combined stress or including contrasting sub-species. Thus, this experiment is an attempted to find out the responses and the underlying mechanisms of salinity and drought stress (alone and combination) tolerance with the following objectives under consideration:

I. To investigate the effect of salinity and drought (alone and combined) on indica (BRRI dhan29; BRRI dhan48) and japonica (Koshihikari) rice cultivars.

II. To understand the mechanism of oxidative stress and antioxidant defense system and glyoxalase system in these sub-species under single and combined stress of salinity and drought.

Chapter II

REVIEW OF LITERATURE

2.1 Rice

Rice (*Oryza* spp.) is a monocarpic crop, therefore blooms once, set seeds after that die. Cultivated rice is a yearly grass and growing to 0.6- 1.8 m with a round, hollow and joints culms, flat leaves and a terminal inflorescence (Kellogg *et al.*, 2013). Rice has good nutritional values. It is mainly used as a source of energy due to quick digestion of proteins than others cereals (Ali *et al.*, 2014). Rice can be used in different food processing industries such as snack food, beverages, bran oil, syrup and it is believed that rice has medicinal value (Ali *et al.*, 2014). In Asia both indica and japonica cultivated rice derived from *Oryza rufipogon* which was domesticated in China about 8,200-13,500 years ago (Wei *et al.*, 2012). After that rice cultivation spread all over the world very rapidly. Bangladesh has a long history of rice cultivation. Bangladesh is an agricultural country. It lies on the Northeastern South Asia with 147,570 km^2 masses of land and cultivable area 8.52 million ha (BBS, 2014). In Bangladesh, about 76% peoples live in rural areas and around 47.5% manpower involved in agricultural activities (Shelley *et al.*, 2016). In Bangladesh agriculture contributes about 19.3% of GDP in which 11.6% comes from rice (Bangladesh Finance Bureau, 2014). Due to the tropical climatic condition Bangladesh is suitable for rice cultivation and cultivated all over the country except southern hilly area. Rice is grown all year round in Bangladesh with three distinct seasons (*Aus*, *Aman* and *Boro*) and grown in four ecosystems namely irrigated (*Boro*), rainfed (transplanted *Aus* and *Aman*), rainfed upland (direct-seeded *Aus*) and deepwater (broadcast *Aman*) (Hussain, 2012). In total rice production *Aus*, *Aman* and *Boro* contribute 6.60%, 38.84% and 54.56% respectively (DAE, 2015). These three rice growing seasons have covered by more than 73% of modern varieties developed by BRRI and 6% hybrid varieties which are marketed by private sectors (Hussain, 2012). On an average population of Bangladesh consumes 6.06% of rice from total global rice production and actual intake is 416 g per capita per day, which makes it

the fourth highest rice consuming nation of the world (Hussain, 2012). About 69%, 50% of the total calorie and total protein respectively come from rice of the 2318 kcal (Ray *et al.,* 2015). In the last few decades the rice production increased due to the great effort of rice researchers and farming innovations. In 1970, the rice production was 1.7 t ha^{-1} (FAO, 2014) however, now it is 3.05 t ha^{-1} (DAE, 2015). Despite the success in rice production still now Bangladesh faces many challenges in agricultural sectors, especially in rice production owing to climate change and growing population. At the same time, every year more than 1% cultivable land decrease because of the construction of the house, industries, factories, roads and highways (Shelley *et al.,* 2016). Moreover, rice is sensitive to abiotic stresses (drought, salinity, high temperature, low temperature etc.) which are occurring frequently in recent due to global climate change (Mohanty *et al.,* 2013). But among all abiotic stresses drought and salinity are the main environmental constraints to production of rice in Bangladesh. Therefore, few stress tolerant varieties are not sufficient to cope with the adverse environmental condition for increasing the rice production to feed the growing population of Bangladesh. There are many studies reported the effect of drought and salinity individually in growth and yield of these rice varieties. But research works related to oxidative stress and antioxidant activities are limited in numbers which caused by drought and salinity alone or in combination compared with different geographical cultivars. Therefore, some of the studies which are relevant to our study and provided constructive information are reviewed in this chapter.

2.2 Plant's response to abiotic stress

In nature, plants are sessile organism; therefore they constantly faced challenges with different environmental stresses. Stress may be defined as an environmental adverse state that can be reduced the plant growth and potential yield of crops. It is usually divided into two groups such as biotic and abiotic stress (Pandey *et al.,* 2017). But abiotic stress is often more damaging than biotic stress. (Pande and Arora, 2017). The duration and magnitude of these abiotic stresses determine the severity of stress on plants through an increase in respiration, changes in electron transport system, imbalance of ionic and osmotic homeostasis, hindrance of photosynthesis and

reduction in biomass (Hasanuzzaman *et al.*, 2012a). However, dependent on duration and magnitude of stress plant can try to adjust with the environmental stress through changes in morphological structure, physiological and biochemical activities. Adaptive responses of plants to abiotic stress include closure the stomata which limit the water loss and initiation of a series of physiological processes for maintaining the integrity of photosynthesis, CO_2 fixation apparatus and increase the antioxidant activities in plants (Pandey *et al.*, 2017). Furthermore, to escape the stress plants can modify cell cycle, alters in the induction of vacuolization and cell wall organization (dos Reis *et al.*, 2012). Indeed, all biochemical components are present in plants which required for stress tolerance, but the strength of these biochemical components shift species to species depending on the magnitude of stress. This difference of the plant's response to stress depends on the signaling insight, transduction and potentiality of defense machinery of plants which respond to these signals (Scheres and Van der Putten, 2017).

2.3 Drought and drought stress adaptation mechanism in rice

Drought is a meteorological term which is commonly characterized as a period without noteworthy rainfall or an insufficiency of water supply. Agricultural drought is the lack of sufficient soil moisture required for the plant growth, development and reproduction (Ahmed *et al.*, 2015). Drought stress is one of the major limitations for rice production under rainfed conditions, affecting 10 million ha of upland and 13 million ha of lowland rice in Asia (Wassmann *et al.*, 2009). In Bangladesh, due to drought 40% in *aus*, 2.32 million ha in T. *aman* and 1.2 million ha in rabi season land damage in different magnitudes (Dey *et al.*, 2011). From 1960 to 1991 in Bangladesh 19 times drought events occurred, but very strong drought hit the country in 1961, 1975, 1981, 1982, 1989, 1994 and 2000 (Dey *et al.*, 2011).

During drought stress, plants molecular, biochemical and physiological perspective changes occur. But under drought condition plants show variety of morphological and physiological responses to survive the stress such as alter the plant growth with lower the height, leaf rolling, reduced leaf area, leaf abscission, closure stomata, osmotic adjustment, less fruits production and the stimulation of root growth by directing nutrients to the ground parts of plants (Farooq *et al.*, 2009). Initially, when

plant exposed to drought stress plants slow down their growth as a survival technique (Zhu, 2002). An increasing number of studies on rice root traits under drought stress identified that deeper root along with higher branching and penetration and the high root-shoot ratio of rice genotype has more ability to tolerate the drought stress (Gowda *et al.,* 2011). According to Henry *et al.* (2012), deeper root of rice has larger xylem diameter therefore xylem sap bleeding rate is low and it facilitates the water uptake capacity of rice plants in drought stress. At the same time Feng *et al.* (2012) reported that root dry mass and length is a good interpreter of rice yield under drought stress. Moreover, plant roots induce a signal cascade in shoot through the xylem. In this root-shoot signaling cascade has been implicated by plant phytohormones such as abscisic acid (ABA), cytokinins, ethylene, malate and other undefined factors (Anjum *et al.*, 2011a). ABA dominated than other phytohormones under drought stress and by this phytohormone plants can close the stomata during drought stress which can reduce the water loss through transpiration.Thus, above mention responses of plants under drought stress improve the water use efficiency and drought stress tolerance capability.

2.4 Salinity and salinity stress adaptation mechanism in rice

Salinity is a term used to describe a condition when the presence of salt concentration is higher in soil and water, such as sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), calcium sulfate ($CaSO_4$) and bicarbonate (HCO_3^-). Salinity affects more than 100 countries, people livelihood security, as it occupies about 830 million hectare land all over the world (FAO, 2015). In Bangladesh, around 20% of costal area covers approximately 30% of the net cultivable region (Shelley *et al.,* 2016).

Generally, salinity causes two main stresses in plants viz. osmotic stress (1^{st} phase) and ionic stress (2^{nd} phase). Osmotic stress occurs when the salt concentration exceeds the tolerance level of plants around root zone in soil, after that salt reaches in older leaves which increase the amount of Na^+ into plants and causes ionic stress, thus interruption the metabolic process and possess cell death (Munns and Tester, 2008; Hasanuzzaman *et al.,* 2013a, b). Moreover, due to salinity many nutrients are unavailable for plant such as nitrogen (N), phosphorous (P), copper (Cu), zinc (Zn)

and decrease potassium (K), calcium (Ca), magnesium (Mg) into plants, causing significant yield loss (Nahar *et al.*, 2016; Shelley *et al.*, 2016). Although rice is a moderate sensitive cereal crop to salinity with threshold electrical conductivity of 3 dS m^{-1} for most cultivated varieties (Mohanty, *et al.*, 2013; Hoang *et al.*, 2016), but normally salinity denote in soil when electrical conductivity above 4 dS m^{-1} (USAD, 2016). Even it was reported that rice yield is reduced by 10% and 50% at 3.5 dS m^{-1} and 7.2 dS m^{-1} of electrical conductivity respectively (Umali, 1993). Variation of salinity stress tolerance among rice genotypes depend on different developmental stages. It has been reported in previous literature that rice shows salt tolerant during germination, active tillering and toward maturity, but at the time of early vegetative stage and reproductive stage it shows susceptibility (Moradi *et al.*, 2003; Singh *et al.*, 2008). However, rice plant can be tolerated salinity stress through three main mechanisms such as osmotic tolerance, ion exclusion and tissue tolerance (Munns, and Tester, 2008). Rice plants tolerate the osmotic stress for drought aspect of salinity by maintained leaf expansion, stomatal conductance, sequestrations of Na^+ in the vacuole and synthesis of compatible solutes (Tester amd Davenport, 2003). Initially, rice plant restricts the entry of salt into the plant by roots. Through symplastic and apoplastic routes of root cell can control the water uptake along with salt ion into a plant (Das *et al.*, 2015; Reddy *et al.*, 2017). Generally, in rice Na^+ transport occur in shoot from roots through apoplastic pathway reported by Krishnamurthy *et al.* (2009), in this pathway casparian strip is a barrier to apoplastic flux. In rice crop, Na^+/ K^+ ratios in the shoot is dominated by Na^+ leakage to xylem through apoplastic route which is also vary with transpiration rate and root development (Peterson, 1988). Hasanuzzaman *et al.* (2017) observed in the salt tolerant plant the transpiration rate is higher compare than salt sensitive plant, therefore Na^+/ K^+ ratios lower in the salt tolerant plant. Moreover, tolerate rice varieties transfer accumulate salt into older leaves and vacuoles more Na^+ than younger leaves which is allowed to produce more photosynthesizing leaves and it helps to produce some flower and seeds (Reddy *et al.*, 2017). Wang *et al.* (2012) evaluated a wide range of physiological and molecular parameters of rice plants and found that under salinity stress higher amount of Na^+ accumulate in older leaf than younger leaf of rice due to up-regulation of OsHKT1;1, OsHAK10 and OsHAK16 and increased expression of OsNHX1 contributes to the Na^+ compartmentalization in older leaf.

2.5 Common responses of plant under combined stress of drought and salinity

On the basis of physiological, biochemical, molecular and genetical, drought and salinity stress show most of the similar result (Sairam and Tyagi, 2004; Ahmed *et al.,* 2015). During salinity stress physiological drought occurs in plants because of higher level of salt concentration in soil decrease the water potential, therefore in spite of large amount of water contain in soil profile plants cannot uptake water from soil (Lee *et al.,* 2004; Reddy *et al.,* 2017). The major difference between the low water potential into the soil environments caused by drought and salinity stress is the total amount of available water. Therefore, despite of lower water potential at the time of drought stress plant can obtain a limited amount of water from the soil profile. However, plants have a mechanism to alter their osmotic potential, which reduces the turgor loss and increase a lower water potential into the cell sap of plant that permits plant to get water from soil profile for growth and development (Taiz and Zeiger, 2006). But at the severe stage of these stresses increase the dehydration of cell, which cause osmotic stress and removed water from the cytoplasam into the intercellular space, thereby reducing the cytosolic and vacuolar volumes. Initial responses of drought and salinity stress are more similar in plant tissues except ionic components under salinity stress. And these similar responses include metabolic processes, e.g., a decrease of photosynthesis or increase of plants hormonal process such as abscisic acid (ABA) (Vishwakarma *et al.,* 2017). But, higher level of sodium (Na^+) and chloride (Cl^-) additionally found in plant cell during salt stress (Tavakkoli *et al.,* 2011; Hasanuzzaman *et al.,* 2013a). In different stresses plants use common pathways to response stress which is known as cross-tolerance (Foyer and Noctor, 2016). Therefore, salt tolerant varieties can drought tolerant or vice versa, and has a similar mechanism to tolerant with those stresses (Ashraf and O'leary, 1996).

2.6 Effect of drought and salinity stress on plant morphological attributes

2.6.1 Effect on growth and development of plant

Plant growth is a function of the interplay between plant sources and sinks (mainly root and shoot) (Anjum *et al.,* 2011a). Growth is the result of new cell production by the cell division through mitosis and the expansion of new cell. But, under drought and salinity stress a common impact occur in plants which is osmotic stress (faster

process); therefore, cell elongation in plants can be inhibited due to the lowering soil water potential and limiting of water flow from xylem to the encompassing cells (Munns, 2002). Thus, drought and salinity stress interrupted the mitosis process which is involved in cell elongation and expansion resulted in decreased growth and yield attributes (Hussain *et al.*, 2008; Ahmad, 2010). Drought stress reduces the number of leaves per plant and leaf size which ultimately inhibit the photosynthesis in plants (Rostami *et al.*, 2016); therefore, translocation of dry matters in productive tissues of the plant is reduced. Moreover, after osmotic stress salinity stress causes ionic toxicity in plants this is a slower process (Munns, 2005). Salt accumulation in plant leaves causes toxicity and as like drought it reduces the total photosynthetic leaf area which decreases the supply of assimilates into growing tissues and this effect on yield (Hasanuzzaman *et al.*, 2013b). There are many literature reported that drought and salinity stress reduced the plant height, leaf area and dry mass in rice (Ashfaq *et al.*, 2012; Ishak *et al.*, 2015), wheat (Wu *et al.*, 2015; Mujtaba *et al.*, 2016), maize (Khan *et al.*, 2015) and barley (Ahmed *et al.*, 2013a).

2.7 Effect of drought and salinity stress on plant physiological attributes

2.7.1 Effect on plant water relations

Relative water content (RWC), leaf water potential, stomatal conductance, transpiration rate, leaf and canopy temperature are the main characteristics of the plant which effect on water relations. RWC is an indicator that used to measure water status of the plant which including water uptake by roots as well as water loss through transpiration, reflecting metabolic activity in plant tissue and used as an important indicator to drought and salt tolerance. Mainly two components are involved in plant water relations namely water potential and hydraulic conductivity (Negrão *et al.*, 2017). Drought and salinity stress increased osmotic effect on plants due to the reduction of soil water potential surrounding the root zone, which interrupts the ability of water uptake from soil to maintain turgor pressure (Sabir *et al.*, 2009, Ahmed *et al.*, 2015). From two different experiments Anjum *et al.* (2011b, c) reported drought stress decreased the relative leaf water content (RLWC) of maize (*Zea mays* L.) and soybean (*Glycine max* L. Merrill.) plants. Furthermore, in the previous study it was suggested that when plants subjected to drought stress

considerably decreased relative water content, leaf water potential and rate of transpiration with increased of leaf temperature (Siddique *et al.,* 2001). It has also been suggested that reduction of leaf water potential, relative water content and osmotic potential of rice (*Oryza sativa* L.) crop depend on different levels of drought stress (Pandey and Shukla, 2015; Basu *et al.,* 2016). Higher intracellular Na^+ concentration is an additional problem in plant under salinity stress, thereby RWC also decreased in plant. Ueda *et al.* (2013) were carried out an experiment with two rice cultivars namely CFX 18 (salt-tolerant) and Juma67 (salt-sensitive). They reported that leaf area, root-shoot fresh and dry weight, leaf water content and leaf water potential significantly decreased in Juma67 than CFX 18 with increased salinity, therefore, CFX 18 considered more salt tolerant. According to Ueda *et al.* (2013), it can be suggested that under higher salinity concentration plants sequester more NaCl in leaf tissue than normally occurs, thus, lower osmotic potentials and reduction in the root hydraulic conductance decreases water flow rate from root to shoot, causing water stress in the leaf tissue. In the combined stress of drought and salinity relative water content, water potential decreased in cultivated barley, whereas, unchanged in Tibetan wild barley compare to control observed by Ahmed *et al.* (2013b).

2.7.2 Effect on photosynthesis

Drought stress mainly reduced the photosynthesis capacity of the plant by decreasing leaf expansion, CO_2 availability through stomata closure, premature leaf senescence, which is associated with reduction of a food production (Wahid and Rasul, 2005). Stomatal closure is one of the major and first responses to drought stress, which decrease the photosynthesis rate through reduction of CO_2 fixation into leaves; thus, photosynthetic carbon assimilation is decreased for photorespiration (Anjum *et al.,* 2011a). Anjum *et al.* (2011b) reported that drought stress in maize (*Zea mays* L.) decreased at 33.22%, 25.54%, 37.84%, 50.87%, 5.86% of net photosynthesis, stomatal conductance, transpiration rate, water use efficiency and intercellular CO_2 respectively compared than well watered plant. In another study it was reported by Sharifi and Mohammadkhani (2016) from their observation that drought stress at reproductive stage decreased net photosynthesis of flag leaves 38% to 57% and

stomatal conductance 0.91% to 55% relative to well watered control plant of wheat (*Triticum aestivum* L.). Also Zhang *et al.* (2016) indicated that drought stress in rice (*Oryza sativa* L.) led to decrease the photosynthesis (45%), stomatal conductance (73%), intercellular CO_2 (24%) and transpiration rate (60%) relative to well water control plant.

As like drought stress, photosynthesis activity impaired in salinity stress due to the lower stomatal conductance, hampered in CO_2 fixation process, photochemical capacity inhibited (Dubey, 1997; Ahmed *et al.*, 2015). In a previous study Muranaka *et al.* (2002) observed that under 100 mM salt stress the photosynthesis rate was decreased in two wheat (*Triticum aestivum* L.) at two stages. At first photosynthesis was decreased slowly without any visible changes in photochemical and after that in second stage photosynthesis was reduced together with impaired the energy generation efficiency of photo-system two (PSII). Furthermore, reduction of photosynthesis in plants due to the salinity stress is related to reduce production of ATP through impairing the electron transport system (Curtiss *et al.*, 2011). Ionic toxicities of Na^+ and Cl^- reduced the growth and photosynthesis of plants through impaired the photosynthetic apparatus reported by Tavakkoli *et al.* (2011). Wang *et al.* (2017) observed in their experiment that specific ionic toxicity of Na^+ and Cl^- decreased the photosynthetic rate, CO_2 concentration and also impaired the electron transport system in rice (*Oryza sativa* L.) crop leaves. Similarly, Mahlooji *et al.* (2017) conducted a field experiment with three barley (*Hordeum vulgare* L.) genotypes viz. Morocco (salt-sensitive); Nosrat (semi-salt-tolerant); Khatam (salt-tolerant) to evaluate the effect of high salinity on net photosynthesis, transpiration rate and stomatal conductance. Under high salinity stress, salt tolerant genotype Khatam had maximum net photosynthesis rate and stomatal conductance than those of Morocco and Nosrat genotypes. According to Mahlooji *et al.* (2017), it was concluded that although high salinity stress decreased the net photosynthesis but salt-tolerance of the Khatam genotype was associated with an avoidance of Na^+ accumulation in aboveground parts and facilitating higher photosynthetic rate.

2.7.3 Effect on photosynthetic pigments

Chlorophyll is a major's chloroplast component of photosynthesis; therefore, chlorophyll contents have a correlation with the photosynthetic rate. Drought stress damages the photosynthetic pigments and also disorganization the thylakoid membranes which cause oxidative stress in plants (Kannan and Kulandaivelu, 2011). For harvesting light and production of reducing powers in plants photosynthetic pigments is played a vital role. It was also shown that the chlorophyll contents would either increase or decrease or remain unchanged during drought stress depend on duration, severity and timing of drought stress in rice plants (Swapna and Shylaraj, 2017). However, the decrease of chlorophyll *a* and chlorophyll *b* are the common phenomenon during drought stress (Farooq *et al.,* 2009). In a study Jayaweera *et al.* (2016) observed that drought stress at the vegetative stage on rice (*Oryza sativa* L.) crop decreased the all three pigments such as chlorophyll *a*, chlorophyll *b* and carotenoid.

As drought stress, salinity stress decreases the chlorophyll contents through the accumulation of Na^+ into oldest leaves. Due to the toxicity of Na^+ into the oldest leaves start to develop chlorosis and senescence (Yang *et al.,* 2011). In some earlier studies on different plant species showed that salinity stress decreased the chlorophyll contents such as wheat (*Triticum aestivum* L.) (Perveen *et al.,* 2010), corn (*Zea mays* L.) (Molazem *et al.,* 2010) and sunflower (*Helianthus annuus* L.) (Akram and Ashraf, 2011). Furthermore, the toxicity of Na^+ into oldest leaves impair the biosynthesis of chlorophyll contents or increase the degradation of photosynthetic pigments. Therefore, during degradation of these photosynthetic pigments may be the chlorophyll *b* convert into chlorophyll *a* and increase the chlorophyll *a* (Eckardt, 2009). Bhusan *et al.* (2016) observed in an experiment that under salinity stress chlorophyll *b* and total chlorophyll contents of rice cultivars decreased but chlorophyll *a* was increased significantly. It has been reported that reduction of chlorophyll contents under salinity stress depend on plant species because of chlorophyll contents increase in salt-tolerant cultivars, decrease in salt-sensitive cultivars (Khan *et al.,* 2009). For this it has been suggested that increase of chlorophyll contents in salt-tolerant plants are a physiological indicator of salinity stress tolerance in rice (*Oryza sativa* L.) (Chunthaburee *et al.,* 2016).

2.7.4 Effect on plant nutrient availability

Nutrient uptake in plant depends on the soil-root-shoot pathway which is disturbed by drought and salinity stress (Farooq *et al.,* 2009; Tavakkoli *et al.*, 2011). Effect of drought and salinity stress on plant nutrients may cause limitation of energy for assimilation of NO_2^-/NH_4^+, PO_4^{3-} and SO_4^{2-}, these ions used as energy dependent process before these ions can be used as for plants growth and development (Grossman and Takahashi, 2001; Tavakkoli *et al.,* 2011). During drought stress transpirational flow is decreased and absorption of inorganic nutrients into plant is reduced (Garg, 2003). Mauad *et al.* (2011) were conducted an experiment with rice (*Oryza sativa* L.) under drought stress and they found that during drought stress nitrogen (N) and potassium (K) content decreased in the plant. Similarlly, Silva *et al*. (2017) were found that drought stress remarkably decreased the nitrogen (N) and potassium (K) content in sugar cane (*Saccharum officinarum* L.) plant. Likewise, phosphorus (P) and phosphate (PO_4^{3-}) contents also decreased in plant tissue because of lowered mobility under drought stress (Peuke and Rennenberg, 2004). Ashraf *et al.* (2013) was carried out a pot experiment with four canola (*Brassica napus* L.) accessions under drought stress viz. Dunkeld, 24177 (drought-tolerant) and 24173, Pakola (drought-sensitive) and they found the reduction of K^+ , Ca^{2+}, N and P in shoot and root was more in sensitive accessions than tolerant accessions at 50% field capacity (FC). Furthermore, drought stress reduced the magnesium (Mg), manganese (Mn) and zinc (Zn) contents in plant (Peuke and Rennenberg, 2011). On the other hand, salinity stress not only limits the nutrients uptake in plant but also increases the Na^+ and Cl^- ions in plants which inhibit the plants growth (Ahmed *et al.,* 2015). Moreover, both of Na^+ and Cl^- ions disturb the specific transport system of nitrate (NO_3^-), phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}) ions into plants (Maathuis, 2006). However, according to Rahman *et al.* (2016a) during salinity stress calcium (Ca), magnesium (Mg), manganese (Mn) and zinc (Zn) content decreased significantly in rice (*Oryza sativa* L.) seedlings. Whereas, Ahmed *et al.* (2013b) reported that accumulation of calcium (Ca), manganese (Mn) and iron (Fe) was increased in wild barley (XZ5) than cultivated barley (CM72) under combined stress of drought and salinity.

2.8 Effect on plant yield

Yield is a quantitative mechanism of plants and so many physiological processes are involved in this mechanism. Drought and salinity stress simultaneously disturb many physiological processes which are involved in the yield-determining process of plants. Yield interact these physiological processes in a complex way; therefore, it is difficult to interpret that how will plants be responded the indefinite physiological processes entire their life cycle. As a result, for yield reduction severity, timing, plants response after stress removal and interaction between stress and other factors during drought and salinity stresses are extremely important (Plaut, 2003; Negrão *et al.,* 2017).

Water deficiency limits the yield traits of the crop by disturbing CO_2 gas exchange into leaf which not only reduced the size of the source and sinks but also impaired the photosynthesis; assimilate translocation and partitioning of dry matter in plants (Farooq *et al.,* 2009). Drought stress applied during pre-anthesis reduced the time of anthesis; whether, at the time of post-anthesis period shortened the grain filling duration in triticale genotypes which was reported by Estrada-Campuzano *et al.* (2008). In previous study Samarah (2005) was observed that drought imposed at the initial timing of grain filling (post-anthesis period) is severely detrimental to grain yield of barley (*Hordeum vulgare* L.) crop. But at the time of flowering drought stress commonly results in barrenness. It was reported by Dixit et al. (2014) that drought stress at the reproductive stage dcreased 65.91% yield in drought-sensitive rice cultivars.

Similarly, salinity stress is affects plant growth at different stages of life cycle. The adverse effect of salinity on rice crop at the different stage was observed by Zeng *et al.* (2001) and they have exposed the rice crop to salinity at the time of seeding, first leaf, third leaf, panicle initiation (PI) and booting stages respectively. They point out that salinity stress before PI reduced shoot dry weight and grain yield. The yield of plants are closely associated with grain number and weakly associated with grain size; therefore, Harris *et al.* (2010) carried out a greenhouse experiment with barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) to observe the effect of salinity on growth and yield. They observed at high salinity stress grain number reduced at 31%, 22% in barley and wheat crop respectively and also grain size

reduced in both crops. Plants yield also correlated with spikelet fertility and grain filling. For this, Ahmed *et al.* (2013a) observed in a greenhouse experiment on barley (*Hordeum vulgare* L.) cultivars that under single or combined stress of drought and salinity during anthesis period reduced spike length and grain filling per spike in CM72 and XZ16 at 22.6%, 27.7% and 36.8%, 19.9% respectively.

2.9 Effect of drought and salinity stress on plant biochemical attributes

2.9.1 Oxidative damages and ROS production

Reactive oxygen species (ROS) are produced normally in the plant for cell metabolism function but when plants exposed to environmental stresses lead to excessive reactive oxygen species (ROS) generation which is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses (Apel and Hirt, 2004). ROS include free radicals ($O_2^{\bullet-}$ and $OH^{\bullet}$) and non-radicals molecules ($^1O_2$ and H_2O_2) (Hasanuzzaman *et al.*, 2013a, b). In plants this ROS generation mainly occurs due to the electron leakage from chloroplasts (when CO_2 limited), mitochondria (over-reduction of electron transport chain), peroxisomes (glycolate oxidized to glyoxylic acid during photorespiration), plasma membranes and various metabolic pathways in cellular compartment under stressful conditions (Miller *et al.*, 2010). Drought and salinity are played a major role to produce excessive ROS in plants like other abiotic stresses. These excessive productions of ROS under drought and salinity stress cause oxidative damage through accelerate the lipid peroxidation, denaturation of protein, mutation of DNA, impair cellular homeostasis and the antioxidant activities (Apel and Hirt, 2004; Miller *et al.*, 2010), thus inhibit the plants growth and development.

However, plants can detoxify this ROS through increasing endogenous enzymatic compounds such as SOD, CAT, APX, MDHAR, DHAR, GR, GPX, GST and POX and as well as non-enzymatic compounds like AsA, GSH, carotenoids, α-tocopherols and proline (Hasanuzzaman *et al.*, 2012a). Moreover, under stress condition higher level of methylglyoxal (MG) compound produced in plants which is highly cytotoxic (Yadav *et al.*, 2005; Hasanuzzamanet *et al.*, 2014b). These higher levels of MG also disturb cellular function and alter the metabolic activities, ultimately leads to plants death (Hossain *et al.*, 2011a). To detoxify the higher level

of MG plant have possessed a defensive mechanism through Gly I and Gly II system; therefore, MG and the glyoxalase are the potential indicators of plants stress tolerance (Nahar *et al.*, 2015a).

9.2 Antioxidant defense system

Plants have evolved an own antioxidant defensive mechanism in order to decrease the deleterious effect of oxidative damage to maintain the metabolic activities under drought and salinity stress (Figure 1). Antioxidants are two types in plants defense system enzymatic (SOD, CAT, APX, MDHAR, DHAR, GR, GPX and GST) and non-enzymatic (AsA, GSH, carotenoids, α-tocopherols and proline) (Hasanuzzamanet *et al.*, 2012a). These antioxidants play a vital role to detoxify the ROS in specific compartments of cell in a particular condition. In general during stress condition SOD act as a first line defense system which is catalyzed the dismutation of two molecules of superoxide into O_2 and H_2O_2. Further, the H_2O_2 convert into H_2O and MDHA through APX, where APX used ascorbate as an electron donor (Foyer and Noctor, 2011; Hasanuzzaman *et al.*, 2012a). As like APX, CAT can also convert H_2O_2 into H_2O, but it has lower affinity to H_2O_2 than APX. However, in plant MDHA is relatively instable; therefore a little portion of MDHA restored into AsA by MDHAR and other portion of MDHA are oxidized to DHA (Lisenbee *et al.*, 2005). After that with the help of MDHAR, DHA can be reduced to AsA by GSH and at the same time GSSG is oxidized to GSH by GR (Hasanuzzaman *et al.*, 2012a). Therefore, Ahmed *et al.*, 2013a suggested increased level of antioxidant activities such as SOD, CAT, APX and POD is related to lower the oxidative damage in Tibetan wild barley than cv. CM72 under drought and salinity stress.

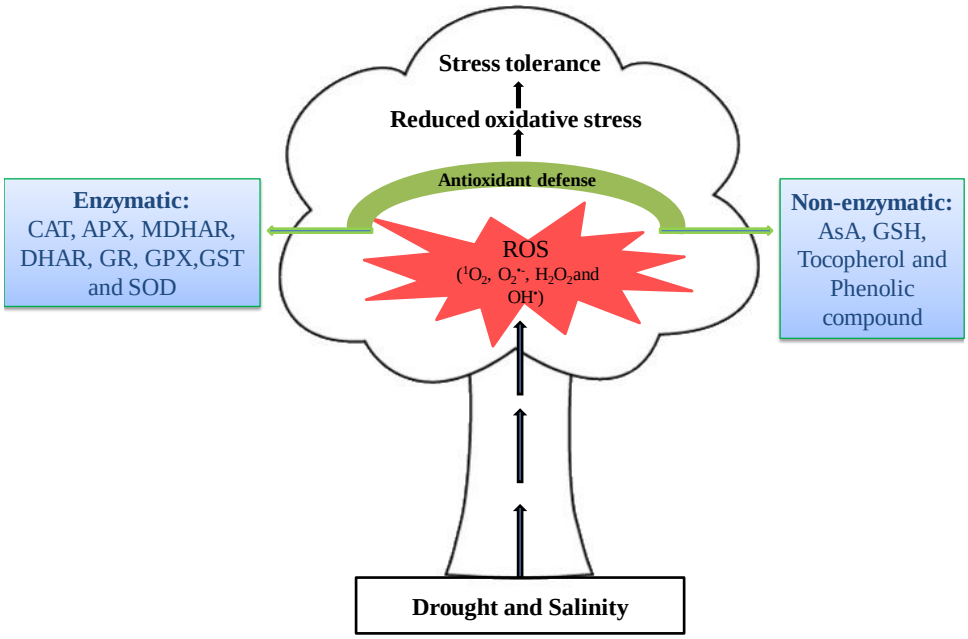

Figure 1. Schematic presentation of ROS scavenging mechanism by antioxidant system

In previous study Yang *et al.* (2009) observed that in 25% field capacity the activity of SOD, APX, CAT and POD increased and lowered the oxidative damage in plants. Whereas, it has been reported by Nahar *et al.* (2015b) an increase in the enzymatic activity of SOD (125%), APX (121%), GR (105%), GST (170%), GPX (106%) and non-enzymatic GSH (184%) in mung bean (*Vigna radiata* L.) seedlings at 25% PEG (PEG-6000) stress compared with control plant. In another study Nahar *et al.* (2016) found SOD, APX, GR, GST and GPX activity increased at 150%, 126%, 132%, 188% and 113% respectively under 200 mM salt stress in mung bean (*Vigna radiata* L.) seedlings. Xia *et al.* (2016) exposed the mustard (*Brassica napus* L. cv. Yangyou 6) and white mustard (*Sinapis alba*) seedlings to drought stress at four leaves stage and observed SOD and POD activity increased at 121% and 436% to 509% respectively. But Kibria *et al.* (2017) observed the opposite results in different rice varieties viz. BRRI dhan29, BRRI dhan47, Binadhan-7 and Binadhan-8 in response of salinity stress. In BRRI dhan47, Binadhan-7 and Binadhan-8 activity of CAT and APX increased but decreased in BRRI dhan29, whereas, POX activity decreased in all varieties. This higher level of CAT and APX in BRRI dhan47, Binadhan-7 and

Binadhan-8 showed a higher level of ROS scavenging ability and protection mechanism under salinity stress. Therefore, the most important role of the antioxidative system in the plant under stress condition is to detoxify the overproduction of ROS to maintain an optimum level of signaling and restore the metabolic activities.

2.9.3 Accumulation of compatible solutes

Under stress condition plants can accumulate different types of organic or inorganic solutes in the cytosol, which are low molecular weight and highly water soluble, even at high concentrations solutes in the cytosol are nontoxic for plants (Ahmed *et al.,* 2015). These solutes lowered the osmotic potential; thereby maintain cell turgor (Farooq *et al.,* 2009). Usually, these solutes protect the plants by means of osmotic adjustment, detoxification of ROS, increased membrane integrity and stabilization of enzymes/proteins (Ashraf and Foolad, 2007). During stress conditions some compatible solutes are played a vital role to osmotic adjustments such as inorganic solute K^+ at the early stage of stress and organic solutes proline (Pro.), glycine betaine (GB) and glucose at the late stress (Nio *et al.,* 2011). Among all compatible solutes proline is a key biochemical solute that accumulates in significant amount when exposed to various stress including drought (Choudhary *et al.,* 2005) and salinity stress (Munns, 2005). After so many studies it has been anticipated that an increase of proline can be protected the plants during abiotic stress through stabilization of subcellular structure to maintain ions homeostasis (Silva-Ortega *et al.,* 2008; Szabados and Savouré, 2010), furthermore it has also suggested being an important protector (act as osmolyte) against higher accumulation of ROS under stress condition by quenching $^1O_2$ and directly scavenging of $OH^{\bullet}$, as a result proline might be able to protect proteins, DNA and membranes from oxidative damages (Smirnoff and Cumbes, 1989). Sharma *et al.* (2011) investigated the function of proline at low water potential and they observed proline could be able to scavenge the overproduction of ROS and improve the ion homeostasis in *Arabidopsis*. According to Solařová *et al.* (2016) and Zu *et al.* (2017) increased Pro. content provides an effective mechanism of cell adaptation for osmotic adjustment in wheat

(*Triticum aestivum* L.) and rice (*Oryza sativa* L.) crops respectively under drought stress.

2.10 Effect of drought and salinity stress on morphological attributes of the rice plants

2.10.1 Effect on growth and development of rice plant

Ashfaq *et al.* (2012) investigated the effect of drought on growth and yield of eight rice varieties. Throughout the experiment they maintained 50% water holding capacity at the vegetative and reproductive stage and after that they observed due to drought plant height decreased 14.79% compared with well watered plant.

Plant growth of rice decreased with the severity of drought stress. Bunnag and Pongthai (2013) experimented in a field condition with seven rice cultivars which grouped as highly drought-tolerant (CT9993), moderate drought-tolerant (KDML 105, IR58821, IR57514, IR52561, BT) and drought-sensitive (IR62266) cultivars. Rice seedlings exposed to drought stress in three different levels of drought regimes at vegetative stage. Where, the major reduction of plant height and 70% leaf injury have occurred in drought-sensitive IR62266 cultivar at higher levels (60 d) of drought stress.

Dixit *et al.* (2014) carried out an experiment under four levels of drought stress at different year in dry season (DS) viz. lowland severe-stress (LSS) (DS2011), lowland moderate stress (LMS I) (DS2012) and lowland moderate stress (LMS II) (DS2013) and upland mild-stress (UMiS) (DS2012) with 365 lines of drought-tolerant upland-adapted indica and drought-sensitive low-adapted indica variety. They observed that due to severely stress plant height remarkably decreased at 18.49% in drought-sensitive rice lines compared with non-stress plants.

It also reported by Kadhimi *et al.* (2016) that the plant height and root length of MR269 genotype rice reduced with different drought regimes. They exposed the seven-day-old rice seedlings to 10% and 20% PEG and after that found plant height, root length reduced at 11%, 25% under 10% PEG and 21%, 50% under 20% PEG respectively.

On the other hand, Li *et al.* (2017) conducted an experiment to investigate the root traits of different indica and japonica subpopulations under drought stress. At the

booting stage these subpopulations of rice plant exposed to drought stress in two cycles. When all the leaves of plants became fully rolled, watering was applied to full capacity and maintained it for one day. After first cycle again drought stress applied to the plant and continued for the rest of the life cycle. They observed the effect of drought on root traits of these subpopulations was complex but deep root weight rate higher in most of the of japonica subpopulations in comparison to indica subpopulations after drought treatment.

Hairmansis *et al.* (2014) investigated the response of rice varieties viz. IR64 and Fatmawati under moderate (0, 50, 75 and 100 mM NaCl) and higher salinity concentration (0, 100, 150 and 200 mM NaCl). They observed after 20 d of salinity treatment the shoot area reduced significantly at 100 mM salinity concentration in Fatmawati and IR64 at 37% and 30% respectively, but in 200 mM salinity stress shoot area reduction pronounced in Fatmawati and IR64 by 49% and 54% respectively. They also found that shoot senescence was higher in Fatmawati (salt-sensitive) about 23% than IR64 compared with non-stress plants.

A higher level of salinity stress decreased the biomass remarkably in plants. According to Ishak *et al.* (2015), high concentration of salinity (300 mM NaCl) reduced the shoot dry weight of wild-type Nippobare up to 57.14%.

In a greenhouse experiment Hussain *et al.* (2016) used eleven diverse rice genotypes to investigate the effect of salinity stress. For this they exposed the rice plant under 200 mM (NaCl) concentration salinity stress at reproductive stage and observed that the reduction of plant height is higher in BRRI dhan56 (7.67%), followed by BRRI dhan40 (7.18%), BRRI dhan41 (6.52%), BRRI dhan53 (5.55%), IR29 (5.06%), Nona Bokra (4.17%), FL-478 (3.99%) and Binadhan-8 (1.50%) with little reduction in Binadhan-7 (0.91%), whereas Binadhan-10 was not affected.

2.11 Effect of drought and salinity stress on physiological attributes of the rice plant

In an experiment Lima *et al.* (2015) rice variety Nagina22 selected as a tasted crop against drought stress. They created the water deficit with three level of PEG (molecular weight 6000) viz. 20%, 25% and 30%. But in 20% PEG solution

Nagina22 did not show any sign of stress; on the other hand in 30% PEG solution Nagina22 did not survive. Therefore, they selected 25% PEG solution for optimizing the water deficit. After that they exposed twenty one-day-old rice seedlings to water deficit for 6 days and observed relative water content (RWC) and chlorophyll content decreased at 44.45%, 40% respectively.

Dinh *et al.* (2016) observed in their experiment that when three-week-old japonica rice (Donjin variety) seedlings exposed to drought stress the chlorophyll *a*, chlorophyll *b* and total chlorophyll content decreased at 28%, 25% and 14.29% respectively.

Similarly, Kadhimi *et al.* (2016) investigated the effect of drought on total chlorophyll content of MR269 genotype rice which is drought-tolerant. For this reason, they treated the seven-day-old rice seedlings with 10% and 20% PEG and found that 40% and 52% of total chlorophyll content decreased under 10% and 20% PEG respectively.

In a hydroponic experiment Swapna and Shylaraj (2017) used 42 high-yielding rice varieties from indica sub-species as tasted crops for evaluating the responses of rice varieties under drought stress. Nine-day-old rice seedlings exposed to drought stress by 15% PEG for 5 days and then consequently, the stress level was increased by substituting 15% with 20% and 25% PEG for 14 days and 4 days respectively. Under drought condition they observed the highest RWC in tolerant check Sahabhagidhan (97.12%) followed by Neeraja (91.52%), Swarnaprabha (89.36%) and Kattamodan (81.43%) compared with control and also these varieties showed the highest Pro. content than others. The increased level of chl *a* was observed 137.35% in Remanika, chl *b* 117.77% in Sahabhagidhan, total chl 105.13% in Sahabhagidhan and carotenoid contents 119.72% in Swarnaprabha respectively. Moreover, Swarnaprabha, Krishnannjana and Kanakam showed lower cell membrane injuries both in root and shoot tissues under drought stress.

For comparative study under drought stress Anupama *et al.* (2018) conducted an experiment on indica rice varieties which comprising Sahabhagidhan as drought-tolerant variety and IR64, MTU 1010 as drought-sensitive varieties. After penicle initiation rice varieties, irrigation was withheld in five sets [-10 kPa, -30 kPa, -50 kPa, -80 kPa and > -80 kPa soil matric potential (SMP)] to impose drought stress.

They observed RWC content decreased in MTU 1010 7.47±1.47 and in IR64 25.08±2.38 compared with Sahabhagidhan which showed RWC decrease 42.27±0.12 at > -80 kPa SMP and proline content increased remarkably up to 4.30±0.10 µmol g^{-1} FW in Sahabhagidhan, 3.52±0.130 µmol g^{-1} FW in IR64 and 3.33±0.33 µmol g^{-1} FW in MTU 1010 respectively under stress condition.

Mostofa *et al.* (2015) conducted an experiment with rice crop (BR11) at different concentration of salinity (150 mM and 250 mM). They exposed fourteen-day-old rice seedlings in salinity stress and observed relative water content (RWC) decreased at 15-25% and total chlorophyll content decreased at 17-38%.

Two varieties of rice namely Giza 177 and Giza 178 used as a tasted crops to evaluate the negative effect of salinity by Abdallah *et al.* (2016). To performance this experiment they comprised three levels of salinity concentration (0, 30, 60 mM NaCl). They reported due to the salinity stress 18% and 24% in shoot fresh weight, 6% and 9% in dry weight and 4% and 5% in relative water content reduced for both varieties respectively as copared with control plant. Whereas, reduction of 12.9% and 10.4% in chlorophyll *a*, 19.8% and 18.8% in chlorophyll *b*, 25.7% and 21.8% in carotenoids and 15.7% and 14.2% in total pigments contents for both varieties respectively compared with controls plants observed by them.

At the same time Rahman *et al.* (2016a) carried out an experiment with rice cultivar BRRI dhan47 to investigate the response rice seedlings under salinity stress. They exposed the thirteen-day-old rice seedling to 200 mM NaCl salinity stress. They evaluated that due to salinity stress fresh weight, dry weight, relative water content, chlorophyll a, chlorophyll b and carotenoid decreased at 31.23%, 21.74%, 19.24%, 33%, 38.36% and 37.5% respectively compared with control plant. They also found salinity stress decrease the uptake of minerals in rice plants such as Ca (7.32% in root and 29.26% in shoot), Mg (48% in root and 13.63% in shoot), Mn (27.78% in root and 37% in shoot) and Zn (31.15% in root and 31.58% in shoot).

Chunthaburee *et al.* (2016) performed an experiment on 12 rice cultivars including salt-tolerant Pokkali and salt-sensitive IR29 cultivars as a standard check in salt screening. They exposed twenty five-day-old rice seedlings to 100 mM NaCl salt stress for 14 days and observed the K^+/Na^+ ratio for IR29 drastically reduced (5.41 folds) wheras Pokkali showed a slightly reduction in the K^+/Na^+ ratio (2.16 folds).

The Pro. accumulation among all cultivars found higher in Pokkali, FL496 and Niewdam Gs.no.00621 was 1.83, 2.57 and 2.19 folds respectively compared to control. Moreover, it was noted that shoot FW (8.61%) and shoot DW (13.48%) slightly decreased in Pokkali, in contrast the FL496 exhibited a dramatic reduction in FW and DW (56.13 and 52.21%, respectively)

To investigate the effect of salinity on growth as well as physiological and biochemical characteristics Kibria *et al.* (2017) conducted an experiment with four rice varieties including one salt-sensitive variety BRRI dhan28 and three salt-tolerant varieties viz. BRRI dhan47, Binadhan-8 and Binadhan-10 and comprised four different salt concentration (0, 20, 40 and 60 mM NaCl). They exposed all rice varieties to salinity stress at five weeks after transplanting and observed that chlorophyll content of BRRI dhan28, BRRI dhan47, Binadhan-8 reduced by 38%, 32% and 42% at 60mM NaCl respectively compared with control and among all varieties Binadhan-10 showed the higher K^+/Na^+ ratio compared than other varieties

2.12 Effect on the yield of rice plant

Drought stress affects on potential yield of plant even drought stress reduces the yield up to 100% in sensitive plants. For this, Dixit *et al.* (2014) conducted an experiment with high yielding varieties but drought susceptible. They comprised four levels of drought stress in different dry season viz. lowland severe-stress (LSS) (DS2011), lowland moderate stress (LMS I) (DS2012) and lowland moderate stress (LMS II) (DS2013) and upland mild-stress (UMiS) (DS2012) to observe the yield reduction percentage and they recorded 57% to 70% yield reduced under drought stress.

An experiment conducted by Monkham *et al.* (2015) with seventy rice genotypes at Chum Phae (CPA; latitude 16°32′2.6″N, 102°6′0″E) and Ubon Ratchathani (UBN; latitude 15°19′52.35″N, longitude 104°40′55.15″E) locations. Throughout the experiment they maintained three water trials viz. flooding, intermittent drought and terminal drought. For creating flooded condition in field conserved water 1-10 cm above from the soil surface, whereas, for intermittent drought water was drained out from the field at 53-54 days after sowing (DAS). After that field was irrigated at 63, 75, 87, 96 and 105 DAS at CPA and at 62, 71, 77, 89, 98 and 107 DAS at UBN

locations until the rice genotypes showed any visual symtom of water stress. And to create terminal drought rice field irrigated up to 67 days then withdrew water at both locations. After completing the treatment period they observed due to terminal drought grain yield reduced by 52% to 55% while it was 10% to 19% for the whole population and in intermittent drought yield reduced by 23% to 33% for early flowering genotypes.

To evaluate the effect of drought stress on rice yield Torres and Henry (2016) conducted a field experiment in different season namely dry season and wet season. They observed during wet season mild stress occurred at the time of flowering stage which is more severely affected rice yield and yield reduced 50% to 63%; whereas, at the time of dry season moderate stress could not affeced the yield of rice as like wet season.

Hakim *et al.* (2014) conducted a glasshouse experiment to observe the effect of salinity on growth and yield of rice. They selected five Malaysian rice genotypes (MR33, MR52, MR211, MR232 and MR219), two susceptible (BRRI dhan29 and IR20) and one tolerant genotype (Pokkali). They comprised four levels of treatment viz. 0, 4, 8, 12 dS m^{-1}) and they treated rice genotypes with four levels of salinity at the reproductive stage and observed the yield reduction varied between 34-96% with the salinity concentration increased. Lowest yield reduction found in MR221 (39%) and highest yield reduction observed in BRRI dhan29 (62%). Furthermore, they found that BRRI dhan29 did not survive at 8 dS m^{-1} salt concentration.

In a greenhouse experiment Hussain *et al.* (2016) reported that higher level of salinity (200 mM) decreased the yield of rice varieties. In their study they tasted 11 rice varieties yield under salinity stress and observed reduction level is higher in IR29 (49.64%).

Similarly, Girma *et al.* (2017) carried out research on rice crop to observe the effect of salinity on yield. For this they selected 15 rice genotypes along with two checks (one tolerant and susceptible) at four levels of salinity concentration (0, 4, 8 and 12 dS m^{-1}). They found that highest reduction of yield occurred in IR 59418, IR 72593, IR 73055 and NERICA 4 its about 59-100% in all salinity levels compared to control. Whereas, at 12 dS m^{-1} concentration of salt IR 70023, IR 71810, IR 71901

and IR 71991 showed salt tolerance and reduction percentage lowered (37-46%) than others genotypes.

2.13 Effect of drought and salinity stress on biochemical attributes of the rice plant

Zain *et al.* (2014) conducted an experiment with MR220 rice variety under drought stress condition. For carrying out this experiment they set four levels of drought stress viz. periodical water stress for 15 d, water stress for 15 d at vegetative stress + 120 kg K_2O ha^{-1}, water stress for 25 d at vegetative stress + 120 kg K_2O ha^{-1} and water stress for 15 d at reproductive stage + 120 kg K_2O ha^{-1}. They found POX activity increased in periodical water stress for 15 d at vegetative stage by 124.59% but 18% decreased in water stress for 15 d at reproductive stage + 120 kg K_2O ha^{-1}, whereas, CAT activity increased in 15 d periodical water stress at 126% and decreased by 26% at reproductive stage for 15 d water stress. They also observed that proline and soluble sugar content increased in plants under 15 d periodical water stress at 223.58% and 125% respectively.

In an experiment Ma *et al.* (2016) observed the response of Liaoxing No. 1 rice seedlings in drought stress. Therefore, they induced 10% PEG (PEG-6000) stress in seven-day-old rice seedlings then after completion of three days treatment period, investigated the physiological and biological responses of rice seedlings. In their investigation they observed due to 10% PEG stress SOD, POD, CAT activities and total sugar content in rice seedlings increased at 128.57%, 122.22%, 132.14% and 187.5% respectively with compared than well watered rice seedlings.

Currently, many researchers have proved that stress tolerance of plants correlated with antioxidant activity; therefore to investigate the leaf traits and antioxidant defense for drought tolerance Mishra and Panda (2017) used three traditional rice landraces namely Kalajeera, Machakanta and Haladichudi along with N22 (drought-tolerant) and IR64 (drought-sensitive) as check varieties. For this they comprised four levels of drought concentration viz. 0, 19.6% PEG, 29.6% PEG and 36% PEG and induced these concentrations of PEG in fifteen-day-old rice seedlings. After that they reported SOD and CAT activities overtly increased in all three landraces along with N22 in 36% PEG stress except Machakanta for CAT activity and APX activity

highly increased in Haladichudi and Machakanta landraces than drought tolerant variety N22 under 36% PEG stress, where GPX activity increased only in Haladichudi at the same stress concentration. On the other hand, above all enzymatic activities decreased in drought sensitive variety IR64 in all concentrations of PEG compared than well watered plants.

Bhattacharjee and Dey (2017) conducted an experiment for screening drought-tolerant indigenous aromatic rice cultivars. They used four aromatic rice cultivars as tested crops namely Jamainadu, Tulaipanji, Sitabhog and Badshabhog. Imbibed seed lots of these cultivars were treated with -0.344, -0.851 and -1.619 MPa PEG-6000 for 7 days. After 7 days of treatment, they analyzed biochemical responses of rice cultivars. They reported that Tulaipanji exhibited maximum up-regulation in APX, GR and GSH but CAT activities decreased in all cultivars. Furthermore, Sitabhog cultivar showed the higher up-regulation in the AsA content and AsA/DHA ratio.

In order to investigate the effect of salinity, Yamane *et al.* (2009) used Nipponbare rice cultivar which is japonica sub-species. Three-week-old seedlings exposed to 200 mM NaCl salt concentration at different durations such as 0, 12, 48 and 72 hours respectively. They observed the SOD activity increased at 48 and 72 hours and the GR activity increased at 24 hours of salt stress. Wheather, the activities of APX, CAT and GR decreaed at 48 and 72 hours of salt stress and the GPX activity did not show any significant change in all durations of salt stress.

Nounjan *et al.* (2012) performed an experiment with one rice cultivar namely KDML 105 which is salt- sensitive. For this, they used 100 mM NaCl as salinity treatment and thirty three-day-old rice seedlings exposed to stress. After completing the treatment period they observed that proline content, SOD, POX, APX and CAT activities increased in rice plants.

Seeds of two bred indica rice cultivars namely Malviya-36 (salt-sensitive) and CSR-27 (salt-tolerant) used by Mishra *et al.* (2013) as tasted crop to observe the biochemical response under salinity stress. Two levels of salinity concentrations comprised viz. 7 dS m^{-1} (moderate) and 14 dS m^{-1} (higher). They treated the plants after 20 days of planting and physiological and biological parameters data was measured at 5 days intervals upto 20 days. After measuring the data they observed that MDA increased significantly in salt-sensitive cultivar Malviya-36 at 27–51% in

both root and shoot for 10 days treated plants, whereas, $O_2^{\cdot -}$ increased 25% to 80% in root and 33% to 160% in the shoot at 14 dS m^{-1} for 15 days treated plants. On the other side, in CSR-27 cultivar MDA and $O_2^{\cdot -}$ did not increase significantly but enzymatic activities increased significantly at 14 dS m^{-1} for 20 days treated plants such as SOD (38-99% in root and 84-142% in shoot), CAT (26% in root and 48% in shoot), APX (42% in root and 48% in shoot), MDHAR (18% in root and 45% in shoot) and DHAR (183% in root and 62% in shoot).

To observe the biochemical responses of rice seedlings under salinity stress Mostofa *et al.* (2015) performed a study. Twelve-day-old seedlings exposed to 150 mM and 250 mM (NaCl) salinity stress for 72 hours. They observed due to salt stress the MDA, H_2O_2, Pro., and LOX activity increased. Moreover, salt stress stimulated the activities of SOD, MDHAR, DHAR, GR, GPX, APX and GSH but decreased the AsA, AsA/DHA ratio and GSH/GSSG ratio.

Rahman *et al.* (2016b) conducted an experiment with a rice cultivar namely BRRI dhan47 under salinity stress which is salt-tolerant. They grew the rice seedlings with commercial hydroponics nutrient solution (Hyponex, Japan) in a growth chamber and renewed the nutrient solution after three days. After twelve days they exposed the rice seedlings to 150 mM NaCl stress and then after three and six days later of treatment they assayed the antioxidant contents and observed enzymatic activities of SOD, MDHAR, DHAR, GR, GPX and Gly II increased in both three and six days, whereas, APX and Gly I activities increased only in six days treatment. Also Pro. content overtly increased under salinity stress in rice seedlings. Moreover, non-enzymatic antioxidants the AsA and the AsA/DHA ratio decreased but DHA content increased, whereas GSH and GSSG increased and GSH/GSSG decreased in rice seedlings.

Two indica rice varieties viz. ASD16 (salt-sensitive) and BR26 (salt-tolerant) used by Jini and Joseph (2017) in their experiment to observe the response of rice plants under salt stress. 200 mM and 400 mM NaCl used to create salinity stress and after completing the treatment period they observed SOD, CAT and POD activity increased in ASD16 at 173%, 163% and 166% compared with control than BR26.

At the same time Vighi *et al.* (2017) performed an experiment to investigate the functional characteristics of antoxidant enzymes in rice plant under salinity stress.

For this, genotypes BRS Bojuru (salt-tolerant) and BRS Pampa (salt-sensitive) were subjected to 150 mM NaCl for 0, 6, 24, 48, and 72 hours at the vegetative stage of rice seedlings. And they observed H_2O_2 content increased in BRS Bojuru genotype and decreased in BRS Pampa genotype compares with control, but in MDA content showed opposite result. In antioxidant enzynes they found no significant difference in the CAT activity for all genotypes at any stress period, whereas the SOD activity increased in BRS Bojuru and the APX activity increased in BRS Pampa genotype for all stress periods. Moreover, the GR activity increased in both genotypes.

CHAPTER III

MATERIALS AND METHODS

This chapter represents a brief description of the experimental time, location, growing condition, planting materials, treatment, experimental design & layout and data collection and statistical analysis of the experiment.

3.1 Experimental location

To study the morpho-physiological, oxidative stress and biochemical responses this experiment was conducted at Laboratory of Plant stress responses, Kagawa University, Kagawa, Japan during the period from March, 2017 to September, 2017.

3.2 Plant materials

Indica rice cultivars viz. BRRI dhan29, BRRI dhan48 collected from Bangladesh Rice Research Institute, Gazipur, Bangladesh and japonica cultivar viz. Koshihikari collects from Dr. MOROKUMA, Masahiro, Kagawa University, Japan. BRRI dhan29 is a late and high yielding variety in *boro* season. Its height around 95 cm and grain medium in size and white in color. About 160 days take to complete life cycle and yield 7.5 t ha^{-1}. BRRI dhan48 is a drought tolerant and early variety in *aus* season. About 105 cm tall and medium grain size, white in color. About 110 days require to complete life cycle and yield 5 t ha^{-1} if get proper management. Koshihikari is a late spring to early summer variety with short grain rice that distinguished by its aroma, sweet flavor and firm texture. It's a semi-dwarf variety about 78.3cm tall. It takes 160 days to complete life cycle and yield 5.6 t ha^{-1}. It has low glycemic, thus it suitable for making sushi.

3.3 Growing condition

Healthy uniform sized seeds of three rice varieties sterilized with 70% ethanol and after 10 minutes thoroughly washed with distilled water then soaked seeds kept in a dark incubator for 48 hours. After 2 days seeds were sown in 250 ml plastic pot (height 7 cm and diameter 6 cm) which is contain 200 ml distilled water and then kept it again in dark incubator for 2 days. After 2^{nd} time incubation each pot with 75 morphologically uniform germinated seedlings was transferred into growth chamber (Iwaki; Asahi Techno Glass, Japan) under controlled conditions (light: 350 µmol

photons m^{-1} s^{-2}; temperature: 25 ± 2 ℃; relative humidity: 65–70%) with a dilute (7500 times) nutrient solution (Hyponex, Japan). This commercial nutrient solution contained 8 % N, 6.43% P, 3.08 % Mg, 0.07 % B, 0.24 % Fe, 0.03 % Mn, 0.0014 % Mo, 0.008 % Zn and 0.003% Cu. The nutrient solution was changed twice in a week.

3.4 Experimental treatments

This experiment treatment was comprised of two factors.

Factor A: Variety

i. BRRI dhan29
ii. BRRI dhan48
iii. Koshihikari

Factor B: Treatment

i. Control
ii. Salt (150 mM)
iii. Drought (15% PEG)
iv. Salt (150 mM) + Drought (15% PEG)

All varieties of rice (18 days old) seedlings were treated with drought and salt separately and combinely and treated rice plant (18 days old) grown for 72 hours, after that data were taken.

3.5 Experimental design and layout

This experiment was laid out in a Completely Randomized Design (CRD) with three replications.

3.6 Data collection parameter

For morphological, physiological and biochemical parameters data were collected after the completion of treatment durations each parameter.

Listed data collection parameters were following:

3.6.1 Seed quality parameter

- Germination percentage

3.6.2 Growth parameters of crop

- Mortality rate
- Plant height (shoots height and root length)
- Fresh weight (FW) plant^{-1}
- Dry weight (DW) plant^{-1}

3.6.3 Physiological parameters of crop

- Relative water content (RWC)
- Photosynthetic pigments
- Na^+ and K^+ contents

3.6.4 Biochemical parameters

- Lipid peroxidation (MDA)
- H_2O_2 content
- Histochemical localization of H_2O_2
- Proline content
- Methylglyoxal content
- Electrolyte leakage
- Ascorbic acid content
- Glutathione content
- Activities of antioxidant enzymes (CAT, APX, MDHAR, DHAR, GR, GPX, Gly I, Gly II, GST SOD, and LOX)

3.7 Germination percentage determination procedure

To determine the germination percentage for each variety ninety seeds were placed in a 250 ml plastic pot (height 7 cm and diameter 6 cm) with 200 ml distilled water. After that to facilitate the seeds germination the pots were placed in a dark incubator maintained 25±1°C for 48 hours. Seeds germination was considered when the radical length at least 2 mm long (Gholami *et al.,* 2015). Seeds germination inspected and the data were collected 48 hours after seed sowing and during data collection period discarded all the abnormal seedlings (short, thick, spiral hypocotyles and stunt roots) (ISTA, 2003). The following formula was used to calculate the germination percentage:

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

3.8 Growth parameters determination and sampling procedure during growing period

3.8.1 Mortality rate

Uniformly germinated 75 seedlings counted before treated the seedlings with salt and PEG which denoted as Ni and after completing the treatment the total number seedlings counted again and it's denoted as Np. The following formula was used to calculate the mortality rate:

$$\text{Mortality rate (\%)} = \frac{Ni - Np}{Ni} \times 100$$

3.8.2 Plant height (shoots height and root length)

After completion of the treatment period the shoot height measured from the jointing point of stem and root to leaf tip and for root length from the jointing point of stem and root to root tip. For this ten seedlings were taken randomly to measure the height for each pot and average the height.

3.8.3 Fresh weight plant^{-1}

For measuring the fresh weight ten seedlings were randomly selected from each pot and washed them with distilled water. After washing the seedlings absorbed surface water by tissue paper then measured the fresh weight in an electric balance and averages them to have fresh weight plant^{-1}.

3.8.4 Dry weight plant^{-1}

Ten sample seedlings were collected from each pot randomly then washed with distilled water. To determine the dry weight all sample seedlings were kept in an oven at 80 °C for 48 hours. After finishing the dry period data were recorded in an electric balance and average then to have dry weight plant^{-1}.

3.9 Physiological parameters determination and sampling procedure during the growing period

3.9.1 Relative water content

According to Barrs and Weatherly (1962), leaf relative water content (RWC) was measured. Ten flag leaves were randomly selected for each pot and weight as fresh weight (FW). After that the leaf samples soaked in Petri dishes and kept in dark place for 24 hours. Then measured the weight again which considered as turgid weight (TW) and these samples were kept in an oven at 80 °C for 48 hours to determine the dry weight (DW).

3.9.2 Photosynthetic pigments

For determining the photosynthetic pigments, a fresh leaf (0.5 g) sample was homogenized from randomly selected seedlings with 10 mL acetone (80% v/v) by using pre-cooled mortar and pestle. After that, the homogenate samples were centrifuged at 10,000 × g for 10 min. The absorbance of the supernatants (after diluting) was measured with a UV- spectrophotometer at 663, 645 and 480 nm for chl a, chl b and carotenoid contents respectively as proposed by Arnon (1949) and expressed as mg g^{-1} DW.

3.9.3 Determination of Na^+ and K^+ contents

To determine the Na^+ and K^+ contents following the method of Rahman *et al.* (2016a). Shoot and root samples were oven dried at 80 °C for a period until weight become constants. 0.1 g shoot and root sample were ground and digested separately with nitric acid and perchloric (5:1) acid mixture at 70 °C for 48 h. After that mineral contents were measured by using an atomic absorption spectrophotometer (Hitachi Z-5000; Hitachi, Japan).

3.10 Biochemical parameters determination and sampling procedure during the growing period

3.10.1 Determination of lipid peroxidation

According to Heath and Packer (1968) with slightly modified by Hasanuzzaman *et al.* (2012b), the malondialdehyde content (MDA, a product of lipid peroxidation) was measured. Randomly selected seedlings leaf samples (0.5 g) were homogenized

with 3 mL 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 11,500 × g for 15min. After that 1mL supernatant was mixed with 4 mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). The reaction mixture was then heated at 95 °C for 30 min in a water bath and then quickly cooled in an ice bath for 15 min and centrifuged again at 11,500 × g for 10 min. The colored supernatant absorbance was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. Extinction coefficient 155 $mM^{-1}cm^{-1}$ was used to calculate the MDA content and expressed as nmol g^{-1} FW.

3.10.2 Measurement of H_2O_2 content

H_2O_2 was assayed according to Yang *et al.* (2007) proposed method. Leaf samples (0.5 g) were homogenized with 3 mL 5% TCA by using ice-cooled mortar and pestle. The homogenate was centrifuged at 4 °C for 15 min at 11,500 × g. The supernatant (500 µL) was mixed with 500 µL 10 mM K-P buffer (pH 7) and 1 mL of 1M KI reagent and at the same time prepared the blank with 5% TCA instead of leaf extract. After that sample with leaf extract and the blank sample was incubated for 1 hour. The absorbance was recorded at 390 nm and calculated the data from the standard curve (0 – 96 µM) and expressed as µmol g^{-1} FW.

3.10.3 Histochemical detection of H_2O_2

In situ localization of hydrogen peroxide (H_2O_2) was detected in rice leaf according to Thordal-Christensen *et al.* (1997) described the method with slightly modification. Leaf samples (collect from the same position in seedlings of the same age) were incubed in 0.1% 3-diaminobenzidine (DAB) at 25 °C. This DAB solution prepared in HCl acidified (pH 3.8) water. The incubation of rice leaves continued 12 h after that leaves were boiled in ethanol (90%) for 15 min to appear the reddish brown spots which produced by the reaction of H_2O_2 and DAB. Photographs were then taken by placing the leaves on the glass.

3.10.4 Determination of proline (Pro.) content

Pro content in leaf tissue was measured by following the methods of Bates *et al.* (1973). Fresh leaf samples (0.25 g) were homogenized with 5 mL of 3% sulfo-salicylic acid by using ice-cooled mortar and pestle and then the homogenate was centrifuged at 11,500 × g for 15 min at 4 °C. The supernatant (1 mL) was mixed with

1 mL of acid ninhydrin (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M phosphoric acid) and 1 mL of glacial acetic acid. The mixture heated in water bath at 100 °C for 60 min. After that the mixtures quickly transfer into test tube kept in ice for 10 min. When the mixtures were cooled, 2 mL of toluene was added and mixed thoroughly by vortex mixture (20-30 sec). The upper layer of chromophore-containing toluene was separated and the absorbance read spectrophotometrically at 520 nm by using toluene as a blank. The Pro. content was calculated from the standard curve of known concentration laboratory grade Pro.

3.10.5 Measurement of methylglyoxal content

Randomly selected Leaf samples were homogenized in 5% perchloric acid and the homogenate was centrifuged at 4 °C for 10 min at 11,000 × g. The supernatant was decolorized by adding charcoal and neutralized by adding a saturated sodium carbonate solution at room temperature. After that the neutralize supernatant was used to determine the MG content by adding sodium dihydrogen phosphate and N-acetyl-L-cysteine to a final volume of 1 mL. After 10 min the formation of the product N-α-acetyl-S-(1-hydroxy-2-oxoprop-1-yl) cysteine absorbance was measured at 288 nm wavelength (Wild *et al.,* 2012) and calculated the MG content from standard curve of known concentration and expressed as µmol g^{-1} FW.

3.10.6 Determination of electrolyte leakage

According to Dionisio-Sese and Tobita (1998) method electrolyte leakage was determined. Shoot (0.2 g FW) and root (0.2 g FW) samples were cut into small pieces and then transferred into test tube containing 20 mL distilled deionized water and closed with cap. The test tube was heated at 40 °C for 60 min in a water bath. After cooling the samples, initial electrolyte conductivity (EC_1) was measured in Eutech CON 700 conductivity meter, Singapore. Again the samples were heated in an autoclave at at 121 °C for 20 min and then cooling the samples at room temperature. After cooling the samples measured the final electrolyte conductivity (EC_2) and calculated as following formula:

$$\text{Electrolyte leakage (\%)} = \frac{EC_1}{EC_2} \times 100$$

3.10.7 Extraction and measurement of ascorbate and glutathione

Fresh leaves (0.5 g) were homogenized in 3 mL ice-cooled 5% meta-phosphoric acid which contained 1 mM ethylenediaminetetraacetic acid (EDTA) using an ice-cooled mortar and pestle. The homogenate was centrifuged at 4 ℃ for 12 min at 11,500 × g. The supernatant was collected to measure AsA and GSH. For determining AsA content following the Huang *et al.* (2005) proposed method with modification. The supernatant was neutralized with 0.5 M K-P buffers (pH 7.0) and the oxidized fraction was reduced by 0.1 M dithiothretitol. AsA content was assayed spectrophotometrically at 265 nm in 100 100 mM K-P buffer (pH 7.0) with 0.5 units of ascorbate oxidase (AO). For quantification of AsA a specific standard curve was used.

According to Yu *et al.* (2003) method the GSH pool was assayed with modifications as proposed by Paradiso *et al.* (2008). The supernatant (0.2 mL) was neutralized with 0.3 mL of 0.5 M K-P buffers (pH 7.0). Based on enzymatic recycle, to measure the oxidized glutathione or glutathione disulfide (GSSG) and total glutathione, GSH is oxidized by 5, 5-dithio-bis (2- nitrobenzoic acid) (DTNB) and reduced by nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of GR, and GSH content was evaluated by the rate of absorption changes at 412 nm of 2-nitro-5-thiobenzoic acid (NTB) generated from the reduction of DTNB. Oxidized glutathione (GSSG) was determined after removing GSH by 2-vinylpyridine derivatization. After that standard curve with known concentration of GSH and GSSG were used. The GSH content was calculated by subtracting GSSG from total GSH.

3.10.8 Determination of protein

Following the described method of Bradford (1976) the protein concentration of each leaf sample was measured by using BSA (Bovin Serum Albumin) as standard.

3.10.9 Enzyme extraction and assays

Leaf tissue (0.5 g) was homogenized in 1 mL of 50 mM ice-cooled K-P buffer (pH 7.0) containing 100 mM KCl, 1 mM AsA, 5 mM β-mercaptoethanol and 10% (w/v) glycerol using ice-cooled mortar and pestle. The homogenate was centrifuged at 0-4

ºC for 15 min at 11,500 × g and the supernatants were used for enzyme determination.

To determine Ascorbate peroxidase (APX; EC: 1.11.1.11) activity, the enzyme extract mixed with reaction buffer containing 50 mM K-P buffer (pH 7.0), 0.5 mM AsA, 0.1 mM H_2O_2, 0.1 mM EDTA. The reaction was started by the addition of H_2O_2 and the APX activity was measured by observing decrease absorbance at 290 nm for 1 min using an extinction coefficient 2.8 mM^{-1} cm^{-1} (Nakano and Asada, 1981).

According to Hossain *et al.* (1984) described method, monodehydroascorbate reductase (MDHAR; EC: 1.6.5.4) was measured. The reaction mixture contained of 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 µL. The reaction was started by the addition of AO and the MDHAR activity was measured by observing decrease absorbance at 340 nm for 1 min using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

Dehydroascorbate reductase (DHAR; EC: 1.8.5.1) activity was assayed following Nakano and Asada (1981) described method. The reaction mixture contained 50 mM K-P buffer (pH 7.0), 2.5 mM glutathione (GSH), 0.1 mM EDTA, and 0.1 mM dehydroascorbate (DHA) and activity was measured from the change in absorbance at 265 nm for 1 min using an extinction coefficient 14 mM^{-1} cm^{-1}.

To determine the Glutathione reductase (GR; EC: 1.6.4.2) activity following the method of Hasanuzzaman *et al.* (2012b). The reaction buffer contained 0.1 M K-P buffers (pH 7.0), 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH, and enzyme extract in a final volume of 1 mL. The activity of GR initiated with GSSG and the decrease in absorbance at 340 nm for 1 min. The activity was calculated an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

Catalase (CAT; EC: 1.11.1.6) activity was assayed following the method as described by Hasanuzzaman *et al.* (2012b) by monitoring the decreased absorbance (by decomposition of H_2O_2) at 240 nm for 1 min. The reaction mixture contained 50 mM K-P buffer (pH 7.0), 15 mM H_2O_2 and enzyme solution in a final volume of 700 µL and CAT activity was calculated using an extinction coefficient 39.4 mM^{-1} cm^{-1}.

Glyoxalase I (Gly I; EC: 4.4.1.5) activity was assayed following the described method of Hasanuzzaman *et al.* (2011a). The assay buffer contained 100 mM K-P buffer (pH 7.0), 15 mM magnesium sulphate, 1.7 mM GSH and 3.5 mM MG and enzyme extract in a final volume of 700 µL. The increased absorbance was observed at 240 nm for 1 min and Gly I activity was calculated using an extinction coefficient of 3.37 mM^{-1} cm^{-1}.

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

According to Elia *et al.* (2003) proposed method glutathione peroxidase (GPX; EC: 1.11.1.9) activity was measured at 340 nm for 1 min. The reaction buffer contained 100 mM K-P buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide (NaN3), 0.12 mM NADPH, 2 mM GSH, 1 unit of GR, 0.6 mM H_2O_2 (as a substrate), and 20 µL of sample solution. The GPX activity was calculated using the extinction coefficient of 6.62 mM^{-1} cm^{-1}.

To calculate lipoxygenase (LOX; EC: 1.13.11.12) activity following Doderer *et al.* (1992) described the method. The activity was assayed by monitoring in absorbance at 234 nm using linoleic acid as a substrate. The LOX activity was calculated using an extinction coefficient of 25 mM^{-1} cm^{-1}.

3.11 Statistical Analysis

The data obtained for different parameters were subjected to analysis of variance (ANOVA) and the mean differences were compared by Fisher's LSD using XLSTAT 2018 software (Addinsoft, 2018). Differences at $P \leq 0.05$ were considered significant.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Total germination percentage

Seeds remain dormant until it gets the proper environment to germinate. After getting proper environment seeds start to germinate which is considered as the beginning of life cycle of plants. Germination percentage of seeds differs depending on species and environmental conditions. When germination process hampers due to the adverse environmental conditions such as drought, salinity, high and low temperature etc. plant growth, development and grain yield are affected. Among these stresses drought and salinity noticeably inhibit the seeds germination. During drought and salinity stress at first create low osmotic potential in seeds, thus preventing the water uptake from soil (Wei *et al.,* 2013; Panuccio *et al.,* 2014). At the later stage of salinity enhances Na^+ concentration in seeds which reduced the germination of seeds through arrest the metabolic activities (Zehra *et al.,* 2013). It has also been reported by Liu *et al.* (2018) that salinity stress inhibits the rice seed germination by reducing α-amylase activity via decreased bioactive gibberellins contents. In the present study, seeds of different varieties of rice namely BRRI dhan29, BRRI dhan48 and Koshihikari exposed to salinity and drought stress separately or in combination at different magnitude. Therefore, due to stress total germination percentage of all rice cultivars seed reduced remarkably (Figure 2). Whereas, total germination percentage was reduced low in Koshihikari under salinity and drought stress 30% and 50% compare with control respectively than other varieties of rice. But in combine stress of salinity and drought total germination percentage was reduced low in BRRI dhan48 (79%) compare with control than BRRI dhan29 (85%) and Koshihikari (89%).

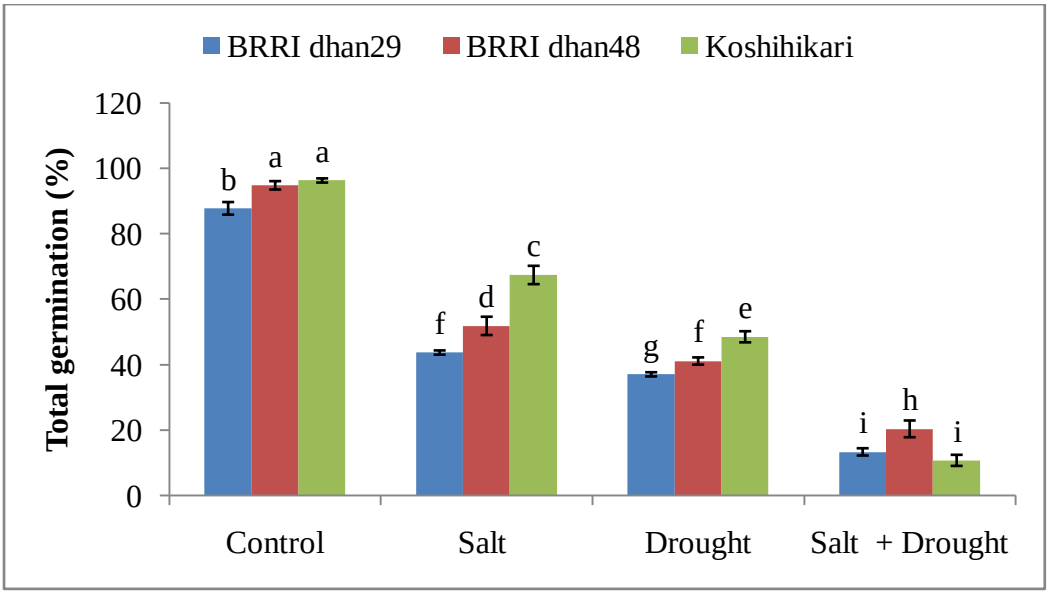

Figure 2. Total germination percentage of rice seedlings under control, salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 2.85). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.2 Growth parameters of rice seedlings

4.2.1 Mortality rate

When plants expose to drought and salinity stress, they face inhibition of CO_2 fixation and photochemical capacity that's why continued demand of carbohydrates decreased the carbohydrate reserves (McDowell *et al.*, 2008; Ahmed *et al.*, 2015). Moreover, excessive accumulation of Na^+ into rice leaves under salinity stress impaired the electron transport system which decreased the photosynthesis activity ultimately this lead to plants death (Wang *et al.*, 2017). In this experiment, it was investigated the mortality rate of rice seedlings at the early vegetative stage. It was observed lowest mortality rate showed BRRI dhan48 under salinity, drought and in combine stress of salinity and drought at 27%, 16% and 61% respectively (Figure 3). On the other hand, Koshihikari showed the highest mortality rate at 51%, 25% and 83% respectively in three treatments (Figure 3). BRRI dhan29 showed moderate mortality under salinity, drought and in combine stress. However, in control

seedlings mortality rate was zero which indicates the reason for seedlings death as salinity and drought stress.

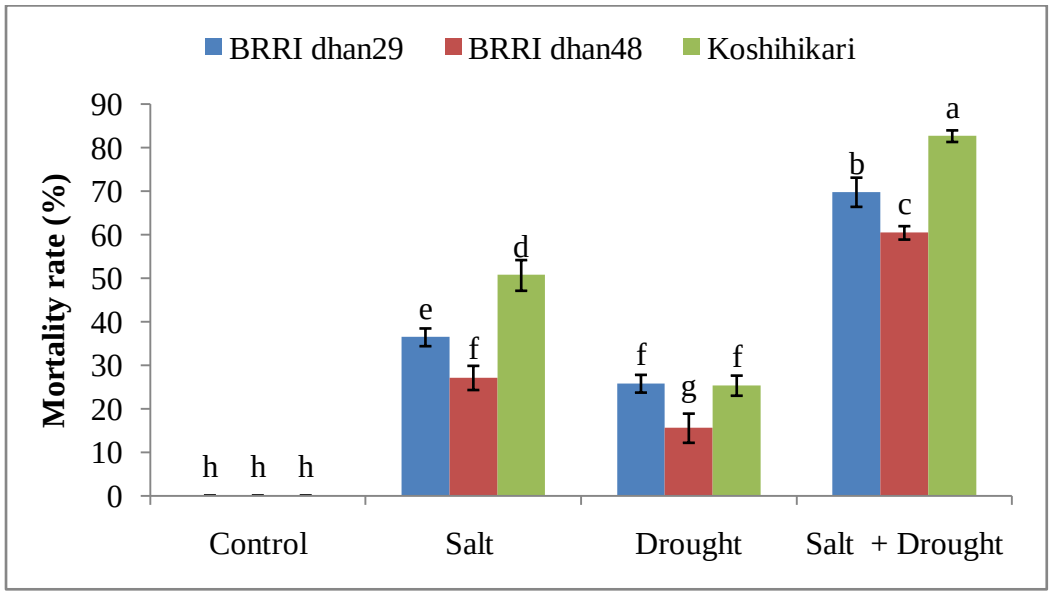

Figure 3. Effect of salinity, drought and combined stress of salinity and drought on rice seedlings mortality at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 3.92). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.2.2 Shoot and root length

Salinity and drought stress decreased the shoot and root length of three varieties of rice seedlings compared with control (Table 1). Shoot and root length of BRRI dhan29 and Koshihikari were reduced markedly at 8%, 10% and 26%, 24% respectively compared with control under combined stress. In contrast, higher reduction of root length was observed in BRRI dhan29 and BRRI dhan48 under salinity stress at 19% and 18% respectively.

Table 1. Effect of shoot and root length of rice seedlings under salinity, drought and combined stress of salinity and drought at the early vegetative stage

	Shoot length (cm)		
Treatment	**BRRI dhan29**	**BRRI dhan48**	**Koshihikari**
Control	18.23 ± 0.08d	20.30 ± 0.51c	24.45 ± 0.30a
Salt	17.23 ± 0.39e	19 ± 0.66d	22.15 ± 0.79b
Drought	16.86 ± 0.27e	18.88 ± 0.46d	22.91 ± 0.22b
Salt + Drought	15.46 ± 0.08f	18.25 ± 0.10d	20.60 ± 0.95c
$LSD_{(0.05)}$	0.79		
CV (%)	2.38		
	Root length (cm)		
Treatment	**BRRI dhan29**	**BRRI dhan48**	**Koshihikari**
Control	8.75 ± 0.51cd	9.40 ± 0.40c	11.33 ± 0.74a
Salt	7.07 ± 0.34f	7.66 ± 0.39ef	10.50 ± 0.24b
Drought	8.27 ± 0.37d	8.49 ± 0.18de	10.69 ± 0.65ab
Salt + Drought	6.16 ± 0.41g	7.77 ± 0.31ef	8.15 ± 0.05de
$LSD_{(0.05)}$	0.71		
CV (%)	4.86		

Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test. Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.2.3 Shoot-root FW and DW

Many research publications have been reported the FW and DW of plants in the response of salinity and drought are reduced significantly (Ahmed *et al.,* 2013a; Al Hassan *et al.,* 2017). In this present study the FW and DW of shoot and root as a percentage of control rice seedlings were reduced under stress conditions in each variety of rice seedlings (Table 2) except the DW of root under drought stress. In

some cases due to water deficit condition the root biomass of rice plants may be increased relative to that of well-water controls which have reported by Li *et al.* (2017). Therefore, this increased DW of root under drought stress in each variety of rice seedlings supported the above mention reports. However, reduction in FW of shoot and root were more pronounced in BRRI dhan29 and Koshihikari at 42%, 41% and 54%, 52% respectively under combined stress of salinity and drought compare with control seedlings. But in case of sole stress of salinity and drought in BRRI dhan29 the FW of shoot and DW of root were higher than Koshihikari. On the other hand, in all three stress treatments higher FW of root was found in BRRI dhan48 than others. Moreover, under salinity, drought and combine stress DW of shoot and root were higher in BRRI dhan48 at 86.83%, 88.40%, 82.13% and 91.28%, 122.56%, 82.05% respectively compared with BRRI dhan29 and Koshihikari. Thus, relative stress tolerance of BRRI dhan48 › BRRI dhan29 › Koshihikari as defined by the degree of stress-induced reduction of the FW and DW of shoot and root.

Table 2. Effect of shoot-root FW and DW of rice seedlings under salinity, drought and combine stress of salinity and drought at the early vegetative stage

	Shoot FW (gm)		
Treatment	**BRRI dhan29**	**BRRI dhan48**	**Koshihikari**
Control	0.060 ± 0.001bc	0.083 ± 0.003a	0.085 ± 0.0008a
Salt	0.057 ± 0.001cd	0.057 ± 0.001bc	0.059 ± 0.0007bc
Drought	0.053 ± 0.001de	0.06 ± 0.002bc	0.061 ± 0.003b
Salt + Drought	0.035 ± 0.0007f	0.0531 ± 0.002de	0.050 ± 0.006e
$LSD_{(0.05)}$	3.88		
CV (%)	3.86		
	Shoot DW (gm)		
Treatment	**BRRI dhan29**	**BRRI dhan48**	**Koshihikari**
Control	0.014 ± 0.0004b	0.016 ± 0.0001a	0.016 ± 0.0005a
Salt	0.013 ± 0.0004e	0.014 ± 0.0003bc	0.013 ±0.0003de
Drought	0.012 ± 0.001cd	0.014 ± 0.0003b	0.013 ± 0.0002cd

Table 2 (cont'd)

Treatment	Shoot DW (gm)		
	BRRI dhan29	**BRRI dhan48**	**Koshihikari**
Salt + Drought	0.009 ± 0.0005g	0.013 ± 0.0008de	0.011 ± 0.0008f
$LSD_{(0.05)}$	0.73		
CV (%)	3.25		
Treatment	**Root FW (gm)**		
	BRRI dhan29	**BRRI dhan48**	**Koshihikari**
Control	0.051 ± 0.0004a	0.053 ± 0.0004a	0.047 ± 0.001b
Salt	0.030 ± 0.003d	0.046 ± 0.0007b	0.027 ± 0.001e
Drought	0.037 ± 0.001c	0.051 ± 0.0003a	0.035 ± 0.001c
Salt + Drought	0.024 ± 0.002f	0.031 ± 0.002d	0.023 ± 0.004f
$LSD_{(0.05)}$	0.0029		
CV (%)	4.45		
Treatment	**Root DW (gm)**		
	BRRI dhan29	**BRRI dhan48**	**Koshihikari**
Control	0.005 ± 0.001ef	0.006 ± 0.0002c	0.007 ± 0.0003b
Salt	0.005 ± 0.0004g	0.006 ± 0.00005d	0.006 ± 0.00005de
Drought	0.006 ± 0.001d	0.008 ± 0.0001a	0.008 ± 0.0001a
Salt + Drought	0.004 ± 0.0004h	0.005 ± 0.0001ef	0.005 ± 0.0001fg
$LSD_{(0.05)}$	0.00043		
CV (%)	4.22		

Mean (±SD) was calculated from three replications for each treatment. Values in column with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test. Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.3 Physiological parameters of rice seedlings

4.3.1 Relative water content

Relative water content (RWC) of plants is an indicator to measure the relative tolerance levels of plants under stress conditions. Salinity and drought both are reduced the soil water potential surrounding the root zone of plants, therefore water uptake from the soil through roots was interrupted to maintain the turgor pressure (Sabir *et al.,* 2009; Pandey and Shukla, 2015). Moreover, plants uptake more Na^+ due to salinity stress and this higher level of Na^+ reduced the osmotic potential of leaf, thus reducing RWC of the leaf (Ueda *et al.,* 2013). The present study showed that the RWC of rice seedlings leaf decreased significantly by salinity and drought either alone or in combination (Figure 4). Compared with control seedlings RWC was decreased by 24.3%, 14.6%, 44.2% in BRRI dhan29; 23.7%, 10.8%, 41.8% in BRRI dhan48; 41.2%, 8.7%, 55.3% in Koshihikari under salinity, drought and combined stress respectively (Figure 4). And as figure 4 shown that higher level of leaf RWC observed in BRRI dhan48 under stress conditions except for drought stress. These results supported by Lima *et al.* (2015) and Mostofa *et al.* (2015), they observed RWC content decreased in rice plants under drought and salinity stress respectively.

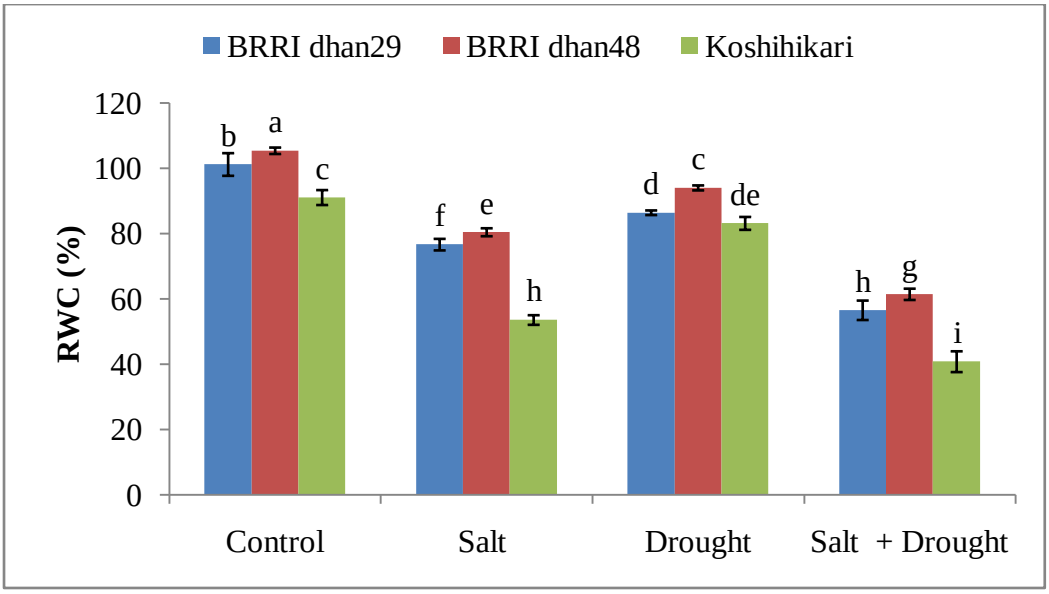

Figure 4. Changes in leaf relative water of rice seedlings under salinity, drought and combine stress of salinity and drought at early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 3.65). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.3.2 Photosynthetic pigments

Plants can produce their own food by the process of photosynthesis. And photosynthetic pigments of plants involved in the photosynthesis process by the harvesting of light and producing reducing power in plants. But this process of plants can be interrupted due to abiotic stresses. Salinity and drought stress breakdown the chlorophyll contents rapidly instead of its slow synthesis. Moreover, during salinity stress plants accumulate the higher level of Na^+ in the oldest leaf which caused toxicity into oldest leaf and start to develop chlorosis and senescence (Li *et al.,* 2010; Yang *et al.,* 2011). Kadhimi *et al.* (2016) and Abdallah *et al.* (2016) have reported that chlorophyll contents of rice seedlings decreased in drought and salinity respectively. Also, it has been observed in this study the chlorophyll contents were decreased in each variety of rice seedlings under three stress treatments in comparison their control (Table 3). Although, compared with controls chlorophyll a (Chl *a*) content was recorded higher in BRRI dhan48 at 89.67%, 82.53% under salinity and in combine stress respectively than others varieties, Koshihikari showed

a higher level of Chl *a* under drought stress (Table 3). Further, chlorophyll b (Chl *b*) content was recorded higher in BRRI dhan29 and BRRI dhan48 in salinity and in combine stress and Koshihikari did not show any significant change in Chl *b* content under drought stress (Table 3). Again it has been observed the similar trend for the total chl (Chl *a* + *b*) content as like Chl *a* and Chl *b* in all varieties of rice seedlings under all stress conditions (Table 3). As like our result of higher chl *a*, Chl *b* and total chl (Chl *a* + *b*) contents for Koshihikari under drought stress, Swapna and Shylaraj, (2017) found the similar result in different varieties of rice under drought stress. Moreover, carotenoids (Car) content was decreased in BRRI dhan29 and Koshikari under stress conditions except Koshihikari in drought stress did not change significantly.

Table 3. Effect of salinity, drought and combined stress of salinity and drought on contents of chl *a*, chl *b*, total chl (chl *a* + *b*) and carotenoids (Car) of rice seedlings at the early vegetative stage

	Chl *a* (%)		
Treatment	**BRRI dhan29**	**BRRI dhan48**	**Koshihikari**
Control	14.23 ± 0.05a	11.19 ± 0.15c	13.03 ± 0.39b
Salt	12.47 ± 0.17b	10.04 ± 0.18d	9.79 ± 0.82d
Drought	12.38 ± 0.66b	11.16 ± 0.99c	13.22 ± 1.16ab
Salt + Drought	9.21 ± 0.39d	9.24 ± 0.65d	7.70 ± 0.02e
$LSD_{(0.05)}$	1.05		
CV (%)	5.55		
	Chl *b* (%)		
Treatment	**BRRI dhan29**	**BRRI dhan48**	**Koshihikari**
Control	4.06 ± 0.24a	3.57 ± 0.17bc	3.76 ± 0.04ab
Salt	3.65 ± 0.06b	3.17 ± 0.19ef	2.85 ± 0.31de
Drought	3.71 ± 0.18ab	3.25 ± 0.22cd	3.80 ± 0.38ab
Salt + Drought	2.60 ± 0.09f	2.63 ± 0.14f	2.68 ± 0.29f
$LSD_{(0.05)}$	0.38		
CV (%)	6.81		

Table 3 (cont'd)

Treatment	Total chl content (Chl *a* + *b*) (%)		
	BRRI dhan29	**BRRI dhan48**	**Koshihikari**
Control	18.29 ± 0.19a	14.76 ± 0.32cd	16.80 ± 0.35b
Salt	16.13 ± 0.11b	13.20 ± 0.37ef	12.63 ± 1.14fg
Drought	16.08 ± 0.84bc	14.41 ± 1.20de	17.02 ± 1.53ab
Salt + Drought	11.81 ± 0.48g	11.86 ± 0.79fg	10.39 ± 0.31h
$LSD_{(0.05)}$	1.36		
CV (%)	5.57		
Treatment	**Carotenoids (Car) (%)**		
	BRRI dhan29	**BRRI dhan48**	**Koshihikari**
Control	4.26 ± 0.17a	3.45 ± 0.02de	3.82 ± 0.24bcd
Salt	3.91 ± 0.01abc	3.22 ± 0.07ef	2.93 ± 0.17f
Drought	3.97 ± 0.48ab	3.58 ± 0.11cde	3.85 ± 0.37bc
Salt + Drought	3.58 ± 0.05cde	3.53 ± 0.10cde	2.98 ± 0.26f
$LSD_{(0.05)}$	0.39		
CV (%)	6.39		

Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test. Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.3.3 Na^+ and K^+ homeostasis

Both salinity and drought stress are disturbed the ion homeostasis of plants. Water deficit condition of soil decreases the mobility of K^+, thus root cannot uptake the K^+ from soil and ultimate plants face intracellular K^+ starvation (Hu and Schmidhalter, 2005). Moreover, water deficit condition increased the cellular lipid peroxidation which is caused K^+ leakage from plants cell (Wei *et al.*, 2013). In this study it has

been observed that K^+ content decreased in shoot and root of all varieties of rice seedlings under drought stress compared with respective control (Figure 5B). Similarly, Zain *et al.* (2014) were found that K^+ content decreased along with increased of drought stress in rice plants. On the other hand, the salinity-induced Na^+ influx into root through plasma membrane non-selective channels (NSCC) which is caused K^+ efflux (Shabala *et al.*, 2007). Therefore, higher Na^+ content disrupted Na^+/ K^+ ratio and ion homeostasis in plants. In this study, salinity and combined stress increased the Na^+ and under three treatments the K^+ decreased in shoot and root of all varieties of rice seedlings compared with non-stressed control seedlings (Figure 5A, B, D, E). Furthermore, higher level of Na^+ content increased the Na^+/ K^+ ratio both in shoot and root of all rice varieties under salinity and combine stress (Figure 5C, E). Ahmed *et al.* (2013a) reported that salinity alone and combine stress of salinity and drought decreased the K^+ content and increased the Na^+/ K^+ ratio in root, leaf and stem of barley plants. Whereas, Chunthaburee *et al.* (2016), Rahman *et al.* (2016a) and Kibria *et al.* (2017) who observed the similar trend in Na^+, K^+ and Na^+/ K^+ ratio of rice seedlings under salinity stress. However, we also observed the higher level of K^+ content and lower ratio of Na^+/ K^+ in the shoot of BRRI dhan48 under salinity alone and combine stress compared with others (Figure 5B, C). Therefore, it might be suggested relative tolerance level under stress conditions higher in BRRI dhan48 than BRRI dhan29 and Koshihikari.

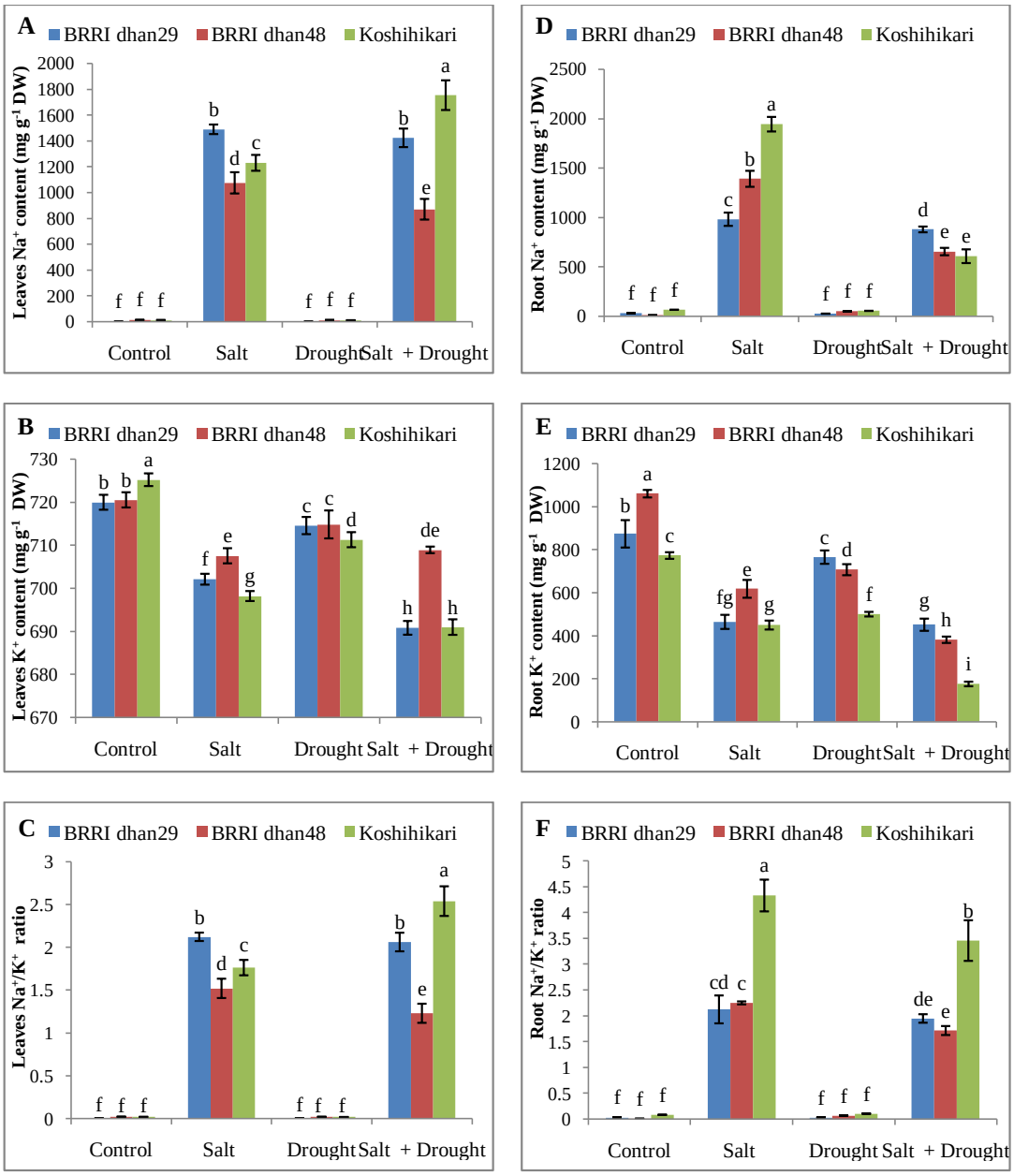

Figure 5. Effect of salinity, drought and combined stress of salinity and drought on Na^+ and K^+ content and their ratio in leaves (A,B,C) and root (D,E,F) of rice seedlings at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 75.70, 2.90, 0.11, 78.29, 43.75 and 0.27 repectively). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.4 Biochemical parameters of rice seedlings

4.4.1 Oxidative stress markers

4.4.1.1 MDA content (product of lipid peroxidation)

Salinity and drought stress leads the accumulation of ROS (reactive oxygen species) in plants which accelerate the lipid peroxidation and impair the cellular homeostasis, thus causes oxidative damage (Miller *et al.,* 2010). In stress conditions, the lipid peroxidation used as an indicator of ROS ($^1O_2$, $O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$) mediated damage to the cell membrane. Where, MDA content is one of the cytotoxic compound and final product of lipid peroxidation which determined the degree of oxidative damage of cell membrane (Gill and Tuteja, 2010).

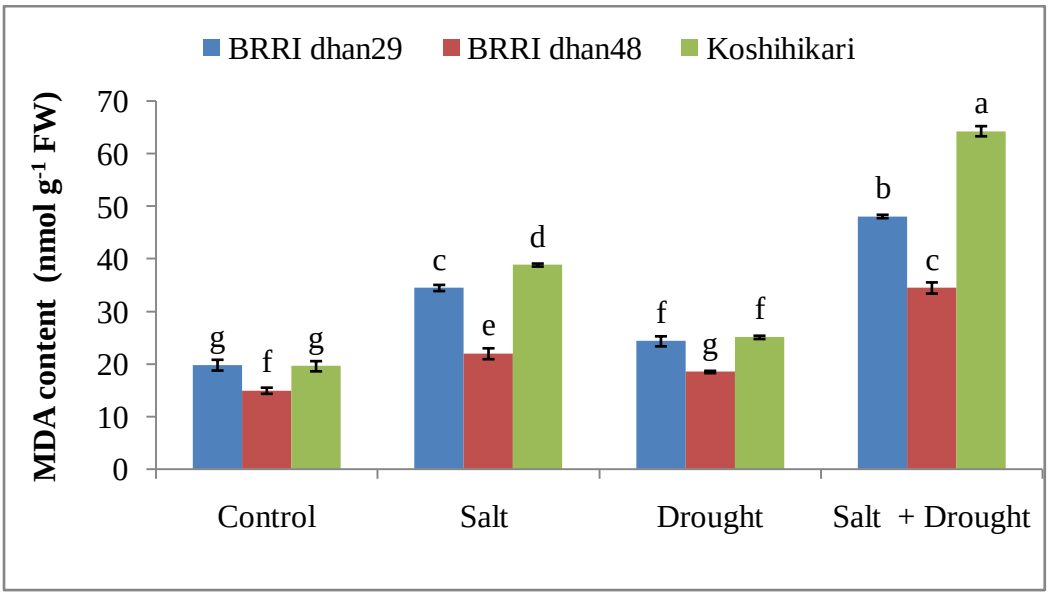

Figure 6. MDA content of rice leaves affected by salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 1.17). Here salt, drought and control, salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

In this experiment, it has been observed that under salinity, drought and combine stress compared with non-stressed control seedlings the MDA content rose higher in Koshihikari at 98.19%, 28.01% and 228.11% respectively and lowest MDA content

was recorded in BRRI dhan48 at 47.13%, 23.89% and 130.89% respectively (Figure 6). Similarly, Mishra *et al.* (2013) and Zu *et al.* (2017) found the increasing level of MDA content in rice seedlings under salinity and drought stress respectively.

4.4.1.2 H_2O_2 content and histochemical localization of H_2O_2

Plant beings are an aerobic organism that's why they use O_2 as a terminal electron acceptor. Therefore, the reduction of O_2 accelerates the production of relatively unstable highly reactive superoxide ($O_2^{\bullet-}$) under stress conditions (Halliwell, 2006). After a short period, this relatively unstable $O_2^{\bullet-}$ leads to the formation of relatively stable hydrogen peroxide (H_2O_2) which can inactive the enzymes by oxidizing their thiol groups (Hasanuzzaman *et al,* 2012a). However, during abiotic stresses the accumulation of H_2O_2 increased in plants cell and increased oxidative damage in the cell membrane (Nahar *et al.,* 2015b; Rahman *et al.,* 2016a). This study showed that the content of H_2O_2 increased significantly in all cultivars of rice seedlings under salinity, drought and combine stress (Figure 7). Compared with control seedlings the higher level of H_2O_2 content was observed 111.34% in BRRI dhan29 and 88.93% in Koshihikari in combine stress treatment and the lowest H_2O_2 content was measured in BRRI dhan48 at 40%, 7.95% and 68.04% under salinity, drought and combined stress respectively (Figure 7). Similarly, Nxele *et al.* (2017) and Vighi *et al.* (2017) were reported from their study that salinity and drought stress increased the H_2O_2 content significantly in sorghum and rice plant respectively.

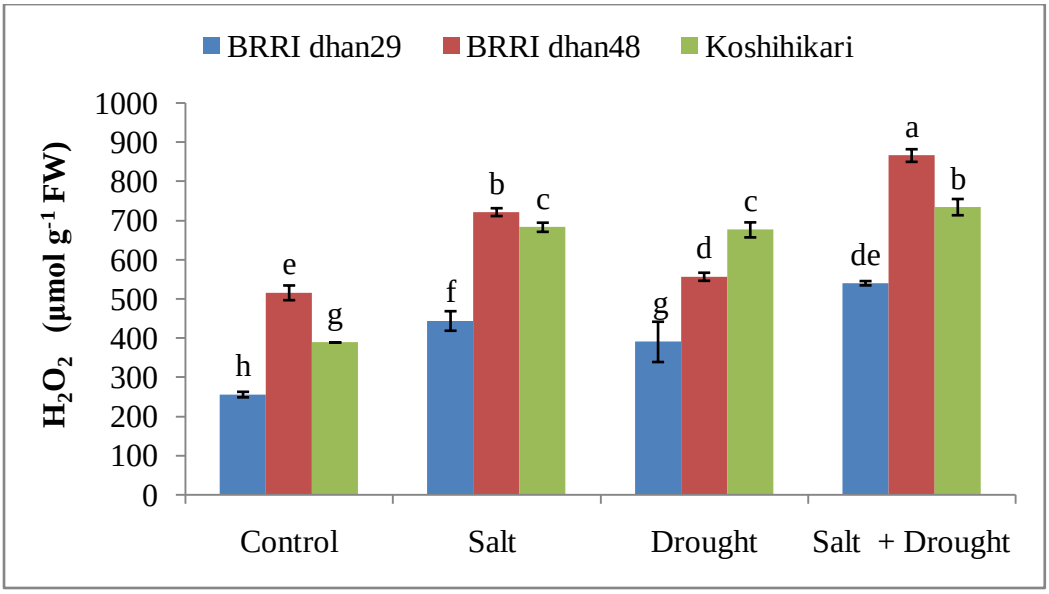

Figure 7. H_2O_2 content of rice leaves affected by salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 32.47). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

In addition, it has been investigated the visual localization of H_2O_2 as a brown spots through histochemical staining of rice seedlings leaves (Plate 1A, B, C, D). As like above mention result, the lowest brown spots were observed in BRRI dhan48 than BRRI dhan29 and Koshihikari compared with control seedlings leaves.

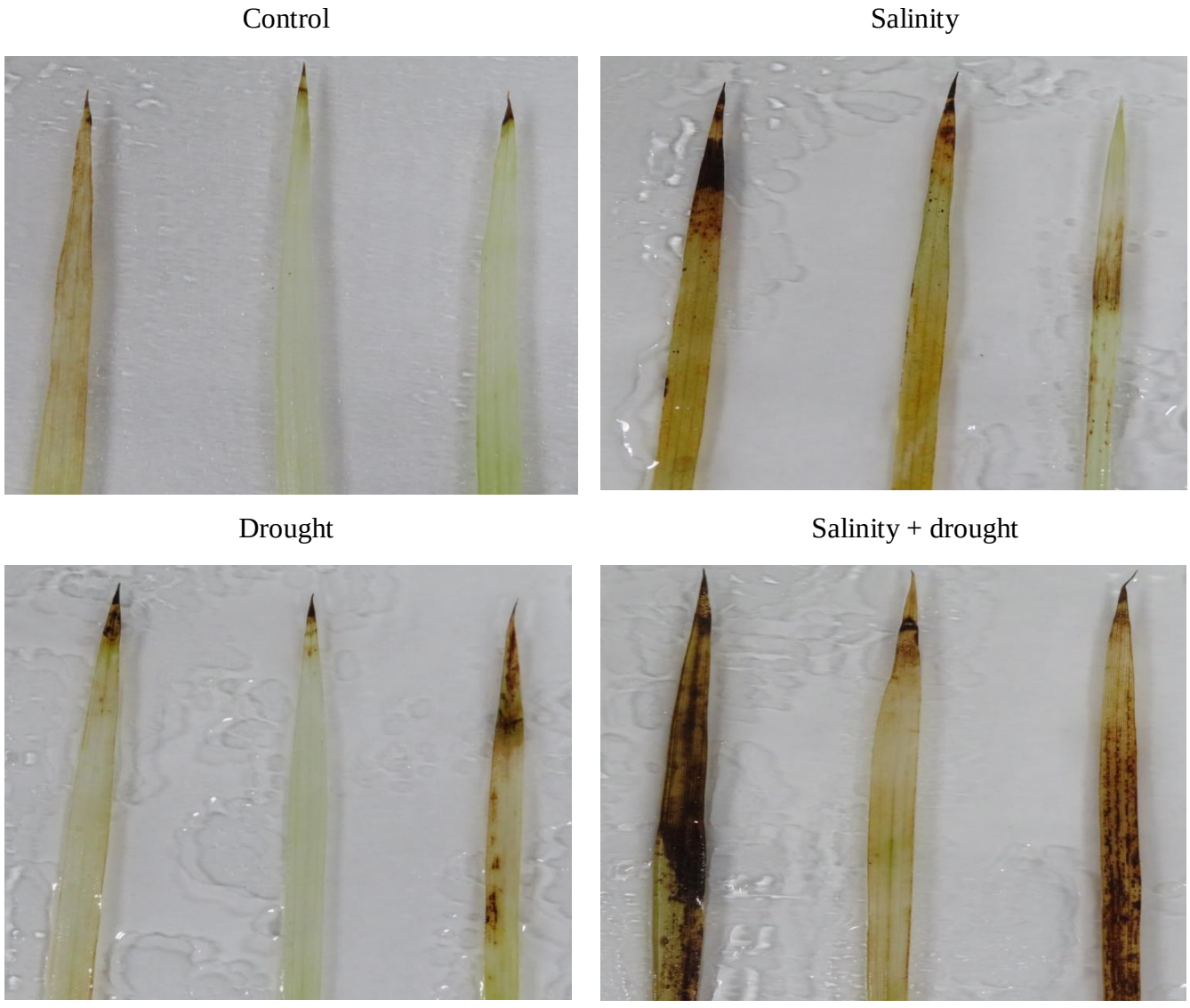

Plate 1. Histochemical detection of H_2O_2 in leaf of rice seedlings under salinity, drought and combine stress of salinity and drought. Here control, salinity, drought and salinity + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG.

4.4.1.3 Lipoxygenase activity

As like MDA content, increased of LOX activity is an indicator event of oxidative burst in the leaf tissues. Therefore, increased LOX activity might be performed to enhance the degree of oxidative damage in the cell membrane through lipid peroxidation in salinity and drought-stressed seedlings (Mostofa *et al.,* 2015; Alam *et al.,* 2014). In our study, we observed the treatment × variety was statistically

significant for the activity of LOX (Figure 8). Because of salinity, drought and combined stress of salinity and drought the highest LOX activity recorded in BRRI dhan29 at 205.46%, 138.09%, 325.14% and in Koshihikari at 285.25%, 152.45%, and 493.46% respectively compared with non-stressed rice seedlings (Figure 8). However, in stress conditions the lowest LOX activity measured in BRRI dhan48 compared with non-stressed rice seedlings than BRRI dhan29 and Koshihikari except salinity stress because in this stress we observed the lower LOX activity in BRRI dhan29 (Figure 8). Similarly, Mostofa *et al.* (2015) and Alam *et al.* (2014) found a clear link between salinity and drought stress induced LOX production and the lipid peroxidation of the membrane in rice seedlings and in *Brassica* species respectively.

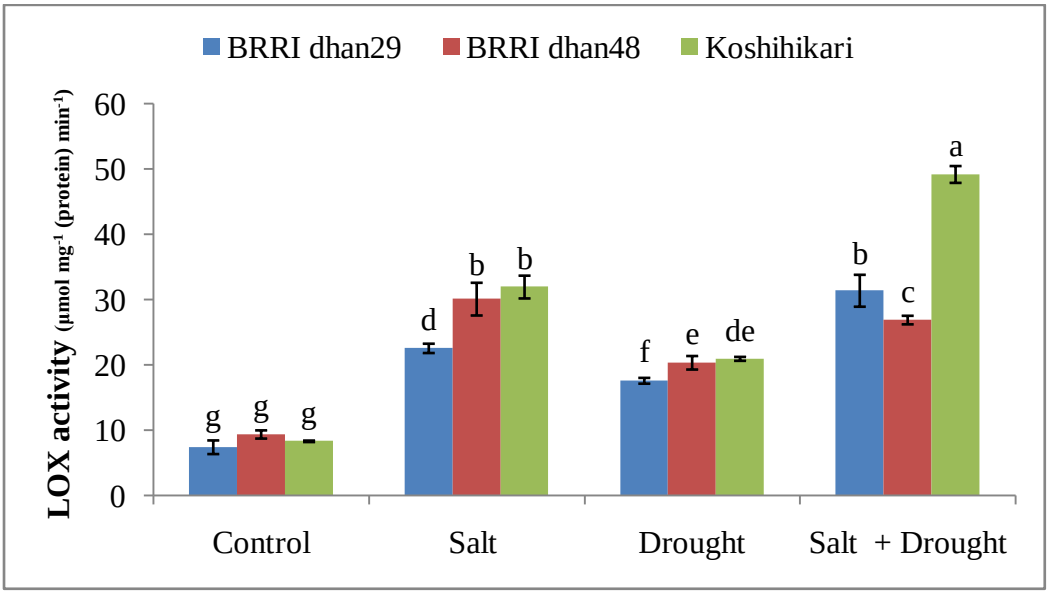

Figure 8. LOX activity of rice leaves affected by salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 2.15). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.4.1.4 Electrolyte leakage

Electrolyte leakage (EL) indicates the degree of cell membrane damage in plants. This leakage is caused by stress resulting K^+ efflux and the counter ions such as Cl^-, HPO_4^{2-}, NO_3^-, $C_6H_5O_7^{3-}$ and $C_4H_4O_5^{2-}$ which are required to balance the positively charged K^+. Moreover, EL occurs in plants due to the activation of K-permeable channels along with cell membrane oxidation in stress conditions (Demidchik *et al.* 2014). In this study, higher EL was observed in combine stress of salinity and drought-treated BRRI dhan29, BRRI dhan48 and Koshihikari seedlings at 190.39%, 115.89%, and 111.97% in root; 317.55%, 226.59% and 138.26% in shoot respectively compared with control seedlings (Figure 9A, B). In contrast, lower EL was observed in drought stress treated BRRI dhan29, BRRI dhan48 and Koshihikari seedlings at 28.14%, 77.13%, and 54.40% in root; 48.40%, 44.40% and 13.41% in shoot respectively compared to their control seedlings (Figure 9A, B).

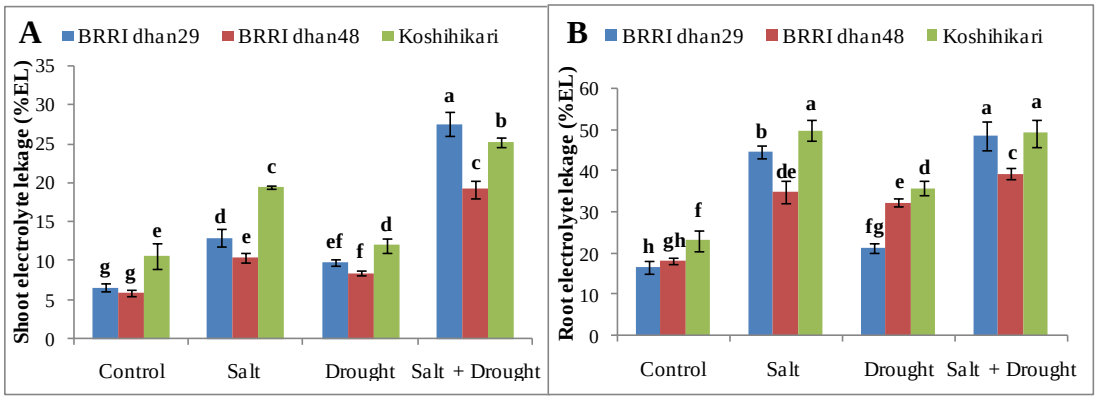

Figure 9. Electrolyte leakage percentage (% EL) of rice shoot and root affected by salinity, drought and combine stress of salinity and drought at early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 1.42 and 3.47). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

This results supported by Hossain *et al.* (2017) where they treated lentil seedlings with 100 mM NaCl and 20% PEG and observed the EL was higher in 100 mM NaCl lentil seedlings than 20% PEG-treated lentils seedlings. It has also been observed in our study that the EL higher in root than shoot under salinity and drought stress, whereas, higher in shoot than root under combine stress of salinity and drought stress

(Figure 9A, B). Furthermore, Swapna and Shylaraj (2017) reported the lower EL occurred in drought-tolerant rice seedlings than drought-sensitive.

4.4.2 Proline content

During stress conditions plants accumulate some compatible solutes [proline (Pro.), glycine betaine (GB) and glucose etc.] to maintain the osmotic adjustment, cell membrane integrity and stabilization of enzymes/proteins (Ashraf and Foolad, 2007). Among all compatible solutes Pro. is a key biochemical solute that act as an osmoprotectant molecule which improved the water status and RWC of plants leaf in stress conditions (Hasanuzzaman *et al.*, 2014a; Nahar *et al.*, 2016). It has been observed in our study that the Pro. content was higher in indica cultivar compared with japonica cultivar under stress conditions (Figure10). But, within two varieties of indica cultivar BRRI dhan48 showed the higher level of proline content in salinity and combined stress compared with control seedlings (Figure 10).

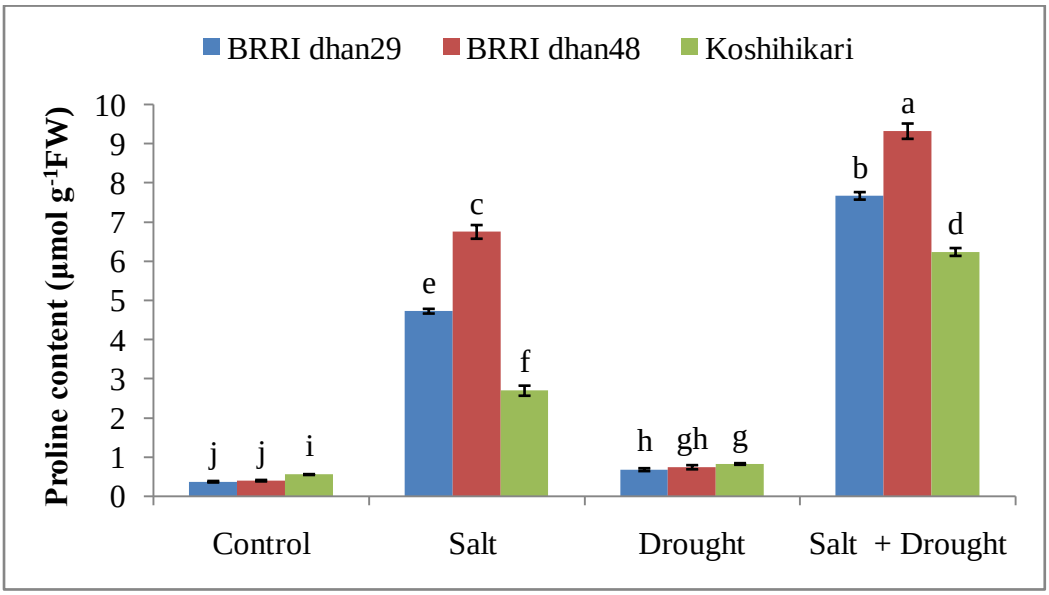

Figure 10. Proline content of rice leaves affected by salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 0.12). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

Moreover, Pro. content in drought stress did not increase as like salinity and combine stress and the increased level of Pro. content among the rice varieties seedling were not statistically significant (Figure 10). Likewise, Filippou *et al.* (2011) and Swapna and Shylaraj (2017) observed in their study the Pro. content did not change significantly in short-term drought stress in *Medicago truncatula* and rice plants respectively.

4.4.3 Antioxidant defense system

4.4.3.1 Ascorbate and glutathione contents

As a non-enzymatic antioxidant AsA is one of the most important antioxidant for plant tissues to maintain the cellular redox state and protecting the plant's from oxidative damage, thus AsA content acts as a salinity and drought stress tolerance marker in plants (Hasanuzzaman *et al.*, 2011a, b; Hasanuzzaman and Fujita, 2011). Mainly, it protects the plants cell membrane from oxidative stress by scavenging the free radicals (H_2O_2, $O_2^{\bullet-}$ and $^1O_2$) and regenerating α-tocopherol and upregulate the enzyme activities (Noctor and foyer, 1998). Moreover, among all antioxidative pathways the AsA-GSH cycle is one of the most important because in this cycle H_2O_2 the most long lived ROS converted into H_2O by APX along with the generation of monodehydroascorbate (MDHA), where AsA used as an electron donor. After that MDHA is converted into AsA or disproportionate into AsA and dehydroascorbate (DHA). In previous study, Rahman *et al.* (2016b) suggested the AsA content and the AsA/DHA ratio are crucial for the maintaining of rice seedlings redox homeostasis. Therefore, in this study we investigated the AsA, DHA and AsA/DHA ratio in rice cultivars seedlings under salinity, drought and combine stress of salinity and drought (Figure 11A, B, C). We observed the AsA content and the AsA/DHA ratio decreased in all rice cultivars seedlings compared with control under salinity and combine stress (Figure 11A, C), whereas the DHA content changed statistically significant in rice cultivars seedlings at 250.65% in BRRI dhan29, 261.53% in BRRI dhan48 and 90.96% in Koshihikari compared with control seedlings under combine stress (Figure 11B). However, among three rice varieties BRRI dhan48 showed the highest AsA content and AsA/DHA ratio in drought stress at 113.87% and 263.18% respectively (Figure 11A, C) which means it showed

relative tolerance in drought stress. These results are consistent with the previous study of Bhattacharjee and Dey (2017) who reported that the AsA and the AsA/DHA ratio increased in drought-tolerant rice cultivars, but decreased in drought-sensitive rice cultivars. For salinity stress our results are supported by Nahar *et al.* (2016) in mung and Rahman *et al.* (2016b) in rice who observed in their study that the AsA and the AsA/DHA ratio decreased seedlings under salinity stress.

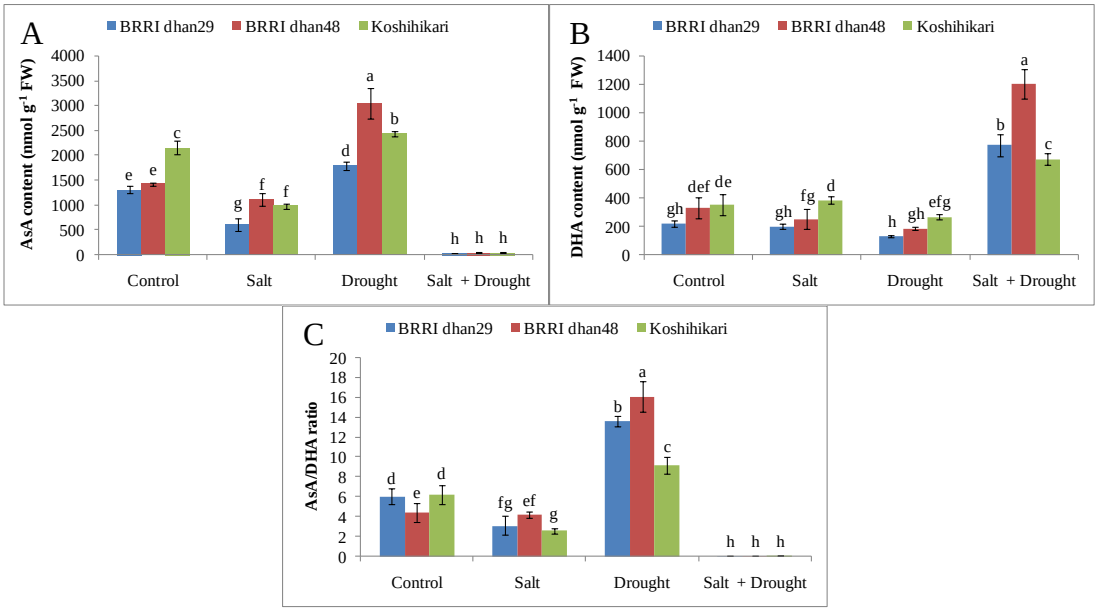

Figure 11. (A) AsA contents, (B) DHA contents and (C) AsA/DHA ratio of rice leaves affected by salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 189.17, 95.96 and 1.24 respectively). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is a non-protein thiol tripeptide component, having a direct role of ROS scavenging and reduced ROS induced oxidative damages through maintaining the redox homeostasis in plants tissue (Gill and Tuteja, 2010). As like AsA, GSH plays a vital role in the cycle of AsA-GSH for scavenging the H_2O_2. Moreover, it can be directly reacted with others free radicals such as $O_2^{\bullet-}$ and $OH^{\bullet}$ for scavenging (May *et al.*, 1998). Therefore, in this ROS scavenging process, the changing ratio of reduced glutathione and oxidized

glutathione (GSH/GSSG) is an important phenomenon in redox signaling pathways (Li and Jin, 2007). With increased research evidence it has been suggested that an increased of GSH in plants tissue under various abiotic stresses indicated as relative tolerance of stress (Hasanuzzaman *et al.*, 2011b; Alam *et al.*, 2014; Nahar *et al.*, 2016; Rahman *et al.*, 2016b). In this study, it has been observed that the GSH content higher in BRRI dhan48 at 154.08% in drought stress (Figure 12A). Moreover, GSH also increased in BRRI dhan29 and BRRI dhan48 under salinity but not much as drought stress. It (GSH) may be used to ROS scavenging, thus the GSSG content recorded lower in BRRI dhan29 (194.14%) and BRRI dhan48 (198.45%) under salinity stress compared with Koshihikari (505.22%) (Figure 12B). We also found that the GSH/GSSG ratio decreased in all rice cultivars seedlings compared with control under salinity and combine stress, but among rice varieties BRRI dhan48 showed higher GSH/GSSG ratio in salinity stress (Figure 12C).

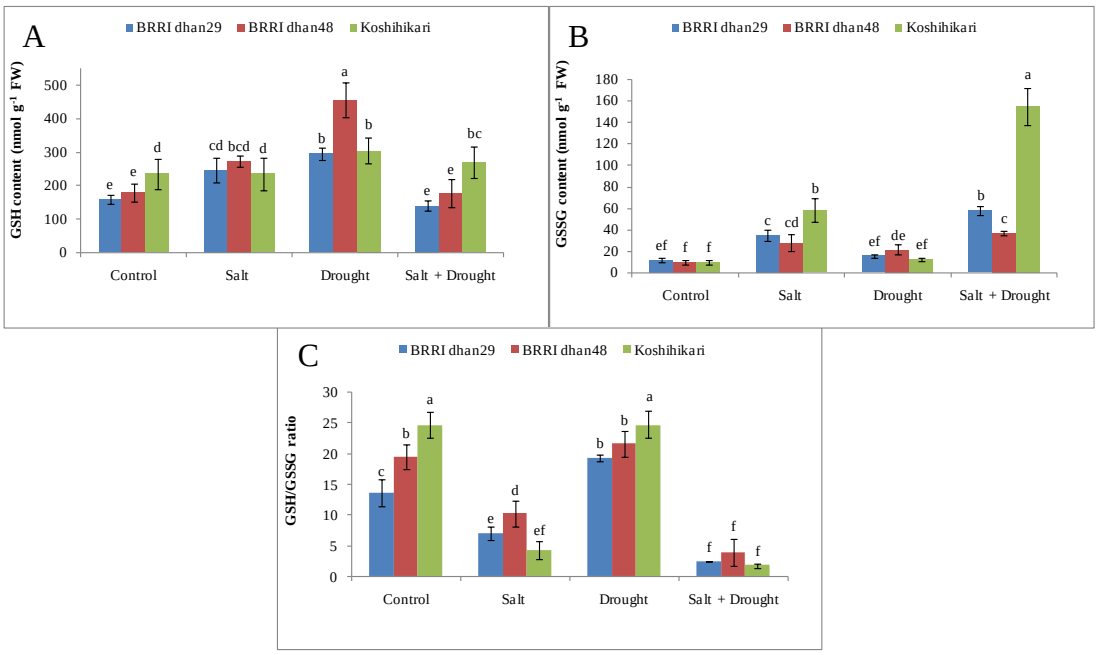

Figure 12. (A) GSH content, (B) GSSG content and (C) GSH/GSSG ratio of rice leaves affected by salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 45.27, 10.97 and 2.89 respectively). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

These results from our study are similar as like Alam *et al.* (2014) and Nahar *et al.* (2016) who found the increased level of GSH content in *Brassica* species under drought stress and the increased level of GSSG content with the lower ratio of GSH/GSSG in mung bean seedlings under salinity stress respectively. However, we have not found any statistically change of GSH content in BRRI dhan29 and BRRI dhan48 under combine stress; GSSG content in BRRI dhan29 and Koshihikari under drought stress; GSH/GSSG ratio in BRRI dhan48 and Koshihikari under drought stress compared with control seedlings (Figure 12A, B, C). These results are suppoted by Hossain *et al.* (2017) who found that 12% PEG did not change the GSH content in lentil seedlings and Alam *et al.* (2014) reported that GSH/GSSG ratio in *Brassica* species did not change compared with control seedlings under drought stress.

4.4.3.2 Ascobate peroxidae activity

The AsA-GSH cycle consists of four vital antioxidant enzymes namely APX, MDHAR, DHAR and GR which are detoxified ROS with the help of AsA and GSH and after that AsA and GSH are recycled (Apostolova *et al.*, 2011). However, in this AsA-GSH cycle the APX performed as a first line enzyme for scavenging the H_2O_2 into the water by using AsA as the substrate (Xu *et al.*, 2008). In this experiment, exposure to salinity and drought stress increased the APX activity in rice seedlings compared to their respective control (Figure 13). As like our study similar trend were also found by Rahman *et al.* (2016a) and Mishra and Panda (2017) in rice seedlings under salinity and drought stress respectively. The higher amount of APX measured in BRRI dhan29 at 83%, 89.2% and 211.01% under salinity, drought and combined stress respectively compared to their control seedlings (Figure 13). This higher APX level in BRRI dhan29 may be due to the higher H_2O_2 and lower AsA content under stress conditions. However, compared with control BRRI dhan48 and Koshihikari have showed lower APX activity than BRRI dhan29 under all three stress treatments (Figure 13).

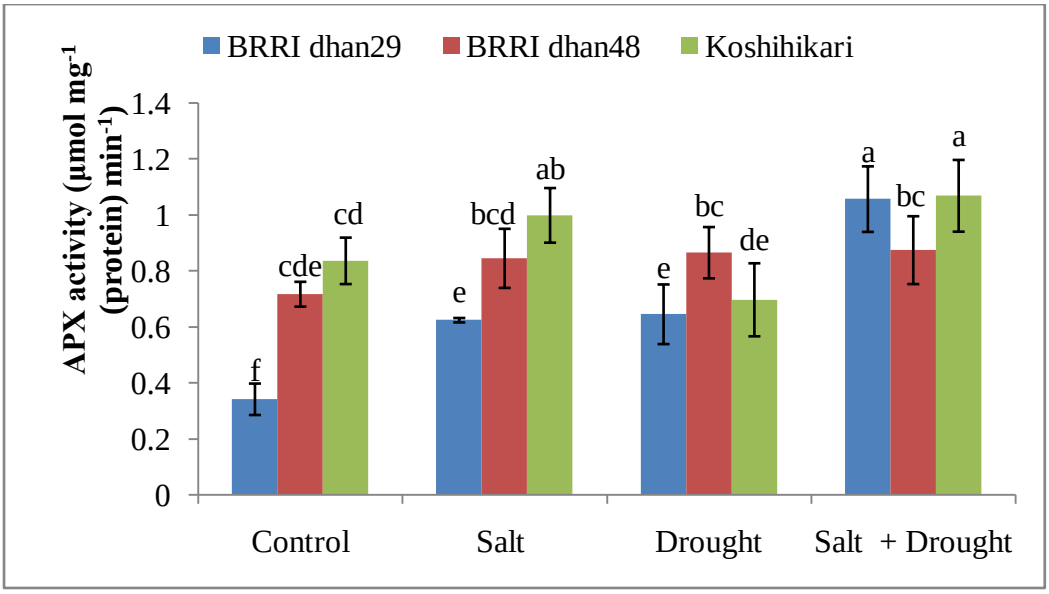

Figure 13. APX activity in rice leaves under rice leaves affected by salinity, drought and combined stress of salinity and drought at early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 0.16). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.4.3.3 Monodehydroascorbate reductase activity

In AsA-GSH cycle monodehydroascorbate reductase (MDHAR) is an important enzyme. At first the univalent oxidation of AsA leads to the formation of MDHA. After that if MDHA is not reduced again to AsA by MDHAR, it will be disproportionate into AsA and DHA. But rapid regeneration of AsA is required to maintain the antioxidative capacity of plants under stress conditions and this regeneration of AsA could be regulated by the NADPH-dependent MDHAR activity (Mittova *et al.*, 2003). Therefore, it is crucial for AsA regeneration and essential to maintain the reduced pool of AsA (Martínez and Araya, 2010). Though, it has also been reported in few studies that the activity of MDHAR in other physiological processes related to oxidative stress, research on various crops in stress conditions revealed the regulatory role of MDHAR during oxidative stress tolerance and acclimation (Hossain *et al.*, 2011b).

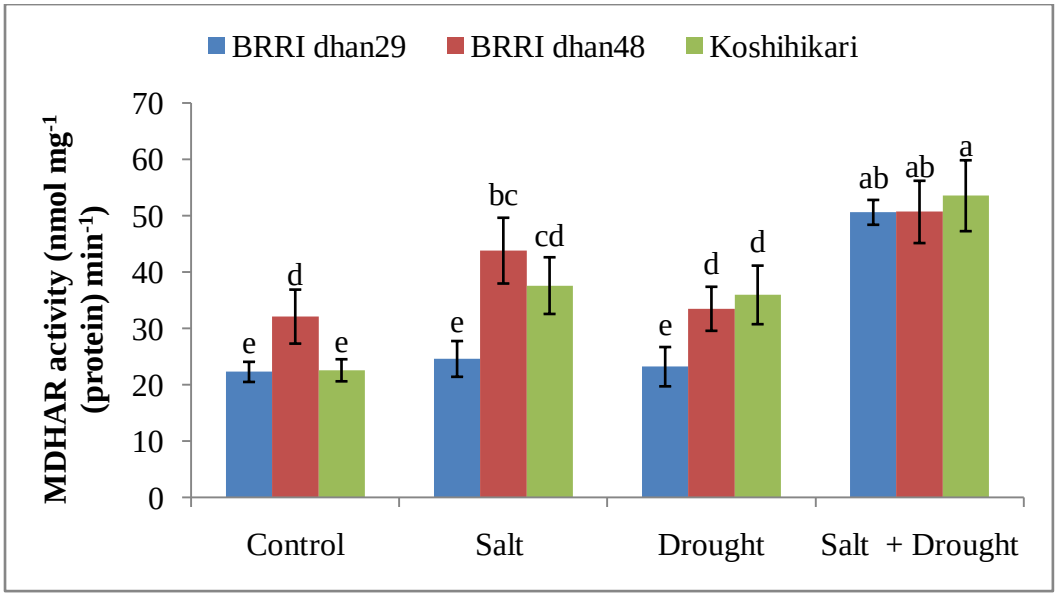

Figure 14. MDHAR activity in rice leaves under salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 7.09). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

In this experiment, it has been observed that the change of MDHAR activity for all rice cultivars seedlings in three stress treatments were not equally significant (Figure 14). Although till now there is no previous reports has been established regarding the activities of MDHAR under combine stress of salinity and drought in rice seedlings, we found the higher activity of MDHAR at 137% in Koshihikari; 126.83% in BBRI dhan29 and 57.81% in BBRI dhan48 compared to their control seedlings under combined stress, moreover under salinity stress MDHAR activity increased at 36.43% in BBRI dhan48 and 66.46% in Koshihikari (Figure 14). Mishra *et al.* (2013) found the increased level of MDHAR activity in rice seedlings under salinity stress. However, this higher level of MDHAR activity might be due to the lowest AsA content in rice seedlings under salinity and combine stress (Figure 11A). In contrast, under drought stress MDHAR activity did not change significantly in rice seedlings, thus it might be due to higher AsA content in rice cultivars seedlings under drought stress (Figure 11A).

4.4.3.4 Dehydroascorbate reductase activity

As like MDHAR, DHAR is equally important to regulate the level of AsA and its redox state during oxidative stress (Eltayeb *et al.,* 2007). DHAR, which is catalyzed the reduction of DHA to AsA with the help of GSH and regulates the AsA redox state in plants cell, thus it is a major component in AsA recycling system and crucial for tolerance to abiotic stresses leading to the production of ROS (Martínez and Araya, 2010). With increased research evidence it has been observed the increased level of DHAR in plants under ROS-inducing stresses like salinity (Rahman *et al.,* 2016b), drought (Hasanuzzaman *et al.,* 2017b) and heavy metal (Sun *et al.,* 2015) stress. Also in this study we found the significantly increased level of DHAR activity in BBRI dhan48 under three stress treatments and in BBRI dhan29 and Koshihikari only for combined stress compared to their respective control (Figure 15). But only the higher activity of DHAR under drought stress at 51.73% in BBRI dhan48 increased the AsA level in rice seedlings (Figure 11A) through decreasing the DHA activity (Figure 11B) with the help of increased level of GSH (Figure 12A), whereas in combine stress for all rice cultivars seedlings higher DHAR activity could not increase the AsA level might be due to highest level of DHA (Figure 11B) and the lowest level of GSH (Figure 12A). On the other hand, under salinity stress the DHAR activity in BBRI dhan29 and Koshihikari could not remarkably change compared with control may be for this reason AsA content decreased in salinity stress; APX and DHA activity in BBRI dhan48 could not remarkably change compared with control may be for this reason AsA content decreased in salinity stress (Figure 11A, B; Figure 15). But in spite of unchanged DHAR activity under drought stress, AsA content increased in BBRI dhan29 and Koshihikari might be due to higher activity of APX. Because of APX scavenges the H_2O_2 into H_2O along with regeneration of MDHA which is disproportionate into AsA and DHA, thus in our study may be this increased or unchanged APX activity could/could not accelerate the AsA content under salinity and drought stress.

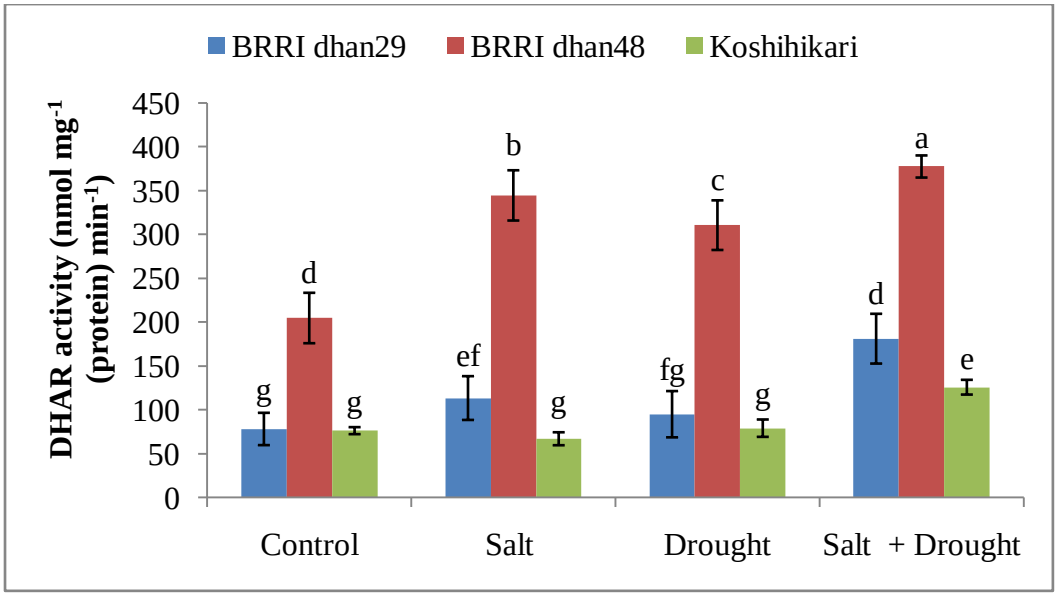

Figure 15. DHAR activity in rice leaves under salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 28.08). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.4.3.5 Glutathione reductase activity

Glutathione reductase (GR) is an important NADPH-dependent enzyme in AsA-GSH cycle because it plays a vital role in defense system against overproduction of ROS in various stress conditions (Romero-Puertas *et al.*, 2006). In plants GR catalyzes the NADPH-dependent reduction of disulfide bond of GSSG into GSH, thus it is essential for maintaining the GSH status, GSH/GSSG ratio and for accelerating the H_2O_2 scavenging pathways under abiotic stress conditions (Pang and Wang, 2010). This study showed that the rice seedlings exposed to salinity, drought and combine stress significantly increased the GR activity compared with their respective control except for BRRI dhan48 in drought stress (Figure 16). Such increased level of GR activity has also been reported by Al Hasan *et al.* (2017) and Vighi *et al.* (2017) in salinity and drought stress of three species of the genus *juncus* and rice genotypes. However, in this study the increased level of GR activity accelerated the higher content of GSH in all rice cultivars seedlings under stress conditions compared to their control (Figure 12A). But under combine stress the

GSH content did not change in BRRI dhan29 and BRRI dhan48 compared to their control (Figure 11A) in respect of higher activity of GR (Figure 16). As a result, AsA content decreased and DHA activity increased in rice seedlings under combine stress (Figure 11A, B) because of DHAR enzyme required a higher amount of GSH content to convert DHA into AsA under stress conditions.

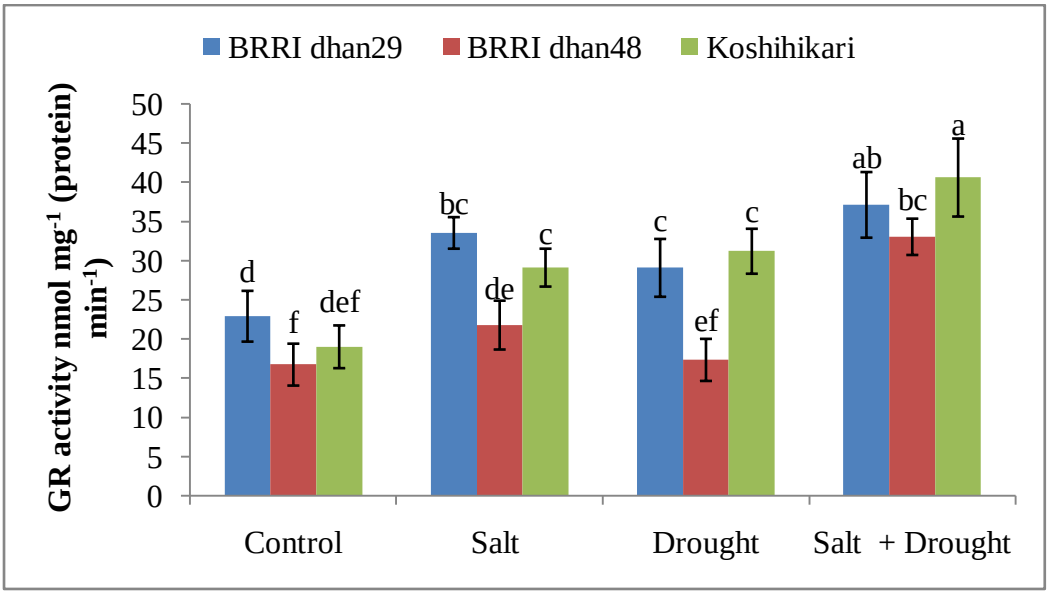

Figure 16. GR activity in rice leaves under salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 4.46). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.4.3.6 Catalase activity

Catalase is a tetrameric heme-containing enzyme which is used H_2O_2 as a substrate to convert it into H_2O and O_2, thus acts as a vital enzyme for ROS detoxification (Sànchez-Casas and Klessig, 1994). In this study, we observed the CAT activity in rice cultivars seedlings have not changed significantly compared with their control under salinity and drought stress (Figure 17). This unchanged CAT activity under salinity and drought stress might not be decreased the higher H_2O_2 in rice cultivars seedlings (Figure 7). Where, the lower activity of CAT was observed in BRRI

dhan48 and Koshihikari and higher in BRRI dhan29 under combine stress (Figure 17). As like our result in stress conditions different trends of CAT activity in various rice genotypes demonstrated by Kibria *et al.* (2017). This diminished CAT activity may be the reason of higher production of H_2O_2 in BRRI dhan48 and Koshihikari (Figure 7). On the other hand, Figure 7 showed that the production for H_2O_2 was higher in BRRI dhan29 (111.34%) under combine stress than BRRI dhan48 (68.04%) and Koshihikari (88.93%) compared to their respective control. Therefore, this increased amount of CAT in BRRI dhan29 could not sufficient to decrease the higher amount of H_2O_2 due to the lowest AsA content under combine stress (Figure 11A).

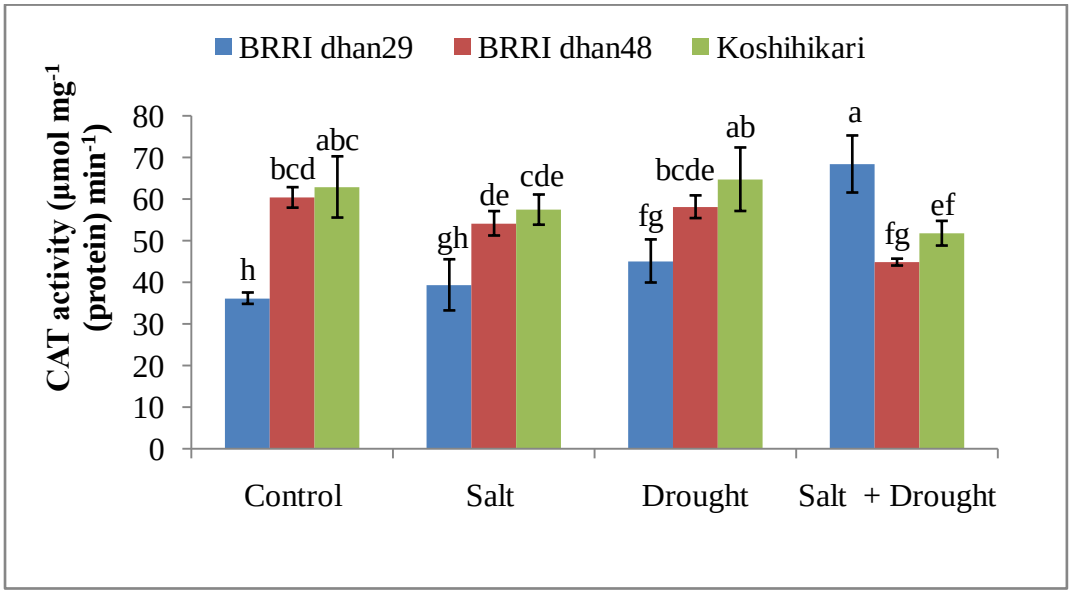

Figure 17. CAT activity in rice leaves under salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 7.18). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.4.3.7 Glutathione peroxidase activity

Glutathione peroxidae (GPX) is consists of multiple isoenzymes that capable of repairing the lipid peroxidation of cellular membrane and acts as a protectant against oxidative damage of membrane (Hasanuzzaman *et al.*, 2012a). Besides scavenging of H_2O_2 using GSH, GPX also functions as an oxidative signal transducer

(Hasanuzzaman *et al.*, 2012a). In previous studies, it has been observed the up-regulate activity of GPX under abiotic stress conditions in rice plants (Mishra and Panda, 2017; Mostofa *et al.*, 2015). The present study resulted in an increased GPX activity under salinity, drought and combine stress among all rice cultivars seedlings compared to their control except for Koshihikari in the sole stress of drought (Figure 18). Although the higher GPX activity showed in BRRI dhan48 at 148.75%, 121.51% and then in BRRI dhan29 at 98.41%, 120.95% under salinity and drought stress respectively with increased GSH activity maintained comparatively lower H_2O_2 content than Koshihikari, but in combined stress despite higher GPX activity the H_2O_2 content increased markedly among all rice cultivars seedlings compared with sole treatment of salinity and drought might be due to the lowest GSH content in combined stress (Figure 12A; Figure 18).

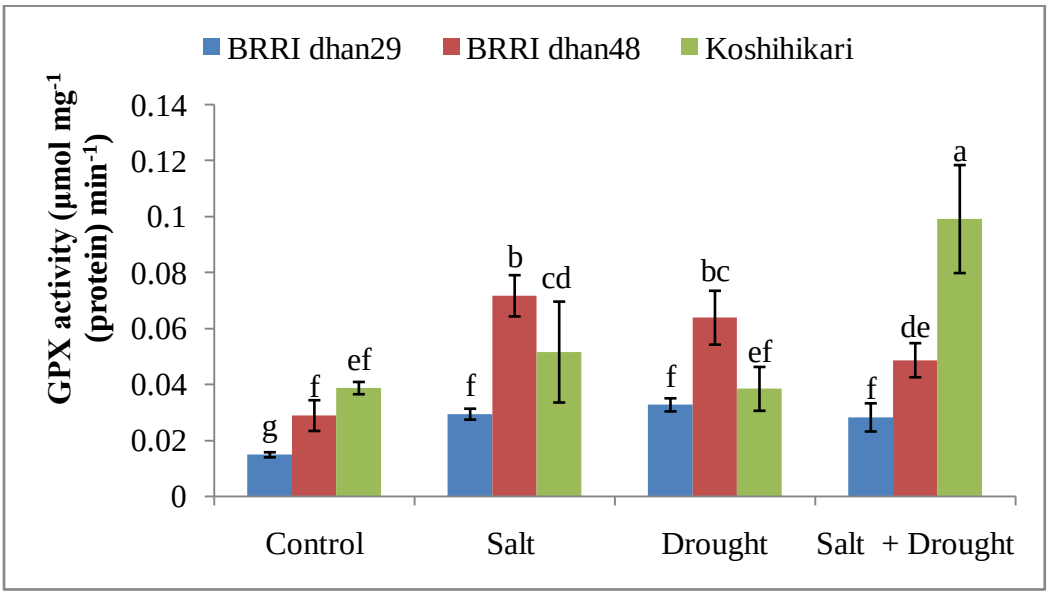

Figure 18. GPX activity in rice leaves under salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 0.01). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

4.4.4 Glyoxalase system enzymes and methylglyoxal detoxification

Methylglyoxal (MG) is highly reactive α-oxoaldehyde cytotoxic compound, in normal growth conditions plant cells contain a lower amount of MG, but with increased the stress severity production of MG gradually increased (Hasanuzzaman *et al.,* 2017a). During stress conditions in plant cells MG formation may be possible due to the metabolism of acetone and aminoacetone through MG synthase (Kaur *et al.,* 2014). However, the plant can be detoxified this cytotoxic compound by the upregulation of glyoxalase system in association with GSH (Yadav *et al.,* 2008). The glyoxalase system consists of two important enzymes, Gly I and Gly II, GSH act coordinately to detoxify MG and which can be occurred in a two-step reaction process. The first step of the glyoxalase system, MG reacts with GSH and produces hemithioacetal, which is later converted to S-D-lactoylglutathione (SLG) catalyzed by the Gly I enzyme. In the second step, Gly II enzyme converts SLG to D-lactate by hydrolysis and at the end of the total reaction GSH is recycled (Kaur *et al.,* 2014). Therefore, the glyoxalase system involved both in detoxifications of MG and in the recycling of GSH which confirms the relative oxidative stress tolerance in plants. There are several reports revealing the increased activity of Gly I or Gly II as well as both reduced the endogenous levels of MG under salinity and drought stress (Rahman *et al.,* 2016a; Nahar *et al.,* 2016). In this study, it has been observed MG content increased significantly in all rice cultivars seedlings compared to control under salinity, drought and combine stress (Figure 19A). The higher MG content was recorded in BRRI dhan29 at 127.91%, 237.46% under salinity and combine stress respectively and in Koshihikari at 37.78% under drought stress; whereas lowest MG content found in BRRI dhan48 at 20.40%, 7.13% and 50.32% under salinity, drought and combine stress respectively compared to their control (Figure 19A). In contrast, we observed that Gly I and Gly II showed the divergent mode of activities in rice cultivars seedlings under all stress conditions. For BRRI dhan48 it has been found that both Gly I and Gly II activity increased in all stress conditions compared to control, thus the MG content was lowest in BRRI dhan48 than BRRI dhan29 and Koshihikari (19A, B, C).

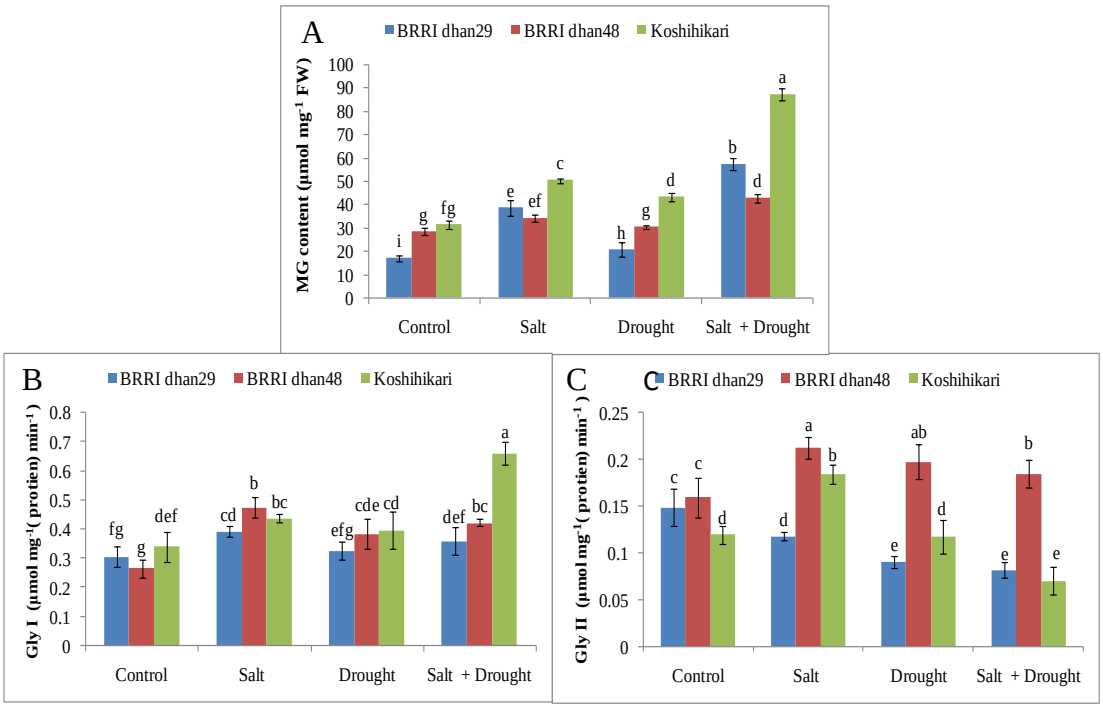

Figure 19. (A) MG content, (B) Gly I and (C) Gly II activities in rice leaves affected by salinity, drought and combined stress of salinity and drought at the early vegetative stage. Mean (±SD) was calculated from three replications for each treatment. Values in a column with different letters are significantly different at P≤0.05 applying the Fisher's LSD test ($LSD_{0.05}$= 3.52, 0.06 and 0.02 respectively). Here control, salt, drought and salt + drought indicate as 0 stress, 150 mM NaCl, 15% PEG and 150 mM NaCl + 15% PEG respectively.

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conduct at Laboratory of Plant Stress Responses of Kagawa University, Kagawa, Japan during the period of March-September, 2017 where we studied the morpho-physiological and biochemical responses of two rice cultivars (BRRI dhan29, BRRI dhan48 from indica and Koshihikari from japonica) as affected by salinity, drought and combined effect of salinity and drought at early vegetative stage (18 days old seedlings).

The experiment was arranged in a Completely Randomized Design (CRD) with three replications. 250 ml plastic pots (height 7 cm and diameter 6 cm) were used to facilitate the development of salinity, drought and combined effect of salinity and drought stresses. This experiment consisted of four treatments: control, salt (150 mM NaCl), drought (15% PEG) and salt + drought (150 mM NaCl + 15% PEG). There were 75 seedlings maintained for each pot.

The treatments were imposed to 18 days old seedlings for considering the early vegetative stage of rice cultivars and data were taken at the completion of stress duration. Total germination percentage, mortality rate, shoot-root length, shoot-root fresh and dry weight were measured for growth. The photosynthetic pigments content, RWC, Na^+ and K^+ homeostasis were measured as physiological parameters. Biochemical parameters included MDA content, H_2O_2, LOX, electrolyte leakage, Pro., AsA, DHA, GSH, GSSG, MG and antioxidant enzymes like APX, MDHAR, DHAR, GR, CAT, GPX, Gly I and Gly II activities.

Total germination (%) of rice reduced with severity of the stress. But Koshihikari showed better performance under salinity and drought stress, whereas BRRI dhan48 under combined stress. Mortality rate (%) of rice seedlings was remarkably reduced in combined stress for each variety compared with control. Shoot and root length of BRRI dhan29 and Koshihikari were reduced markedly at 8%, 10% and 26%, 24% respectively compared with control under combined stress. In contrast, higher reduction of root length was observed in BRRI dhan29 and BRRI dhan48 under salinity stress at 19% and 18% respectively. Moreover, under salinity, drought and combine stress DW of shoot and root were higher in BRRI dhan48 at 86.83%,

88.40%, 82.13% and 91.28%, 122.56%, 82.05% respectively compared with BRRI dhan29 and Koshihikari.

RWC was decreased by 24.3%, 14.6%, 44.2% in BRRI dhan29; 23.7%, 10.8%, 41.8% in BRRI dhan48; 41.2%, 8.7%, 55.3% in Koshihikari under salinity, drought and combined stress respectively compared with control seedlings. Although, compared with controls chlorophyll a (Chl *a*) content was recorded higher in BRRI dhan48 at 89.67%, 82.53% under salinity and in combine stress respectively than others varieties, Koshihikari showed a higher level of Chl *a* under drought stress. Moreover, carotenoids (Car) content was decreased in BRRI dhan29 and Koshikari under stress conditions except Koshihikari in drought stress did not change significantly. Higher level of Na^+ content increased the Na^+/ K^+ ratio both in shoot and root of all rice varieties under salinity and combine stress.

This study revealed salinity, drought and combined stress of salinity and drought disrupted antioxidant defense system through increased MDA content, H_2O_2, LOX, electrolyte leakage which reduced AsA content, AsA/DHA and GSH/GSSG ratio. Activities of APX, MDHAR, DHAR, GR, CAT, GPX and Pro. content were not increased as a similar trend in rice cultivars seedlings under salinity and drought stress. However, in combined stress, these enzymes activities and Pro content increased significantly in all rice cultivars seedlings, whereas BRRI dhan48 showed the highest enzymes activities and Pro. content compared with others. Additionally, MG content increased significantly in all rice cultivars seedlings under three stress treatments. The increased GSH content, upregulating Gly I and Gly II activities indicated to detoxify this raised content of cytotoxic MG in BRRI dhan48. In contrast, Gly II activity was not boosted in BRRI dhan29 and Koshihikari, thus these two rice varieties seedlings got severely damaged at three stress treatments. These higher activities of both Gly I and Gly II in BRRI dhan48 under all stress treatment can be a promising phenomenon to further investigation at Gly I and Gly II gene level to identify the stress tolerance mechanism of BRRI dhan48.

References

Abdallah, M.M.S., Abdelgawad, Z.A. and El-Bassiouny, H.M.S. (2016). Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties. *S. Afr. J. Bot*. **103**: 275–282.

Addinsoft. (2018). XLSTAT v. 2015: Data analysis and statistics software for Microsoft Excel. Addinsoft, Paris, France.

Ahmad, P. (2010). Growth and antioxidant responses in mustard (*Brassica juncea* L.) plants subjected to combined effect of gibberellic acid and salinity. *Arch. Agron. Soil Sci.* **56**: 575–588.

Ahmed, I. M., Nadira, U. A., Bibi, N., Zhang, G. and Wu, F. (2015). Tolerance to combined stress of drought and salinity in barley. In combined stresses in plants physiological, molecular, and biochemical aspects R. Mahalingam, (ed.). Springer, Basel, Switzerland. pp. 93–121.

Ahmed, I.M., Cao, F., Zhang, M., Chen, X., Zhang, G. and Wu, F. (2013a). Difference in yield and physiological features in response to drought and salinity combined stress during anthesis in Tibetan wild and cultivated barleys. *PloS ONE.* **8**(10): e77869.

Ahmed, I.M., Dai, H.X., Zheng, W., Cao, F.B., Zhang, G.P., Sun, D.F. and Wu, F.B. (2013b). Genotypic differences in physiological characteristics in the tolerance to drought and salinity combined stress between Tibetan wild and cultivated barley. *Plant Physiol. Biochem.* **63**: 49–60.

Akram, M.S. and Ashraf, M. (2011). Exogenous application of potassium dihydrogen phosphate can alleviate the adverse effects of salt stress on sunflower (*Helianthus annuus* L.). *J. Plant Nutr.* **34**: 1041–1057.

Al Hassan, M., Chaura, J., Donat-Torres, M.P., Boscaiu, M. and Vicente, O. (2017). Antioxidant responses under salinity and drought in three closely related wild monocots with different ecological optima. *AoB PLANTS*. **9**: plx009.

Alam, M.M., Nahar, K., Hasanuzzaman, M. and Fujita, M. (2014). Trehalose-induced drought stress tolerance: A comparative study among different Brassica species. PLANT OMICS. **7**(4): 271–283.

Ali, M.N., Ghosh, B., Gantait, S. and Chakraborty, S. (2014). Selection of rice genotypes for salinity tolerance through morpho-biochemical assessment. *Rice Sci*. **21**(5): 288−298.

Anjum, S.A., Xie, X., Wang, L., Saleem, M.F., Man, C. and Lei, W. (2011a). Morphological, physiological and biochemical responses of plants to drought stress: a review. *Afr. J. Agric. Res*. **6**: 2026–32.

Anjum, S.A., Wang, L.C., Farooq, M., Hussain, M., Xue, L.L. and Zou, C.M. (2011b). Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. *J. Agron. Crop Sci*. doi:10.1111/j.1439-037X.2010.00459.x

Anjum, S.A., Wang, L.C., Farooq, M., Khan, I. and Xue, L.L. (2011c). Methyl jasmonate-induced alteration in lipid peroxidation, antioxidative defense system and yield in soybean under drought. *J. Agron. Crop Sci.* doi:10.1111/j.1439-037X.2010.00468.x .

Anupama, A., Bhugra, S., Lall, B., Chaudhury, S. and Chugh, A. (2018). Assessing the correlation of genotypic and phenotypic responses of indica rice varieties under drought stress. *Plant Physiol. Biochem.* doi: 10.1016/j.plaphy.2018.04.001

Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Ann. Rev. Plant Biol.* **55**: 373–99.

Apostolova, P., Szalai, G., Kocsy, G., Janda, T. and Popova, L. P. (2011). Environmental factors affecting components of ascorbate-glutathione pathway in crop plants. In: Oxidative Stress in Plants: Causes, Consequences and Tolerance. N.A. Anjum, S. Umar and A. Ahmad, (ed.). I. K. International, New Delhi. pp. 52–70.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **24**: 1–15.

Ashfaq, M., Haider, M.S., Khan, A.S. and Allah, S.U. (2012). Breeding potential of the basmati rice germplasm under water stress condition. *Afr. J. Biotechnol.* **11**(25): 6647–6657.

Ashraf, M. and Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **59**: 206–16.

Ashraf, M. and O'leary, J. (1996). Responses of some newly developed salt-tolerant genotypes of spring wheat to salt stress: 1. yield components and ion distribution. *J. Agron. Crop Sci.* **176**:91–101.

Bangladesh Finance Bureau (2014). Agricultural Statistics. Ministry of Agriculture, Government of the People's Republic of Bangladesh.

Barrs, H.D. and Weatherley, P.E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* **15**: 413 – 428.

Basu, S., Ramegowda, V., Kumar, A., and Pereira, A. (2016). Plant adaptation to drought stress. F1000Research. 5:F1000 Faculty Rev–1554. doi: 10.12688/f1000research.7678.1

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil.* **39**: 205–207.

BBS (Bangladesh Bureau of Statistics) (2014). http://www.bbs.gov.bd

Bhattacharjee, S. and Dey, N. (2017). Redox metabolic and molecular parameters for screening drought tolerant indigenous aromatic rice cultivars. *Physiol. Mol. Biol. Plants.* doi:10.1007/s12298-017-0484-1

Bhusan, D., Das, D.K., Hossain, M., Murata, Y. and Hoque, M.A. (2016). Improvement of salt tolerance in rice (*Oryza sativa* L.) by increasing antioxidant defense systems using exogenous application of proline. *Aust. J. Crop Sci.* **10**(1): 50–56.

Borah, P., Sharma, E., Kaur, A., Chandel, G., Mohapatra, T., Kapoor, S. and Khurana, J. (2017). Analysis of drought-responsive signalling network in two

contrasting rice cultivars using transcriptome-based approach. *Sci. Rep.* doi:10.1038/srep42131

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**(1-2): 248–254.

Bunnag, S. and Pongthai, P. (2013). Selection of rice (*Oryza sativa* L.) cultivars tolerant to drought stress at the vegetative stage under field conditions. *Am. J. Plant Sci.* **4** (9): 1701–1708.

Choudhary, N.L., Sairam, R.K. and Tyagi, A. (2005). Expression of delta1-pyrroline-5-carboxylate synthetase gene during drought in rice (*Oryza sativa* L.). *Indian J. Biochem. Biophys.* **42**: 366–370.

Chunthaburee, S., Dongsansuk, A., Sanitchon, J., Pattanagul, W. and Theerakulpisut, P. (2016). Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. *Saudi J. Biol. Sci.* **23**(4): 467–477.

Curtiss, J., Rodriguez-Uribe, L., Stewart, J.M., Zhang, J. (2011). Identification of differentially expressed genes associated with semigamy in pima cotton (*Gossypium barbadense* L.) through comparative microarray analysis. *BMC Plant Biol.* **11**: 49.

DAE (2015). http://www.bbs.gov.bd/annual report (accessed on 4 November, 2017).

Das, P., Nutan, K.K., Singla-Pareek, S.L. and Pareek, A. (2015). Oxidative environment and redox homeostasis in plants: Dissecting out significant contribution of major cellular organelles. *Front. Environ. Sci.* **2**: 70.

Demidchik, V., Straltsova, D., Medvedev, S.S., Pozhvanov, G.A., Sokolik, A. and Yurin, V. (2014). Stress-induced electrolyte leakage: the role of K+-permeable channels and involvement in programmed cell death and metabolic adjustment. *J. Exp. Bot.* **65**: 1259–1270.

Dey, N.C., Alam, M.S., Sajjan, A.K., Bhuiyan, M.A., Ghose, L., Ibaraki, Y. and Karim, F. (2011). Assessing environmental and health impact of drought in the northwest Bangladesh. *J. Environ. Sci. Nat. Res.* **4**(2): 89–97.

Dinh, S.N., Sai, T.Z. T., Nawaz, G., Lee, K. and Kang, H. (2016). Abiotic stresses affect differently the intron splicing and expression of chloroplast genes in coffee plants (Coffea arabica) and rice (*Oryza sativa*). *J. Plant physiol*. **201**: 84–95.

Dionisio-Sese, M.L. and Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* **135**: 1–9.

Dixit, S., Singh, A., Sta Cruz, M.T., Maturan, P.T., Amante, M. and Kumar, A. (2014). Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genet.* **15**: 16. doi:10.1186/1471-2156-15–16

Doderer, A, Kokkelink, I., van der Veen, S., Valk, B.E, Schram, A.W. and Douma, A.C. (1992). Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochim. Biophys. Acta.* **1120**: 97–104.

dos Reis, S.P., Lima, A.M. and de Souza, C.R. (2012). Recent molecular advances on downstream plant responses to abiotic stress. *Int. J. Mol. Sci.* **13**: 8628–8647.

Dubey, R. (1997). Photosynthesis in plants under stressful conditions. *Handb. Photosynth.* **2**: 717–37.

Eckardt, N.A. (2009). A new chlorophyll degradation pathway. *Plant Cell.* **21**: 700. doi:10.1105/tpc.109.210313

Elia, A.C., Galarini, R., Taticchi, M.I., Dorr, A.J.M. and Mantilacci, L. (2003). Antioxidant responses and bioaccumulation in Ictalurus melas under mercury exposure. *Ecotoxicol. Environ. Saf.* **55**(2): 162–167.

Eltayeb, A.E., Kawano, N., Badawi, G.H., Kaminaka, H., Sanekata, T., Shibahara, T., Inanaga, S. and Tanaka, K. (2007). Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. *Planta.* **225**: 1255–1264.

Estrada-Campuzano, G., Miralles, D.J. and Slafer, G.A. (2008). Genotypic variability and response to water stress of pre- and post-anthesis phases in triticale. *Eur. J. Agron.* **28**(3): 171–177.

FAO (2014). http://www.fao.org/faostat/en/#data/QC (accessed on 16 November, 2017).

FAO (2015). www.fao.org/docrep/003/x6905e/x6905e04.htm (accessed on 4 December, 2017).

FAOStat (Food And Agricultural Organization of The United Nations) (2017). http://www.fao.org/economic/est/publications/rice-publications/rice-market-monitor-rmm (accessed on 20 October, 2017).

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management: a review. *Agron. Sustain. Dev.* **29**: 185-212.

Feng, F.J., Xu, X.Y., Du, X.B., Tong, H.H., Luo, L.J. and Mei, H.W. (2012). Assessment of drought resistance among wild rice accessions using a protocol based on single-tiller propagation and PVC-tube cultivation. *Aust. J. Crop Sci.* **6**: 1205–1211.

Filippou, P., Antoniou, C. and Fotopoulos, V. (2011). Effect of drought and rewatering on the cellular status and antioxidant response of *Medicago truncatula* plants. *Plant Signal. Behav.* **6**: 2270–277.

Foyer, C.H. and Noctor, G. (2011). Ascorbate and glutathione: the heart of the redox hub. *Plant Physiol.* **155**: 2–18.

Foyer, C.H. and Noctor, G. (2016). Stress-triggered redox signalling: what's in pROSpect? *Plant, Cell Environ.* **39**: 951–964.

Frouin, J., Languillaume, A., Mas, J., Mieulet, D., Boisnard, A., Labeyrie, A., Bettembourg, M., Burea, C., Lorenzini, E., Portefaix, M., Turquay, P., Vernet, A., Périn, C., Ahmadi, N., Courtois, B. (2018). Tolerance to mild salinity stress in japonica rice: A genome-wide association mapping study highlights calcium signaling and metabolism genes. *PLoS ONE*. **13**(1): e0190964. doi:10.1371/journal.pone.0190964

Garg, B.K. (2003). Nutrient uptake and management under drought: nutrient-moisture interaction. *Curr. Agric.* **27**: 1–8.

Gholami, M., Mokhtarian, F. and Baninasab, B. (2015). Seed halopriming improves the germination performance of black seed (*Nigella sativa*) under salinity stress conditions. *J. Crop Sci. Biotech.* **18**(1): 21–26.

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **48**(12): 909–930.

Girma, B.T., Ali, H.M. and Gebeyaneh, A.A. (2017). Effect of salinity on final growth stage of different rice (*Oryza sativa* L.) genotypes. *Asian J. Agri. Res.* **11**: 1–9.

Gowda, V.R.P., Henry, A., Yamauchi, A., Shashidhar, H.E. and Serraj, R. (2011). Root biology and genetic improvement for drought avoidance in rice. *Field Crops Res.* **122**(1): 1–13.

Grossman, A. and Takahashi, H. (2001). Macronutrient utilization by photosynthetic eukaryotes and the fabric of interactions. *Ann. Rev. Plant Biol.* **52**: 163–210.

Hairmansis, A., Berger, B., Tester, M. and Roy, S.J. (2014). Image-based phenotyping for non-destructive screening of different salinity tolerance traits in rice. *Rice.* **7**: 16. doi:10.1186/s12284-014-0016-3

Hakim, M.A., Juraimi, A.S. and Hanafi, M.M. (2014). The effect of salinity on growth, ion accumulation anr yield of rice varieties. *J. Anim. Plant Sci.* **24**: 874–885.

Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol.* **141**: 312–322.

Harris, B.N., Sadras, V.O. and Tester, M. (2010). A water-centred framework to assess the effects of salinity on the growth and yield of wheat and barley. *Plant Soil.* **336**: 377–89.

Hasanuzzaman, M. and Fujita, M. (2011). Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers

enhanced tolerance to drought stress in rapeseed seedlings. *Biol. Trace Elem. Res.* **143**: 1758–1776.

Hasanuzzaman, M., Hossain, M.A. and Fujita, M. (2011a). Selenium-induced upregulation of the antioxidant defense and methylglyoxal detoxification system reduces salinity-induced damage in rapeseed seedlings. *Biol. Trace Elem. Res.* **143**(3): 1704–1721.

Hasanuzzaman, M., Hossain, M.A. and Fujita, M. (2011b). Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. *Plant Biotechnol. Rep.* **5**(4): 353–365.

Hasanuzzaman, M., Davies, N.W., Shabala, L., Zhou, M., Brodribb, T.J. and Shabala, S. (2017). Residual transpiration as a component of salinity stress tolerance mechanism: a case study for barley. *BMC Plant Biol.* **17**: 107.

Hasanuzzaman, M., Hossain, M.A., da Silva, J.A.T. and Fujita, M. (2012a). Plant response and tolerance to abiotic oxidative stress: Antioxidant defense is a key factor. In: Crop stress and its management: Perspectives and strategies. B. Venkateswarlu, A.K. Shanker, C. Shanker and M. Maheswari, (ed.). Springer, Dordrecht. pp. 261–315.

Hasanuzzaman, M., Nahar, K., Alam, M.M. and Fujita, M. (2012b). Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by modulating the antioxidant defense and glyoxalase system. *Aust. J. Crop Sci.* **6**: 1314–132.

Hasanuzzaman, M., Nahar, K., Fujita, M., Ahmad, P., Chandna, R., Prasad, M.N.V. and Öztürk, M. (2013a). Enhancing plant productivity under salt stress—relevance of poly-omics. In: Salt stress in plants: Omics, signaling and responses. P. Ahmad, M. M. Azooz, and M.N.V Prasad, (ed.). Springer, Berlin, Germany. pp. 113–156.

Hasanuzzaman, M., Nahar, K. and Fujita, M. (2013b). Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ecophysiology and responses of plants under salt stress. P. Ahmad, M. M. Azooz, and M.N.V Prasad, (ed.). Springer, New York. pp. 25–87.

Hasanuzzaman, M., Alam, M.M., Rahman, A., Hasanuzzaman, M., Nahar, K. and Fujita, M. (2014a). Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *Biomed. Res. Int.* 2014: 757219.

Hasanuzzaman, M., Nahar, K., Alam, M.M. and Fujita, M. (2014b). Modulation of antioxidant machinery and methlglyoxal detoxification system in selenium-supplemented *Brassica napus* seedlings confers tolerance to high temperature stress. *Biol. Trace. Elem. Res.* **161**: 297–307.

Hasanuzzaman, M., Nahar, K., Hossain, M.S., Mahmud, J.A., Rahman, A., Inafuku, M., Oku, H. and Fujita, M. (2017a). Coordinated actions of glyoxalase and antioxidant defense systems in conferring abiotic stress tolerance in plants. *Int. J. Mol. Sci.* **18**(1): 200. doi:10.3390/ijms18010200

Hasanuzzaman, M., Nahar, K., Hossain, M.S., Anee, T.I., Parvin, K. and Fujita, M. (2017b). Nitric oxide pretreatment enhances antioxidant defense and glyoxalase systems to confer PEG-induced oxidative stress in rapeseed. *J. Plant Interact.* **12**: 1, 323–331.

Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125**: 189–198.

Henry, A., Cal, A.J., Batoto, T.C., Torres, R.O. and Serraj, R. (2012). Root attributes affecting water uptake of rice (*Oryza sativa*) under drought. *J. Exp. Bot.* **63**(13): 4751–4763.

Hoang, T., Tran, T., Nguyen, T., Williams, B., Wurm, P., Bellairs, S., Mundree, S. (2016). Improvement of salinity stress tolerance in rice: challenges and opportunities. *Agronomy*. **6**: 54.

Hossain, M.A., Teixeira da Silva, J.A., and Fujita, M. (2011a). Glyoxalase system and reactive oxygen species detoxification system in plant abiotic stress response and tolerance: an intimate relationship In: Abiotic Stress in Plants-Mechanisms and Adaptations. A. Shanker and B. Venkateswarlu, (ed.). INTECH-Open Access Publisher, pp. 235–266.

Hossain, M.A., Hasanuzzaman, M. and Fujita, M. (2011b). Coordinate induction of antioxidant defense and glyoxalase system by exogenous proline and glycinebetaine is correlated with salt tolerance in mung bean. *Front. Agric. China.* **5**: 1–14.

Hossain, M.A., Nakano, Y. and Asada, K. (1984). Monodehydroascorbate reductase in spinach chloroplasts and its participation in the regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiol*. **25**(3): 385–395.

Hossain, M.S., Alam, M.U., Rahman, A., Hasanuzzaman, M., Nahar, K., Mahmud, J.A. and Fujita, M. (2017). Use of iso-osmotic solution to understand salt stress responses in lentil (*Lens culinaris* Medik.). *S. Afr. J. Bot.* **113**: 346–354.

Huang, C., He, W., Guo, J., Chang, X., Su, P. and Zhang, L. (2005). Increased sensitivity to salt stress in an ascorbate-deficient *Arabidopsis* mutant. *J. Exp. Bot.* **56**(422): 3041–3049.

Hussain, B.M.N., Akram, S., Raffi, S.A., Burritt, D.J. and Hossain, M.A. (2016). Exogenous glutathione improves salinity stress tolerance in rice (*Oryza sativa* L.), *Plant Gene and Trait.* **7**(11): 1–17.

Hussain, M., Malik, M.A., Farooq, M., Ashraf, M.Y. and Cheema, M.A. (2008). Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* **194**: 193–199.

Hussain, S.G. (2012). Role of Rice in Food Security of Bangladesh (Chapter 5). In: Food Security: Quality Management, Issues and Economic Implications.M.A. Jones and F.E. Hernandez, (ed.). Nova Science Publishers. pp. 127–148.

Hu, Y. and Schmidhalter, U. (2005). Drought and salinity: A comparison of their effects on mineral nutrition of plants: an review. *J. Plant Nutr. Soil Sci.* **168**: 541–549.

IRRI (2016). Climate change-ready rice, http://irri.org/our-impact/tackling-climatechange/ developing-drought-tolerant-rice (accessed 17 November 2017).

Ishak, N.K., Sulaiman, Z. and Tennakoon, K.U. (2015). Comparative study on growth performance of transgenic (over-expressed OsNHX1) and wild-type Nipponbare under different salinity Regimes. *Rice Sci.* **22**(6): 275–282.

ISTA. (2003). International Seed Testing Association, ISTA Handbook on Seedling Evaluation, 3rd ed.

Jayaweera, J.K.P.T.P., Herath, H.M.V.G., Jayatilake, D.V., Udumulla, G.S. and Wickramasinghe, H.A.M. (2016). Physiological, biochemical and proteomic responses of rce (*Oryza sativa* L.) varieties godaheenati and pokkali for drought stress at the seedling stage. *Tropical Agricultural Research.* **27** (2): 159– 170.

Jini, D. and Joseph, B. (2017). Physiological mechanism of salicylic acid for alleviation of salt stress in rice. *Rice Sci.* **24**(2): 97–108.

Kadhimi, A.A., Zain, C.R.C.M., Alhasnawi, A.N., Isahak, A., Ashraf, M.F., Mohamad, A., Doni, F. and Yusoff, W.M.W. (2016). Effect of irradiation and polyethylene glycol on drought tolerance of MR269 genotype rice (*Oryza sativa* L.). *Asian J. Crop Sci.* **8**: 52–59.

Kannan, N.D. and Kulandaivelu, G. (2011). Drought induced changes in physiological, biochemical and phytochemical properties of *Withania somnifera* Dun. *J. Med. Plants Res.* **5**: 3929–3935.

Kaur, C., Singla-Pareek, S.L. and Sopory, S.K. (2014). Glyoxalase and methylglyoxal as biomarkers for plant stress tolerance. *Crit. Rev. Plant Sci.* **33**: 429–456.

Kavitha, P.G., Miller, A.J., Mathew, M.K. and Maathuis, F.J.M. (2012). Rice cultivars with differing salt tolerance contain similar cation channels in their root cells. *J. Exp. Bot.* **63**(8): 3289–3296.

Kellogg, E.A., Camara, P.E.A.S., Rudall, P.J., Ladd, P., Malcomber, S.T., Whipple, C.J. and Doust, A.N. (2013). Early inflorescence development in the grasses (Poaceae). *Front. Plant Sci.* **4**: 250.

Khan, M.A., Shirazi, M.U., Khan, M.A., Mujtaba, S.A.I., Islam, E., Mumtaz, S., Shereen, A., Ansari, R. and Ashraf, M.Y. (2009). Role of proline, K/Na

ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pak. J. Bot.* **41**: 633–638.

Khan, M.B., Hussain, M., Raza, A., Farooq, S. and Jabran, K. (2015). Seed priming with CaCl2 and ridge planting for improved drought resistance in maize. *Turk. J. Agric. For.* **39**: 193–203.

Kibria, M.G., Hossain, M., Murata, Y., and Hoque, M.A. (2017). Antioxidant defense mechanisms of salinity tolerance in rice genotypes. *Rice Sci.* **24**(3): 155–162.

Krishnamurthy, P., Ranathunge, K., Franke, R., Prakash, H.S., Schreiber, L. and Mathew, M.K. (2009). The role of root apoplastic transport barriers in salt tolerance of rice (Oryza sativa L.). *Planta.* **230**(1): 119–134.

Lee, G., Duncan, R.R. and Carrow, R.N. (2004). Salinity tolerance of seashore paspalum ecotypes: shoot growth responses and criteria. *Hort. Sci.* **39**: 1138–42.

Levy, D., Coleman, W.K., and Veilleux, R.E. (2013). Adaptation of potato to water shortage: irrigation management and enhancement of tolerance to drought and salinity. *Am. J. Potato Res.* **90**: 1–21.

Li, J.M. and Jin, H. (2007). Regulation of brassinosteroid signaling. *Trends Plant Sci.* **12**: 37–41.

Li, T., Zhang, Y., Liu, H., Wu, Y., Li, W. and Zhang, H. (2010). Stable expression of Arabidopsis vacuolar Na^+/H^+ antiporter gene AtNHX1 and salt tolerance in transgenic soybean for over six generations. *Chinese Sci. Bull.* **55**: 1127–1134.

Li, X., Guo, Z., Lv, Y., Cen, X., Ding, X., Wu, H., Li, X., Huang, J. and Xiong, L. (2017). Genetic control of the root system in rice under normal and drought stress conditions by genome-wide association study. *PLoS Genet.* **13**(7): e1006889.

Lima, J.M., Nath, M., Dokku, P., Raman, K.V., Kulkarni, K.P., Vishwakarma, C., Sahoo, S.P., Mohapatra, U.B., Mithra, S.V.A., Chinnusamy, V., Robin, S., Sarla, N., Seshashayee, M., Singh, K., Singh, A.K., Singh, N.K., Sharma,

R.P. and Mohapatra, T. (2015). Physiological, anatomical and transcriptional alterations in a rice mutant leading to enhanced water stress tolerance. *AoB PLANTS*. **7**: plv023. doi:10.1093/aobpla/plv023

Lisenbee, C.S., Lingard, M.J. and Trelease, R.N. (2005). Arabidopsis peroxisomes possess functionally redundant membrane and matrix isoforms of monodehydroascorbate reductase. *Plant J.* **43**: 900–914.

Liu, L., Xia, W., Li, H., Zeng, H., Wei, B., Han, S. and Yin, C. (2018). Salinity inhibits rice seed germination by reducing α-amylase activity via decreased bioactive gibberellin content. *Front. Plant Sci.* **9**: 275. doi:10.3389/fpls.2018.00275

Ma, L.J., Wang, L.L., Mei, Y.X., Zhang, S.W., Wei, W., Wang, J.Y. and Zhang, Y.L. (2016). Cross adaptation tolerance in rice seedlings exposed to PEG induced salinity and drought stress. *Int. J. Agric. Biol.* **18**: 535–541.

Maathuis, F.J. (2006). The role of monovalent cation transporters in plant responses to salinity. *J. Exp. Bot.* **57**: 1137–47.

Mahlooji, M., Sharifi, R.S., Razmjoo, J., Sabzalian, M.R. and Sedghi, M. (2017). Effect of salt stress on photosynthesis and physiological parameters of three contrasting barley genotypes. *Photosynthetica.* doi:10.1007/s11099-017-0699-y

Martínez, J.P. and Araya, H. (2010). Ascorbate–glutathione cycle: enzymatic and non-enzymatic integrated mechanisms and its biomolecular regulation. In: Ascorbate glutathione pathway and stress tolerance in plants. N.A. Anjum, M.T. Chan, and S. Umar, (ed.). Springer, Dordrecht. pp. 303–322.

Mauad, M., Crusciol, C.A.C. Grassi Filho, H. (2011). Production of dry mass and nutrition of rice cultivars of upland areas under water deficit and fertilization conditions silicate. Semina: Agrarian Sciences, Londrina. **32**: 939–948.

May, M.J., Vernoux, T., Leaver, C., Van Montagu, M. and Inze, D. (1998). Glutathione homeostasis in plants: implications for environmental sensing and plant development. *J. Exp. Bot.* **49**: 649–667.

McDowell, N.G., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. and Yepez, E.A. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.* **178**: 719–739.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S. and Mittler, R. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.* **33**: 453–467.

Mishra, P., Bhoomika, K. and Dubey, R.S. (2013). Differential responses of antioxidative defense system to prolonged salinity stress in salt-tolerant and salt-sensitive indica rice (*Oryza sativa* L.) seedlings. *Protoplasma.* **250**: 3–19.

Mishra, S. S. and Panda, D. (2017). Leaf traits and antioxidant defense for drought tolerance during early growth stage in some popular traditional rice landraces from Koraput, India. *Rice Sci.* **24**(4): 207-217.

Mittova, V., Tal, M., Volokita, M. and Guy, M. (2003). Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to saltinduced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii. Plant Cell Environ.* **26**: 845–856.

Mohanty, S., Wassmann, R., Nelson, A., Moya, P. and Jagadish, S.V.K. (2013). Rice and climate change: Significance for food security and vulnerability. IRRI Discussion Paper Series No. 49. Los Baños.

Molazem, D., Qubanov, E. M. and Dunyamaliyev, S.A. (2010). Role of proline, Na and chlorophyll content in salt tolerance of corn (*Zea mays* L.). *American-Eurasian J. Agric. Environ. Sci.* **9** (3): 319–324.

Monkham, T., Jongdee, B., Pantuwan, G., Sanitchon, J., Mitchell, J.H. and Fukai, S. (2015). Genotypic variation in grain yield and flowering pattern in terminal and intermittent drought screening methods in rainfed lowl and rice. *Field Crops Res.* **175**: 26–36.

Moradi, F., Ismail, A.M., Gregorio, G. and Egdane, J. (2003). Salinity tolerance of rice during reproductive development and association with tolerance at seedling stage. *Indian J. Plant Physiol.* **8**: 105–116.

Mostofa, M.G., Hossain, M.A. and Fujita, M. (2015). Trehalose pretreatment induces salt tolerance in rice seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems. *Protoplsasma.* **252**: 461–475.

Mujtaba, S.M., Faisal, S., Khan, M.A., Mumtaz, S. and Khanzada, B. (2016). Physiological studies on six wheat (*Triticum aestivum* L.) genotypes for drought stress tolerance at seedling stage. *Agri. Res. Tech.* **1**(2): ARTOAJ.MS.ID.55559

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant Cell Environ.* **25**: 239–250.

Munns, R. (2005). Genes and salt tolerance: bringing them together. *New Phytol.* **167**:645–663.

Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **59**: 651–681.

Muranaka, S., Shimizu, K., Kato, M. (2002). A salt-tolerant cultivar of wheat maintains photosynthetic activity by suppressing sodium uptake. *Photosynthetica.* **40**: 509–515.

Nahar, K., Hasanuzzaman, M., Alam, M.M. and Fujita, M. (2015a). Roles of exogenous glutathione in antioxidant defense system and methylglyoxal detoxification during salt stress in mung bean. *Biol. Plant.* **59**: 745–756.

Nahar, K., Hasanuzzaman, M., Alam, M.M. and Fujita, M. (2015b). Glutathione-induced drought stress tolerance in mung bean: coordinated roles of the antioxidant defence and methylglyoxal detoxification systems. *AoB Plants.* **7**: plv069. doi; 10.1093/aobpla/plv069

Nahar, K., Hasanuzzaman, M., Rahman, A., Alam, M.M., Mahmud, J. A., Suzuki, T. and Fujita, M. (2016). Polyamines confer salt tolerance in mung bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis,

antioxidant defense, and methylglyoxal detoxification systems. *Front. Plant Sci.* **7**: 1104.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbatespecific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **22**(5): 867–880.

Negrão, S., Schmöckel, S.M. and Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Ann. Bot.* **119**: 1–11.

Nio, S., Cawthray, G., Wade, L. and Colmer, T. (2011). Pattern of solutes accumulated during leaf osmotic adjustment as related to duration of water deficit for wheat at the reproductive stage. *Plant Physiol. Biochem.* **49**: 1126–37.

Noctor, G. and Foyer, C.H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **49**: 249–279.

Nounjan, N., Nghia, P.T. and Theerakulpisut, P. (2012). Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. *J. Plant Physiol.* **169**(6): 596–604.

Nxele, X., Klein, A. and Ndimba, B.K. (2017). Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. *S. Afr. J. Bot.* **108**: 261–266.

O'Toole, T.C. (2004). Rice water: The final frintier First International Conference on Rice for the Future. Aug. 31–Sept. 2, Bangko, Thailand.

Pande, A. and Arora, S. (2017). Molecular strategies for development of abiotic stress tolerance in plants. *Cell Cellular Lif. Sci. J.* **2**(2): 000112.

Pandey, S., Fartyal, D., Agarwal, A., Shukla, T., James, D., Kaul, T., Negi, Y.K., Arora, S. and Reddy, M.K. (2017). Abiotic stress tolerance in plants: Myriad roles of ascorbate peroxidase. *Front. Plant Sci.* **8**: 581.

Pandey, V. and Shukla, A. (2015). Acclimation and tolerance of rice under drought stress. *Rice Sci.* **22**(4): 147-161.

Pang, C.H. and Wang, B.S. (2010). Role of ascorbate peroxidase and glutathione reductase in Ascorbate–Glutathione cycle and stress tolerance in plants. In: Ascorbate-Glutathione pathway and stress tolerance in plants. N.A. Anjum, M.T. Chan and S. Umar, (ed.). Springer, Dordrecht. pp. 91–112.

Panuccio, M.R., Jacobsen, S.E., Akhtar, S.S. and Muscolo, A. (2014). Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. *AoB PLANTS.* **6**: plu047. doi:10.1093/aobpla/plu047

Paradiso, A., Berardino, R., de Pinto, M., di Toppi, L., Storelli, S. and de Gara, F.T. (2008). Increase in ascorbate-glutathione metabolism as local and precocious systemic responses induced by cadmium in durum wheat plants. *Plant Cell Physiol.* **49**: 362–374.

Perveen, S., Shahbaz, M. and Ashraf, M. (2010). Regulation in gas exchange and quantum yield of photosystem II (PSII) in salt stressed and non-stressed wheat plants raised from seed treated with triacontanol. *Pak. J. Bot.* **42**: 3073–3081.

Peterson, C.A. (1988). Exoderrnal casparian bands: Their significance for ion uptake by toots. *Physiol. Plant.* **72**(1): 204–208.

Peuke, A. and Rennenberg, H. (2004). Carbon, nitrogen, phosphorus, and sulphur concentration and partitioning in beech ecotypes (*Fagus sylvatica* L.): phosphorus most affected by drought. *Trees*. **18**: 639–48.

Peuke, A.D. and Rennenberg, H. (2011). Impacts of drought on mineral macro- and microelements in provenances of beech (*Fagus sylvatica* L.) seedlings. *Tree Physiol.* **31**(2): 196–207.

Plaut, Z. (2003). Plant exposure to water stress during specific growth stages. Encyclopedia of Water Science. CRC Press. New York. pp. 673–675.

Principato, G.B., Rosi, G., Talesa, V., Govannini, E. and Uolila, L. (1987). Purification and characterization of two forms of glyoxalase II from rat liver and brain of Wistar rats. *Biochem. Biophys. Acta.* **911**: 349–355.

Rahman, A., Nahar, K., Hasanuzzaman, M. and Fujita, M. (2016a). Calcium supplementation improves Na^+/K^+ ratio, antioxidant defense and glyoxalase

systems in salt-stressed rice seedlings. *Front. Plant Sci.* **7**. doi:10.3389/fpls.2016.00609

Rahman, A., Hossain, M.S., Mahmud, J., Nahar, K., Hasanuzzaman, M. and Fujita, M. (2016b). Manganese-induced salt stress tolerance in rice seedlings: Regulation of ion homeostasis, antioxidant defense and glyoxalase systems. *Physiol. Mol. Biol. Plants.* doi:10.1007/s12298-016-0371-1

Ray, S., Sarkar, M.A.R., Paul, S.K., Islam, A.K.M.M. and Yeasmin, S. (2015). Variation of growth, yield and protein content of transplant aman rice by three agronomic practices. *Agric. Biol. Sci. J.* **1**(4): 167–176.

Reddy, I.N.B.L., Kim, B-K., Yoon, I-S., Kim, K. and Kwon, T. (2017). Salt tolerance in rice: Focus on mechanisms and approaches. *Rice Sci.* **24**(3): 123–144.

Romero-Puertas, M.C., Corpas, F.J., Sandalio, L.M., Leterrier, M., Rodriguez-Serrano, M., del Rio, L.A. and Palma, J.M. (2006). Glutathione reductase from pea leaves: response to abiotic stress and characterization of the peroxisomal isozyme. *New Phytol.* **170**: 43–52.

Rostami, L., Sadeghi, H. and Hosseini, S. (2016). Response of caper plant to drought and different ratios of calcium and sodium chloride. *Planta Daninha*. **34**: 259–266.

Sabir, P., Ashraf, M., Hussain, M. and Jamil, A. (2009). Relationship of photosynthetic pigments and water relations with salt tolerance of proso millet (*Panicum miliaceum* L.) accessions. *Pak. J. Bot.* **41**: 2957–2964.

Sairam, R. and Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.* Bangalore. **86**: 407–21.

Samarah, N.H. (2005). Effects of drought stress on growth and yield of barley. *Agron. Sustain.* Dev. **25**: 145–149.

Sànchez-Casas, P. and Klessig, D.F. (1994). A salicylic acid-binding activity and a salicylic acid-inhibitable catalase activity are present in a variety of plant species. *Plant Physiol.* **106**: 1675–1679.

Scheres, B. and Van der Putten, W.H. (2017). The plant perception connects environment to development. *Nature.* **543**: 337–345.

Shabala, S., Cuin, T.A., and Pottosin, I. (2007). Polyamines prevent NaCl-induced K^+ efflux from pea mesophyll by blocking non-selective cation channels. *FEBS Lett.* **581**: 1993–1999.

Sharifi, P. and Mohammadkhani, N. (2016). Effects of drought stress on photosynthesis factors in wheat genotypes during anthesis. *Cereal Res. Commun.* **44**(2): 229–239.

Sharma, S., Villamor, J.G. and Verslues, P.E. (2011). Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. *Plant Physiol.* **157**: 292–304.

Shelley, I., Takahashi-Nosaka, M., Kano-Nakata, M., Haque, M. and Inukai, Y. (2016). Rice cultivation in Bangladesh: present scenario, problems, and prospects: a review. *J. Intl. Cooper. Agric. Dev.* **14**: 20–29.

Siddique, M.R.B., Hamid, A. and Islam, M.S. (2001). Drought stress effects on water relations of wheat. *Bot. Bull. Acad. Sin.* **41**: 35–39.

Silva, T.R. da, Cazetta, J.O., Carlin, S.D. and Telles, B.R. (2017). Drought-induced alterations in the uptake of nitrogen, phosphorus and potassium, and the relation with drought tolerance in sugar cane. *Sci. Agrotec.* **41**(2): 117–127.

Silva-Ortega, C.O., Ochoa-Alfaro, A.E., Reyes-Aguero, J.A., Aguado-Santacruz, G.A. and Jimenez-Bremon, J.F. (2008). Salt stress increases the expression of p5cs gene and induces proline accumulation in cactus pear. *Plant Physiol. Biochem.* **46**: 82–92.

Singh Grewal, H. (2010). Response of wheat to subsoil salinity and temporary water stress at different stages of the reproductive phase. *Plant Soil.* **330**: 103–113.

Singh, R.K, Gregorio, G.B. and Ismail, A.M. (2008). Breeding rice varieties with tolerance to salt stress. *J. Ind. Soc. Coastal Agric. Res.* **26**:16–21.

Smirnoff, N. and Cumbes, Q.J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry.* **28**: 1057–60.

Solařová, E., Holková, L., Bradáčová, M. and Smutná, P. (2016). Osmotic adjustment and activity of stress-related genes in wheats of different origin exposed to water stress. *Russ. J. Plant Physiol.* **63**: 532–541.

SRDI. (2010). Saline Soils of Bangladesh. Ministry of Agriculture, Bangladesh: 1–60.

Sun, C., Liu, L., Yu, Y., Liu, W., Lu, L., Jin, C. and Lin, X. (2015). Nitric oxide alleviates aluminum-induced oxidative damage through regulating the ascorbate-glutathione cycle in roots of wheat. *J. Integr. Plant Biol.* **57**: 550–561.

Swapna, S. and Shylaraj, K.S. (2017). Screening for osmotic stress responses in rice varieties under drought condition. *Rice Sci.* **24**(5): 253–263.

Szabados, L. and Savouré, A. (2010). Proline: a multifunctional aminoacid. *Trends Plant Sci.* **15**: 89–97.

Taiz, L. and Zeiger, E. (2006). Plant Physiology. Fourth Edition. Sinauer Associates: Los Angeles.

Tavakkoli, E., Fatehi, F., Coventry, S., Rengasamy, P. and McDonald, G.K. (2011). Additive effects of Na^+ and Cl^- ions on barley growth under salinity stress. *J. Exp. Bot.* **62**: 2189–2203.

Tester, M. and Davenport, R. (2003). Na^+ tolerance and Na^+ transport in higher plants: A review. *Ann. Bot.* **91**: 503–527.

Thordal-Christensen, H., Zhang, Z., Wei, Y. and Collinge, D.B. (1997). Subcellular localization of H_2O_2 in plants, H_2O_2 accumulation in papillae and hypersensitive response during barley powdery mildew interaction. *Plant J.* **11**: 1187-1194.

Torres, R.O. and Henry, A. (2016). Yield stability of selected rice breeding lines and donors across conditions of mild to moderately severe drought stress. *Field Crops Res.* doi:10.1016/j.fcr.2016.09.011

Ueda, A., Yahagi, H., Fujikawa, Y., Nagaoka, T., Esaka, M., Calcaño, M., González, M.M., Martich, J.D.H., Saneoka, H. (2013). Comparative physiological analysis of salinity tolerance in rice. *Soil Sci. Plant Nutri.* **59**(6): 896–903.

Umali, D.L. (1993). Irrigation-Induced Salinity a Growing Problem for Development and the Environment; The Word Bank, Washington, DC, USA.

United States Department of Agriculture (USDA). (2016). Bibliography on Salt Tolerance. Fibres, Grains and Special Crops; Brown, G.E., Jr., Ed.; Salinity Laboratory United States Department of Agriculture Research Service: Riverside, CA, USA. Available online: http://www.Ars.Usda.Gov/services/docs.Htm?Docid=8908 (accessed on 20 August 2016).

Vighi, I.L, Benitez, L.C, Amaral, M.N., Moraes, G.P., Auler, P.A., Rodrigues, G.S., Deuner, S., Maia, L.C. and Braga, E.J.B. (2017). Functional characterization of the antioxidant enzymes in rice plants exposed to salinity stress. *Biol. Plant.* **61**(3): 540–550.

Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R.K., Kumar, V., Verma, R., Upadhyay, R.G., Pandey, M. and Sharma, S. (2017). Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. *Front. Plant Sci.* doi:10.3389/fpls.2017.00161

Wahid, A. and Rasul, E. (2005). Photosynthesis in leaf, stem, flower and fruit. In: Pessarakli, M. (ed.). Handbook of Photosynthesis, 2nd ed., CRC Press, Florida. pp. 479–497.

Wang, H., Zhang, M.S., Guo, R., Shi, D.C., Liu, B., Lin, X.Y. and Yang, C.W. (2012). Effects of salt stress on ion balance and nitrogen metabolism of old and young leaves in rice (*Oryz sativa* L.). *BMC Plant Biol.* **12**: 194.

Wang, X., Wang, W., Huang, J., Peng S. and Xiong, D. (2017). Diffusional conductance to CO_2 is the key limitation to photosynthesis in salt-stressed leaves of rice (*Oryza sativa*). *Physiol. Plant.* doi:10.1111/ppl.12653

Wassmann, R., Jagadish, S.V.K., Heuer, S., Ismail, A., Redona, E., Serraj, R., Singh, R.K., Howell, G., Pathak, H. and Sumfleth, K. (2009). Climate change affecting rice production: The physiological and agronomic basis for possible adaptation strategies. *Adv. Agron*. **101**: 59–122.

Wei, J., Li, C., Li, Y., Jiang, G. and Cheng, G. (2013). Effects of external potassium (K) supply on drought tolerances of two contrasting winter wheat cultivars. *PLoS ONE*. **8**(7): e69737. doi:10.1371/journal.pone.0069737

Wei, X., Wang, R., Cao, L., Yuan, N., Huang, J., Qiao, W., Zhang, W., Zeng, H. and Yang, Q. (2012). Origin of Oryza sativa in China inferred by nucleotide polymorphisms of organell DNA. *PLoS ONE.* **7**(11): e49546.

Wild, R., Ooi, L., Srikanth, V. and Münch, G. (2012). A quick, convenient and economical method for the reliable determination of methylglyoxal in millimolar concentrations: the N-acetyl-L-cysteine assay. *Anal. Bioanal. Chem.* **403**: 2577–2581.

Wu, D., Cai, S., Chen, M., Ye, L., Chen, Z., Zhang, H., Dai, F., Wu, F.B., and Zhang, G.P. (2013). Tissue metabolic responses to salt stress in wild and cultivated barley. *PLoS ONE*. **8**: e55431.

Wu, H., Shabala, L., Liu, X., Azzarello, E., Zhou, M., Pandolfi, C., Chen, Z-H., Bose. J., Mancuso, S. and Shabala, S. (2015). Linking salinity stress tolerance with tissue specific Na^+ sequestration in wheat roots. *Front. Plant Sci.* **6**: 71. doi:10.3389/fpls.2015.00071

Xia, L.J., Yang, L.Q., Sun, N.L., Li, J., Fang, Y.J. and Wang, Y.P. (2016). Physiological and antioxidant enzyme gene expression analysis reveals the improved tolerance to drought stress of the somatic hybrid offspring of *Brassica napus* and *Sinapis alba* at vegetative stage. *Acta Physiol. Plant.* **38**: 1–10.

Xu, W.F., Shi, W.M., Ueda, A. and Takabe, T. (2008). Mechanisms of salt tolerance in transgenic Arabidopsis thaliana carrying a peroxisomal ascorbate peroxidase gene from barley. *Pedosphere.* **18**: 486–495.

Yadav, S.K., Singla-Pareek, S.L. and Sopory, S.K. (2008). An overview on the role of methylglyoxal and glyoxalases in plants. Drug Metabol. *Drug Interact.* **23**: 51–68.

Yadav, S.K., Singla-Pareek, S.L., Ray, M., Reddy, M.K. and Sopory, S.K. (2005). Methylglyoxal levels in plants under salinity stress are dependent on glyoxalase I and glutathione. *Biochem. Biophys. Res. Commun.* **337**: 61–67.

Yamane, K., Mitsuya, S., Kawasaki, M., Taniguchi, M. and Miyake, H. (2009). Antioxidant capacity and damages caused by salinity stress in apical and basal regions of rice leaf. *Plant Prod. Sci.* **12**(3):319–326.

Yang, F., Xu, X., Xiao, X. and Li, C. (2009). Responses to drought stress in two poplar species originating from Water & nitrogen use efficiency in plants and crops. Marston, Lincolnshire, UK. pp. 15–16.

Yang, J.Y., Zheng, W., Tian, Y., Wu, Y. and Zhou, D.W. (2011). Effects of various mixed salt-alkaline stresses on growth, photosynthesis, and photosynthetic pigment concentrations of Medicago ruthenica seedlings. *Photosynthetica.* **49**: 275–284.

Yang, S., Wang, L. and Li, S. (2007). Ultraviolet-B irradiation-induced freezing tolerance in relation to antioxidant system in winter wheat (*Triticum aestivum* L.) leaves. *Environ. Exper. Bot.* **60** (3): 300-307.

Yousfi ,S., Serret, M.D., Márquez, A.J., Voltas, J. and Araus, J.L. (2012). Combined use of δ13C, δ18O and δ15N tracks nitrogen metabolism and genotypic adaptation of durum wheat to salinity and water deficit. *New Phytol.* **194**: 230–44.

Yu, C.W., Murphy, T.M. and Lin, C.H. (2003). Hydrogen peroxide induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. *Func. Plant Biol.* **30**(9): 955–963.

Zain, N.A.M., Ismail, M.R., Puteh, A., Mahmood, M. and Islam, M.R. (2014). Drought tolerance and Ion accumulation of rice following application of additional potassium fertilizer. *Commun. Soil Sci. Plant Anal.* doi:10.1080/00103624.2014.932374

Zehra, A., Gul, B., Ansari, R., Alatar, A.A., Hegazy, A.K. and Khan, M.A. (2013). Interactive effect of salt, light and temperature on seed germination and recovery of a halophytic grass- *Phragmites karka*. *Pak. J. Bot*. **45**: 725–736.

Zeng, L., Shannon, M.C. and Lesch, S.M. (2001). Timing of salinity stress affects rice growth and yield components. *Agric. Water Manag.* **48**: 191–206.

Zhang, Z.F., Li, Y.Y. and Xiao, B.Z. (2016). Comparative transcriptome analysis highlights the crucial roles of photosynthetic system in drought stress adaptation in upland rice. *Sci. Rep.* **6**: 19349. doi:10.1038/srep19349

Zhu, J. K. (2002). Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.* 53: 247–273.

Zu, X., Lu, Y., Wang, Q., Chu, P., Miao, W., Wang, H. and La, H. (2017). A new method for evaluating the drought tolerance of upland rice cultivars. *Crop J.* **5**(6): 488–498.

APPENDICES

Appendix I. Mean square values of germination %, mortality %, shoot height and root length of BRRI dhan29, BRRI dhan48 and Koshihikari seedlings as influenced by salinity and drought (alone or in combined)

Source of variation		Mean square value of			
	df	Germination	Mortality	Shoot length	Root length
Replication	2	5.90	1.53	0.4354	0.1774
Variety	2	325.14	579.01	95.0571	21.5587
Treatment	3	9474.93	8015.87	12.5077	10.0773
Variety × Treatment	6	116.08	105.60	0.6599	0.9050
Error	22	2.83	5.36	0.2166	0.1780

Appendix II. Mean square values of shoot-root fresh weight (FW) and dry weight (DW) of BRRI dhan29, BRRI dhan48 and Koshihikari seedlings as influenced by salinity and drought (alone or in combined)

Source of variation		Mean square value of			
	df	Shoot FW	Shoot DW	Root FW	Root DW
Replication	2	3.58	0.2133	9.013×10^{-6}	7.225×10^{-7}
Variety	2	615.67	9.3446	5.134×10^{-4}	7.981×10^{-6}
Treatment	3	1361.86	25.9459	9.840×10^{-3}	1.055×10^{-5}
Variety × Treatment	6	93.37	1.1090	4.302×10^{-5}	4.294×10^{-7}
Error	22	5.25	0.1865	2.847×10^{-6}	6.311×10^{-8}

Appendix III. Mean square values of RWC (%), Chl *a*, Chl *b*, Chl *a* + Chl *b* and Car of BRRI dhan29, BRRI dhan48 and Koshihikari seedlings as influenced by salinity and drought (alone or in combined)

Source of variation	df	Mean square value of				
		RWC	Chl *a*	Chl *b*	Chl *a* + Chl *b*	Car
Replication	2	0.83	0.0265	0.00292	0.0470	0.00166
Variety	2	1054.91	8.6853	0.38550	12.7289	1.05952
Treatment	3	3711.80	30.1867	2.32601	49.2506	0.64162
Variety × Treatment	6	61.96	3.4878	0.18795	4.8569	0.21892
Error	22	4.65	0.3822	0.05077	0.6466	0.05253

Appendix IV. Mean square values of MDA, H_2O_2, shoot-root EL (%) and shoot-root Na^+/K^+ of BRRI dhan29, BRRI dhan48 and Koshihikari seedlings as influenced by salinity and drought (alone or in combined)

Source of variation	df	Mean square value of					
		MDA	H_2O_2	Shoot EL	Root EL	Shoot Na^+/K^+	Root Na^+/K^+
Replication	2	5.16	1216	2.014	10.65	0.0310	0.0557
Variety	2	251.83	262449	101.832	234.50	0.5477	3.7763
Treatment	3	1473.51	121334	464.021	1356.31	10.4234	20.4356
Variety × Treatment	6	174.61	9049	15.743	78.31	0.3500	1.1732
Error	22	0.48	368	0.70	4.19	0.0042	0.0256

Appendix V. Mean square values of LOX, proline, MG, Gly I and Gly II of BRRI dhan29, BRRI dhan48 and Koshihikari seedlings as influenced by salinity and drought (alone or in combined)

Source of variation		Mean square value of				
	df	LOX	Proline	MG	Gly I	Gly II
Replication	2	3.08	0.057	3.58	0.00438	0.00035
Variety	2	201.71	8.948	1511.29	0.03942	0.02143
Treatment	3	1251.28	109.389	2293.35	0.05352	0.00536
Variety × Treatment	6	100.89	3.523	249.46	0.01664	0.00272
Error	22	1.61	0.005	4.31	0.00125	0.00019

Appendix VI. Mean square values of AsA, DHA, AsA/DHA, GSH, GSSG and GSH/GSSG of BRRI dhan29, BRRI dhan48 and Koshihikari seedlings as influenced by salinity and drought (alone or in combined)

Source of variation		Mean square value of					
	df	AsA	DHA	AsA/ DHA	GSH	GSSG	GSH/ GSSG
Replication	2	21019	457	0.886	5751.8	94.59	3.645
Variety	2	886075	80136	8.872	13797.6	4162.5	43.967
Treatment	3	9294963	897040	269.574	48351.9	9856.3	765.90
Variety × Treatment	6	376689	71951	10.847	11348.5	2845.1	34.170
Error	22	1248	3212	0.536	714.7	41.96	2.920

Appendix VII. Mean square values of APX, MDHAR, DHAR, GR, CAT and GPX of BRRI dhan29, BRRI dhan48 and Koshihikari seedlings as influenced by salinity and drought (alone or in combined)

Source of variation	df	Mean square value of					
		APX	MDHAR	DHAR	GR	CAT	GPX
Replication	2	0.01609	35.83	2288	45.212	72.884	3.780×10^{-4}
Variety	2	0.17027	311.84	174703	263.996	434.16	3.363×10^{-3}
Treatment	3	0.21896	1129.97	18007	465.311	56.00	1.582×10^{-3}
Variety × Treatment	6	0.07050	71.60	3516	27.238	412.98	1.077×10^{-3}
Error	22	0.00894	17.52	275	6.935	17.991	5.780×10^{-5}

df = degree of freedom