OSMOLYTE-INDUCED WATER DEFICIT STRESS MITIGATION DURING PANICLE INITIATION STAGE IN AMAN RICE

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*This is to certify that thesis entitled, "**OSMOLYTE-INDUCED WATER DEFICIT STRESS MITIGATION DURING PANICLE INITIATION STAGE IN AMAN RICE**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN AGRONOMY**, embodies the result of a piece of bona-fide research work carried out by **MD. NURJAMAL ISLAM**, Registration no. 18-09193 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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ABSTRACT

A field experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka-1207 during aman season of the year of 2018 to observe the osmolyte induced water deficit stress mitigation during panicle initiation stage in zinc fortified aman rice. This experiment was carried out of one rice varieties i.e. BRRI dhan72. The experiment was carried out two factors one was water deficit stress viz. D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5 days; D_2= Water deficit at panicle stage, 10days; D_3= Water deficit at panicle stage, 15 days and another was osmolytes viz. 10 mM Proline spray (Pro) and 10 mM trehalose spray (Tre). There were significant difference observed for various kinds of parameters. Water stress drastically reduced the RWC percentage in main and flag leaf, chlorophyll content in main and flag leaf. Several yield contributing characters such as effective tillers hill^{-1}, panicle length, panicle number hill^{-1}, fertile grain panicle^{-1}, 1000 grain weight, grain yield plant^{-1}, and straw yield plant^{-1} including harvest index also reduced. But under the exogenous application of osmolytes like Pro and Tre significantly increased the all kinds of physiological and yield contributing parameters. By the enhancement of osmolytes application with concomitantly decreased the non-effective tillers hill^{-1} and unfertile grain panicle^{-1} but under stress condition these parameter increased significantly.

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LIST OF ABBREVIATION

APX	Ascorbate peroxidase
AsA	Ascorbic acid (ascorbate)
BBS	Bangladesh Bureau of Statistics
BRRI	Bangladesh Rice Research Institute
BINA	Bangladesh Institute of Nuclear Agriculture
CAT	Catalase
Chl	Chlorophyll
DHA	Dehydroascorbate
DHAR	Dehydroascorbate reductase
DNA	Deoxyribonucleic acid
DMSP	Dimethyl Sulfoniopropionate
DW	Dry Weight
ETC	Electron Transport System
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate
GB	Glycinebetaine
Gly I	Glyoxalase I
Gly II	Glyoxalase II
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione

LIST OF ABBREVIATION (Cont'd)

GSSG	Glutathione disulfide
GST	Glutathione *S*-transferase
IRRI	International Rice Research Institute
LSD	Least Significant Difference
MDA	Malondialdehyde
MDGs	Millennium Development Goals
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate reductase
MG	Methylglyoxalase
NADPH	Nicotinamide adenine dinucleotide phosphate
$O_2^{\bullet}$	Superoxide radical
OH•	Hydroxyl radical
OSP	Osmoprotectants
PAR	Photosynthetically active radiation
POD/POX	Peroxidase
Pro	Proline
PWP	Peduncle water potential
ROS	Reactive oxygen species
RuBisCo	Ribulose-1, 5-bisphosphate carboxylase
RWC	Relative water content
SOD	Superoxide dismutase

LIST OF ABBREVIATION (Cont'd)

SRDI	Soil Resource Development Institute
Tre	Trehalose
USDA	United States Department of Agriculture

Chapter I

INTRODUCTION

It's also known as global grainmodel crop, under the family Poaceae and genus *Oryza*. After wheat rice is also known as second most vital crop in the whole world but 90% of rice production occurred in Asian countries. The genus *Oryza* includes two main species of rice which are *Oryzasativa* and *Oryza glaberrima* out of 24 spp. where 22 are wild (Roschevicz, 1931). Different kinds of tropical and subtropical countries of agro ecological zone of rice can be grown a wide range of area. During the year of 2015-16, 169.64 million hectares of land was used to produce 483.3 million metric tons of rice which was the highest of 4.5 million metric tons than the last years (USDA, 2016). The adverse environmental conditions like drought or salinity, population pressure, exchange of global climate change and enhancement of natural calamities are the major causes for diminished of agricultural land (Hasanuzzaman *et al*., 2013).

Drought or moisture stress is a meteorological term that is commonly defined as the period of insufficient water availability including scarcity of storage capacity for soil moisture and significant amount of rainfall. Worldwide the rice production are severely impairs where 23 million hectares of rice land are greatly affected by drought (Pandey and Shukla, 2015). In the whole world, especially in developing countries rice provides more nutrients than the other crops (Phillips *et al*., 2005). It has been determined that the drought stress increased day by day resulting the global rice crop production decreased up to 30% by 2025 (Per *et al*., 2017).

The environmental scenario has been changed due to increasing the severity of drought stress and caused the crop production as well as land area become drastically reduced as fit for agricultural purposes. Water deficit stress greatly affects the plant physiological process resulting various kinds of morphological exchange occurs in the plant such as leaf rolling, reduced number of leaf, small leaf size and also stunted growth of the plant (Rahdari and Hoseini, 2012). Drought is primarily affects the plants consequences the osmotic balance significantly disrupted where the physiological and metabolic disorders are gradually developed (Nahar *et al*., 2015). Drought or water stress causes for primarily reduced the growth of plants which is mostly dependent on cell elongation, cell division and differentiation and also complex interaction including morphological, physiological and biological events (Hasanuzzaman and Fujita, 2013).

Drought stress have the most detrimental effect on crop plants from abiotic stress (Cattivelli *et al.*, 2008; Farooq *et al.*, 2009a; Pennisi, 2008) and limits the global agricultural productivity nearly about 50% (Reynolds *et al.*, 2007; Comas *et al.*, 2013) by shoot production, plant growth and development (Ehdaie *et al.*, 2008, 2012). All the plant tissue are cumulatively affected by the drought stress through impairing the morphological, biochemical, physiological, metabolic and agronomic traits and eventually diminished the yield of the plant (Cochard *et al.*, 2002). Water deficit stress greatly mitigate the stomatal conductance and leaf respiration, water use efficiency (WUE) and carboxylation, photosynthetic activity, higher transpiration, carbon dioxide (CO_2) diffusion and enzymatic activity (Demirevska *et al.*, 2010; Hasanuzzaman *et al.*, 2013). It was estimated that the double food production needed for the enormous population by 2050 when the population reached up to 9-10 billion (Waraich *et al.*, 2011). Different kinds of biotic and abiotic stress causes for the crop productivity is greatly damaged. Drought or water deficit stress caused biomass production, survival and crop yield was diminished significantly (Amtmann *et al.*, 2004; Agarwal *et al.*, 2006). Among the different kinds of abiotic stresses, water deficit stress causes for greatly losses of crop production owing to its significant position and destructive nature (Kusvuran *et al.*, 2011). Various kinds of environmental adversities also faced by plants viz. drought, extreme temperature, salinity, toxic metal etc. which accounts every year more or less yield reduction up to 50%. Abiotic stress have a pernicious impact on biomass production, plant survival and yield (Mantri *et al.*, 2012).

Water deficit stress induced breakdown of membrane structure, enzymatic activity, disorganization of cellular components, cationic and anion imbalance are the main causes for the altering of plant physiological and biochemical process (Hasanuzzaman *et al.*, 2018b). Water deficit stress leads to the generation of reactive oxygen species (ROS), superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and also singlet oxygen ($_1O^2$) are the likely to be common species which also be created due to the iron-catalyzed Fenton reaction causes for the activities of peroxidases (POX), xanthine oxidase, NADPH oxidase and lipoxygenase where the ROS causes for substantial hampers of cell constituents and even cell death (Hasanuzzaman *et al*., 2014). Reactive oxygen species produced in a natural way which is also associated with photosynthetic activity and respiratory metabolism in the cell of plants (Miyake, 2010). The oxidative injury is imparted by the cumulative reaction of ROS to the inevitable biomolecules like lipids, proteins, nucleic acids along with the damage of cell membrane (Ahmed *et al*., 2010).

In crop plants ROS is one of the major toxic radicles which causes demolition of some significant biomolecules such as proteins, lipids, DNA and also leads to the development of metabolic disorder and finally death of the plants cell (Ahmad *et al*., 2016; Nahar *et al.,* 2016a). Nonetheless, significantly thought that the powerful ROS production source is peroxisome where the ROS generating process includes the photochemical reaction and electron transport chain (ETC). Plant health is damaged due to several major reasons such as oxidation of photosynthetic pigments, proteins, nucleic acids and membrane proteins which is also happens owing to the drought stress induced ROS generation leading to the exchange of cellular redox condition (Nahar *et al*., 2015).

Different kinds of strategies applied to reduce the environmental stress like drought by the developing of tolerance mechanism of crop plants with the help of exogenous application of plant growth regulators, inorganic and organic nutrients, osmoprotectants which are effective and environmentally sound approaches (El Sabagh *et al.,* 2019; Hasanuzzaman *et al.,* 2017a). Plants are responsible for one of the common causes that to mitigate the drought stress by the accumulation and synthesis of osmoprotectants or compatible solutes such as proline (Pro), glycinebetaine (GB), choline, trehalose (Tre), sugars and polyols (Per *et al*., 2017). Drought or water deficit stress has been minimized by the foliar application of GB, Tre and so on (Farooq *et al*., 2009b). By the using of osmolytes and the tolerance mechanism of plants remarkably developed against stress condition. The role of osmoprotectants, betaine, Pro and Tre might likely to be performed as a defensive function by the scavenging of ROS. In addition to, Pro has an ability to stop ROS and specially the hydroxyl radicle (OH•) convincingly demonstrated (Signorelli *et al*., 2013). Scavenging the free radicles with the help of phenolic compounds and defending the plants in opposition to the harmful effect of enhancing ROS levels owing to the drought and salt stresses (Petridis *et al.*, 2012).

Many plant scientist work on different kinds of plant growth regulator such as salicylic acid, jasmonic, gibberellic acid etc. as an exogenous type of protectants (Hasanuzzaman *et al.*, 2013). The cell of the plants have well developed glyoxalase and antioxidant defense system for protecting themselves from toxic methylglyoxal (MG) and ROS, respectively. The antioxidant defensive system also comprising of some enzymatic components superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione *S*-transferase (GST), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), catalase (CAT) and glutathione peroxidase (GPX) and non-enzymatic components glutathione (GSH), ascorbic acid (AsA), non-protein amino acids, alkaloids, α-tocopherol and phenolic compounds plays a momentous and direct role for mitigating the adverse environmental stresses like drought, salinity via scavenging of ROS by the application of four important enzymes such as DHAR, MDHAR, GR and APX (Hasanuzzaman *et al.*, 2019). Usually, superoxide dismutase protects frontline from ROS of the antioxidant defense system of plants via changing O_2 to H_2O_2. Interestingly, the plants used two detoxification mechanisms such as glyoxalase and antioxidant systems and reveal the adaptive cellular responses to repair and overcome the damaging status of the plant (Hasanuzzaman *et al.*, 2018a; Hasanuzzaman *et al.*, 2017a, 2017b). To check the oxidative stress hampers, crop plants has been gradually improved the potential and complicated chain of antioxidant defence mechanism which is composed of the up regulation of both non-enzymatic and enzymatic components (Gupta *et al.*, 2009; Hasanuzzaman and Fujita, 2013).

Organic compatible solutes like and Pro, Tre play significant roles under various abiotic stresses (Farooq *et al.*, 2010). Osmolyte like Pro performs various role in plant system such as osmotic adjustment, effectively ROS scavenging, make activate the detoxification process, maintain cell redoxcondition, stabilize the subcellular compositionand membranes which associates with photosystem II and also serve as a signaling molecules in the plant (Hayat *et al.*, 2012; Szabados and Savoure, 2010). Kamran *et al.* (2009) observed that, when Pro used as pre sowing seed treatment under drought stressed condition, seedlings showed that the development of plant root including shoot dry and fresh weights, total grain production and shoot elongation under in case of both stress and non-stress states of rice plant. Trehalose play an important role to develop the water deficit stress tolerance condition in case of Super-basmati variety of rice (*Oryza sativa* L.) plant where it showed that the water stress significantly decreased the growth of plants where the spraying of Tre developed it under this stress situation (Farooq *et al.,* 2008).

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

I. To investigate the effect of water deficit stress on rice plant during panicle initiation stage.
II. To investigate the protective role of proline and trehalose under drought stress of rice plant.
III. To find out the most effective osmoprotectants.

Chapter II

REVIEW OF LITERATURE

2.1 Rice: Botany and production aspects

Rice (*Oryza sativa* L.) is used as a major cereal crop in Bangladesh where the total rice (aus, aman and boro) production in arable land area approximately 80%. But among these, the largest rice production area (5.71 million hectares) covered by T. aman rice which is 11.24 million tons (Zubaer *et al*., 2007). Rice crop is mainly grown in the sub-tropical areas and their favorable temperature range from 21^0 C to 31^0 C where the pH range from 5.5 to 7.0 but the drought stress greatly impedes the plant growth and development, and also decrease the total crop yield significantly (Timung and Bharali, 2020). On the basis of morphology, *indica*, *japonica* and *javanica* are the three major species of rice in the whole world (Purseglove, 1985). Asian rice or *Oryza sativa* and African rice or *Oryza glaberrima* are the two important species of rice crop and both species have a great domestication histories. Tropical and sub-tropical climate are suitable for *indica* rice production in a wide range of area and under this sub-species different kinds of rice cultivars grown in Bangladesh (Alim, 1982). Globally, per capita energy 21% and protein 15% respectively, also provided by rice plant (IRRI, 2012). Worldwide, 740 metric tons of rice production in 2014 (FAOSTAT, 2016), is nearly more than half of the population considered rice as a major cereal crops (Chauhan *et al*., 2017). In Bangladesh, about 150 million people directly uptake rice as a staple food crop and contribution to the agricultural GDP is nearly about 58.3 percent (total GDP of 9.1 percent) (Ahiduzzaman, and Sadrul, 2009).

Rice crop is a monocot plant which is grown annually but it can alive as a perennial plant in the tropical area and also ratoon crops is produced up to 30 years. The favorable rice crop cultivation temperature range from 20^0 C to 30^0 C and these critical temperature change with the genotype, period of critical temperature, physiology and diurnal changes of plant (Nahar *et al*., 2009). Paddy field crop like rice is mainly susceptible to drought stress. Drought stress is a serious problem of agriculture and it has a great impact on quality and total grain production of rice. It was estimated that 40 to 43 % of total world land area affected by drought and every year 12 million hectares of land were lost (Shereen *et al*., 2017). Worldwide the rice production is affected by drought nearly about 50% (Mostajeran and Rahimi-Eichi, 2009). Rice was the most important cereal crop which was significantly grown nearly about 78.16 percent of the total crop production area (BBS, 2014). At this time, Bangladesh had moved MDGs to SDGs targeting eliminated the impoverishment and appetite, ameliorated the nutritional status and acquired food security, improved the sustainability of agriculture by 2030 (Nahar, 2016). In Bangladesh, the major food grain was collected from rice crops which was grown nearly about 80 percent cultivated area where 43.72 million metric tons of rice produced from 11.59 million hectares of land annually (Hasanuzzaman *et al*., 2010).

Rice crop is considered as the main source of calories, which provides nearly about 40 percent of world population (Haffman, 1991). Worldwide rice is considered as the largest staple food crop which supplies calories for daily human consumption. Rice crop cultivation is regard as a yearly grass which growing up to 0.6 to 1.8 meters, hollow, rounds with joints culms and appears the terminal inflorescence (Kellogg *et al*., 2013). Rice plant has a great nutritional values. Rice is considered as a good source of energy owing to rapid protein digestion than the other cereal crops (Ali *et al*., 2014). Rice gives the average yields is about 3.90 tons per hectare in Bangladesh (BRRI, 2007), which is less than 50 percent of the worldwide average yield (Hossain *et al*., 2008). Rice was consumed approximately 90 percent in Asia. Globally, the rice production area greatly affected by drought which was more or less 70 million hectares of land (Ahmad *et al*., 2014). Due to the physical water scarcity, 15 million hectares of rice crop production was affected by 2025 (Shereen *et al*., 2017).

2.2 Effect of water deficit condition on rice plant

The cultivation of rice plants is threatened by abiotic stress where water deficit stress is the most common.The response of rice plants to water deficit stress is the most complex which involves in the changes of plants morphological and physiological process.

Total crop productivity was reduced by the effect of water deficit stress which was the most devastating and complex global scale (Pennisi, 2008), and the consequences of climate change was enhanced and also increased the water crisis (Harb *et al*., 2010; Ceccarelli *et al*., 2010). Expansion of leaf, injure the photosynthetic machinery, CO_2 uptake and leaf senescence prematurely including the total crop production was greatly reduced by the effect of drought or water deficit stress. Flowering and terminal period of rice plant was affected by the water deficit stress causes for the floret initiation was interrupted resulting the grain weight significantly decreased. Rice plant is more sensitive to water stress particularly at different phases like anthesis, panicle initiation and grain filling period (Mishra and Singh, 2010). Globally, water stress severely decreases the crop production and it is considered as the major barrier for crop cultivation (Jaleel *et al*., 2009, Hasanuzzaman *et al*., 2012a).

Sabetfar *et al.* (2013) concluded that water stress have a detrimental effect on effective tillers number, filled and unfilled grain per panicle, panicle number per unit area, total plant height and important effect on grain yield including 1000-grain weight. The leaf pubescence, leaf size and shape was reduced under water deficit condition as well as yellow color of leaf was observed. Furthermore, the initiation of new tillers, new leaves and stem elongation was slow owing to the availability of limited amount of water. Severe drought or water deficit stress ends the leaf drying process and ultimately the plants become death.

Water deficit stress causes for the changes of various physiological characters in plants such as reduction of transpiration rate, photo synthetically active radiation (PAR), photosynthetic rate, stomatal conductance and relative water content (RWC) resulting the growth of plants and WUE is decreased before the plant senescence (Akram *et al*., 2013; Chaves and Oliveira, 2004; Cattivelli *et al*., 2008). Phenological development may delayed due to the water stress in rice plants and also hampers the physiological process such as photosynthesis, transpiration, respiration and assimilates translocation to the grain.

Samarah *et al.* (2009) said that the photosynthetic pigments was decreased due to water deficit stress which was the major causes for the reduction of photosynthesis in rice plant. In the plants, stomata are the process that release of water and absorbing capability of CO_2 where the stomatal closer is the first response to water stress which ultimately reduced the production of photosynthesis. Decreased the photosynthetic pigments such as chl. a, chl. b, and total chl.contents including carotenoids under water stress has been reported in some species of plants like *Oryza* sp., *Triticum* sp., *Avena* sp., and *Gossypium* sp. (Pandey *et al*., 2012). Similarly, the total photosynthetic rate was decreased 22% and 75%, respectively contrast to their control condition by the effect of water or drought stress (Xu *et al*., 2009).

It is considered that the stomatal closer is the major determinant for the reduction of photosynthesis from the range of mild to moderate water stress. Alam *et al*. (2013) stated that the leaf RWC, tissue water content, chl. content and photosynthetic carbon assimilation was decreased by the effect of water deficit stress in crop plants.

Several important attributes of plant water relations such as leaf water potential, RWC, osmotic potential and transpiration rate was significantly hampered under water deficit stress due to reduce in water supply (Kirkham, 2005). The enhancement of water stress causes for the tissue water content was reduced (Reddy *et al*., 2004). Leaf water potential, stomatal conductance, turgor pressure, cell elongation and finally growth reduction was occurred due to the effect of water stress (Jaleel *et al*., 2007). Cell division and cell elongation was decreased due to the water deficit stress where the turgidity of plant cell become lower.

The plant diffusion rate was affected by the limited water availability and ultimately hampers the concentration and composition of soil solution (Singh and Pandey, 2011). Uptake of plant nutrients was decreased due to the shortage of water availability and also slow down the diffusional rate of nutrients from soil matrix to root surface. Plants nutrient uptake process was totally rely on soil-root-shoot pathway which was disturbed by the abiotic stresses where most common was water deficit stress (Farooq *et al*., 2009b).Turk *et al*. (2004) said that water deficit causes for the germination of seed become delayed.

2.3 Effect of water deficit stress on growth and development of rice

The water deficit stress is the worse and progressive stress among the various kinds of abiotic stresses which significantly hampers the plant growth and development and reduces the total crop production than the other ecological constituents and rely on the genotype, intensity and duration, and also the stage of plant development (Anjum *et al.*, 2017).The growth and developmental process of plant was altered owing to water stress which associated with the plant height, diameter of stem, leaves number, area and size of leaf, partitioning and production of dry matter, production of flower and fruit and maturity. The cell division and development one of the crucial process for the plants growth and development. Osmotic stress is a common effect of plants which is occurred in plants by the effect of water stress; therefore, the cell enlargement can be hampered owing to lessen the water availability and lowering the water flow from xylem to surrounding the cells (Munns, 2002).The mitosis process was inhibited by the water stress which associated with the expansion and elongation of cell division resulting the growth was significantly decreased (Hussain *et al*., 2008).

Growth of plants was activated in some region of cells is known as meristems. In meristematic region, nearly all kinds of cytokinesis and mitosis occurs which prompting the cell elongation is called primary growth where the secondary growth of the plant starts and causes for the growing of cells (Taiz and Zeiger, 2006). Additionally, it is thought that the turgor one of the water deficit sensitive physiological process that causes for the reduction of growth and cell expansion. The stream of water infringe from xylem to adjacent meristem cells which impedes the expansion of cell in plants (Jaleel *et al*., 2009). Due to the severe water stress causes for the termination of photosynthesis, impair the metabolism process, lessen the turgidity and finally the cell become death.

The germination process and the poor standard establishment was impaired by the water stress which was considered as the first and foremost effects. The period of developmental process such as spike development was decreased after water stress which severely influences the grain number, size and weight. Nevertheless, when the water stress occurs after the panicle initiation or flower developmental phase resultingthe period increases from seed set to the seed development (Prasad *et al*., 2008). Different kinds of developmental process of plants are hampered by water stress such as leaf senescence, good plant source relationship, phenological development that finally affects the plant growth process and productivity. Cytokinins play a vital role for the source sink regulation and translocation (Jnandabhiram and Sailen Prasad, 2012).

Generally, when the plant faced water deficit condition, the root and shoot ratio was increased because the shoots are more sensitive than the roots to growth restriction by the limited amount of water (Anjum *et al*., 2011). There were many kinds of literature showed that the water deficit stress decreased the plant growth, development, height, total leaf area, and dry mass production in rice plant (Ashfaq *et al*., 2012; Ishak *et al*., 2015).

2.4 Water deficit stress and growth stages of rice

The cultivation of rice plants is most susceptible to water stress at the period of vegetative and reproductive phase. The whole life cycle of rice plants classified into mainly three growing stages that is i) vegetative stage ii) reproductive stage and iii) ripening stage. But these stages are also subdivided into several other stages such as the vegetative stages composed of emergence, seedling development, tillering and stem elongation; reproductive stages comprised of panicle initiation, booting or heading and flowering stages; ripening stage composed of milk, dough and mature stages. But under water stress condition all the growth stages are greatly affected. Rice plant needs more water for their better growth and yield but it is a problematic to cope with this moisture stress owing to the rice shallow root system and decrease water absorption at the period of water stress condition (Dash *et al*., 2017).

Bunnag and Pongthai, (2013) stated that in several areas during the early season water stress condition the seedlings emergence, transplanting, tillering and the increases of direct seeded rice growth was greatly hampered. On the other hand, late season water stress develops premature stage of crops especially in rice cultivation. Moreover, the vegetative phase is the most important determinant for the growth and effectively mature of paddy rice plants. It reduces the leaf enhancement, tillering including the midday photosynthetic process and leaf area owing to the early senescence of the plants

(Munns, 2002). In addition, water stress enhanced the ROS formation resulting denaturation of proteins, peroxidation of lipids and nucleic acids significantly affected but in extreme condition total metabolism process damaged causes for the yield reduced drastically.

Under the vegetative stage, the availability of nutrients and growth status is affected by water stress. Water deficit stress designated as the most crop limiting factors under any kind of growth stages and threaten for successful crop cultivation. The germination of seeds and growth of seedlings responses to the water stress considered as an important stage for the development of the plants (Siopongco *et al.*, 2005). And the tillering stage is the most critical growth stage for obtaining better grain production. Noticeably, the protein synthesizing components and effectively protein synthesis drastically reduced under water deficit condition. In embryonic axis, the total amount of free amino acid reduced with concomitantly enhanced the water stress **(**Jha and Singh, 1997).

Yang *et al*. (2019) stated that when plants incapable to absorb of adequate amount of water during the vegetative and flowering stage resulting the inhibition of floret emergence, height of plants, tiller number per plants, area of leaf, grain filling and finally lower grain production. The turgidity of plant cells is very less resulting the cell division and cell expansion become reduced and finally, retarded the growing status of the plants. Moreover, reduction of chlorophyll contents and lessen the photosynthetic activity leading to decrease the plants growth.

Dramatically reduction of total grain yield when water deficit coincides with the unchanging reproductive development of rice plants (Pantuwan *et al*., 2002). Ji*et al*. (2012) concluded that notoriously paddy rice plants is susceptible to the period of water deficit stress owing to its small roots, swift stomatal closing and small circular wax. Minimization of photosynthetic activity, accumulation of osmolytes and organic solutes, and alters the metabolism rate of carbohydrates are the main biological and physiological responses to water deficit stress.

Patakas (2012) said that, in contrasting with several more crops, paddy rice is most sensitive to water deficit condition particularly at the critical growing stage such as emergence of panicles, anthesis and also grain filling process. Water stress occurred during the growing season from panicle initiation to flowering and the intensity of this stress rely on the frequency and time of water stress. Markedly, the accumulation of sugar was decreased in grains and in leaves at soft dough stage which was greater at the period of panicle emergence stage (Bartels and Sunkar, 2005).

Jahan *et al*. (2014) stated that when the reproductive stage of rice plants was affected by water deficit resulting changed the various physiological parameters such as decreased chlorophyll contents, transpirational rate, photosynthesis, pigment, RWC, stomatal conductance, WUE and finally reduced the growth of plants. The scarcity of water in the crop growing stage particularly at the reproductive phase leading to severe reduction of rice yield. Under water deficit condition, at the period of grain filling, nearly 40% of filled grain was reduced and individually the grain mass minimized by 20% (Boonjung and Fukai, 1996). Due to severe stress causes for the production of toxic substances such as ROS at the period of respiration and photosynthesis which is together with nucleic acids, fats and proteins resulting in harms the denaturation of proteins, plants cells, peroxidation of lipids and also mutation of DNA. Responses of this stress for paddy rice largely rely on some factors such as the severity and timing of stress, plant genotype and plant growth phase. Some researches on paddy rice interpreted that reduction of panicle number per plant, panicle length, and grain weight per plant with concomitantly enhancing the water stress (Sokoto and Muhammad, 2014; Khairi *et al*., 2015).

Ripening stage is a very important stage of rice plants. When this stage affected the water deficit stress leading to drastically reduce the total grain production. The occurrences of water deficit damages the plants physiological activity like transpiration and photosynthesis leading to decrease the plant growth and grain yield (Samonte *et al*., 2001). Carlos *et al*. (2008) stated that water was very essential elements for development of plant tissue which reagent for various chemical reactions and also solvent for the metabolites translocations including minerals and important constituents for the cell elongation and enlargement via enhancing the cell turgor pressure. For various kinds of annual crops especially rice, total grain production significantly affected when water stress occurred during the flowering and formation of yield stages (Pandey and Bhandari, 2007).

Zaman *et al.* (2018) showed that when this stress affected the flowering stage and ripening stage, did not seriously hampered the panicle number and 1000 grain weight of MR253 variety, but drastically decreased the total grain production. Due to this stress resulting the reduction of flowering and yield nearly about 50% and 21%, respectively.

2.5 Effect of water deficit stress on panicle initiation stage of rice

In the reproductive stage the panicle initiation is known as the first reproductive growth stage of rice plants. The physiological and biochemical process was inhibited by water stress at the panicle initiation period and enzyme activities severely decreased the plant stomatal conductance, pigments of chlorophyll causes for lessen the PAR, photosynthesis, RWC and transpirational rate.

Akram *et al*. (2013) stated that especially the rice plant is more sensitive during the period of water stress at various developmental growth stage like period of panicle initiation, grain filling and anthesis. At the growth of reproductive stage particularly booting stage the flower initiation and terminal period significantly inhibits the emergence of florets resulting the spikelet becomes sterile under the water stress condition. Due to this stress the PAR was fall down at the whole growth stage of rice plants. At the panicle initiation stage the panicle length was importantly reduced by the effect of water stress where no considerable changes was observed at the growth stage of grain filling and anthesis for panicle length. This study also showed that in case of panicle initiation the PAR values was recorded for 24.63% which was more pronounced than the other stages such as anthesis 23.13% and grain filling 15.57%, respectively.

Boonjung and Fukai, (1996) stated that when the mild water deficit stress formed at the early panicle emergence period, approximately 30% of total production was decreased owing to the minimization of spikelet number per panicles. Most harmful effect of water stress happened during this stress development of mid-panicle emergence period. Generally, if the stress development occurred from mid-panicle to anthesis period resulting empty filled grain was observed. Cruz and O'Toole (1984) also said that around 73% of spikelet becomes sterile by the effects of water stress at the stage of flowering. He also investigated that nearly 30% of sterile spikelet linked with the poor exertion of panicles.

Majeed *et al*. (2011) reported that the water stress reduced the panicle size, grains per panicle number at the developmental panicle initiation stage.This stress causes the protein content, sugar content was markedly decreased during the emergence of panicle.

Guan *et al*. (2010) said that water stress hampers the development of female and male reproductive phase. There are two stage is very sensitive in rice plants of water deficit stress which mark at the developmental period of reproductive phase where one is meiosis of pollen and another is anthesis or flowering. Drought or water stress prevents the developmental process of pollen under meiosis stage which associates with the shedding of pollen, germination of pollen and anther dehiscence. In secondary branches higher abortion rate of pre-flowering occurred during the mild water stress under meiosis stage resulting nearly 40-45 % panicle number per spikelets was decreased. When the flowering stage was affected by water stress causes for the delayed of flowering period including reduction of fertile panicle number and also spikelet fertility.

Kumar *et al*. (2006) stated that when the water stress prevents the exsertion of panicle that causes for the reduction of peduncle length and also the sterility of spikelets occurred up to 70-75%. Several studies proved that under this stress condition the peduncle water potential (PWP) linked with the delayed flowering and decreased the exsertion of panicle which significantly affected the number of fertile panicle numbers and grains.Water stress was more harmful at the stage of grain filling following the initiation of panicle phase regarding the spikelets number per panicle, fertile grain per panicle (Sharifunnessa and Islam, 2017).

Rehman *et al*. (2002) stated that in rice plant the most detrimental stage is grain filling following by the booting or flowering stages. Water stress influenced the photosynthetic activity which modulated by the closing of stomata with concomitantly reduced the stream of CO_2 to cellular mesophyll tissue and inhibited the metabolic process which ultimately affected the panicle initiation stage (Prasad *et al*., 2008). The synthesis of carbohydrate was delayed due to the moisture stress and also unable to the sink at this reproductive phase.

2.6 Effect of water deficit stress on yield of rice

Water stress or drought is one of the most vital abiotic stress which influenced the total production of rice. It also hampers the grain quality, planting area and potentiality of genetic makeup which ultimately affects the total agricultural productivity.

Pandey *et al*. (2014) reported that when the low soil moisture levels are available, milled content of rice become recovered and the total protein content dramatically increased, but the unripen percentage of rice grain is diminished, meanwhile the amylose percentage is reduced in milled rice. However, the ratio of head rice is enhanced under water stress condition. When water stress occurred at the time of ripening might be considered as a beneficial factor which help to decrease the breaking of milled rice associated with the higher altitude of head whole. The cellular protein contents interlinked with the grain production criterions like ratio of head rice, milled grains quality and also parameters of viscosity.

Ndjiondjop *et al*. (2010) stated that the important barrier for rice crop production and cultivation of water deficit stress at the time of lower rainfall which hampers the vegetative phase, reproductive phase and total grain production. Approximately, 50% of the earth rice production is affected by moisture stress. This stress causes for the rice spikelets turn to sterile and grain becomes unfilled. Usually, water stress at the period of grain filling induced early senescence and lessen the time for grain filling which ultimately affected the total yield of rice (Tao *et al*., 2006).

Fofana *et al*. (2010) reported that the water is a very important components of plant tissue and act as a reagent for biochemical reactions and helps in minerals and metabolites translocation associates with the other essential cell constituents which contributes to the cell elongation via enhancing the turgor pressure but without this water the whole process greatly affected which finally hampers the total grain production. Severe water stress affected the biomass accumulation, limiting the total crop productivity concurrently with yield due to the decreasing of photosynthetic activity, respiration and also influenced the biomass partitioning in the harvesting plants.

Bunnag and Pongthai (2013) reported that the moisture stress have a detrimental effects on plant growth and developmental process which ultimately affects the grain quality of rice. Rice production responses to soil moisture condition which alters with different growth phase and regarding as a most sensitive at the stage of panicle initiation, booting or heading and flowering. More grain yield reduction occurred during the water deficit stress at the stage of flowering resulted by the minimization of filled grain per panicle percentage.

Zain *et al.* (2014) Different kinds of functional and structural disturbance was observed under water stress condition in the reproductive parts resulting the fertilization become fail or prematurely abortion of crop seeds. Early senescence, shortening of grain-filling time, decrease photosynthetic process and enhance the remobilization of soluble sugars from crop grains to various vegetative organs when water deficit stress affects the plant reproductive stages. Remobilizations process of carbohydrate or sugars totally rely on the strength of sink and activity of source which might be altered with varieties (Shahruddin *et al*., 2014).

Zaman *et al*. (2018) investigated that when the potentiality of leaf water was decreased, leading to canopy elongation and phase of reproductive growth drastically reduced resulting adversely affected the total yield. Water stress significantly hampers the plant physiological process via minimization of gas exchanged especially in photosynthetic pigments, stomatal conductance including total plant water relations which ultimately affects the grain production of rice.

Pirdashti *et al*. (2004) investigated that total grain production dramatically reduced when the water stress occurred during the emergence of flowering. He added that the total grain production was decreased by 21%, 50% and 21%, respectively when the moisture stress affected the plant at the period of vegetative and flowering stages associated with grain filling. Moreover, the 1000-grain weight was decreased 17.36 % contrast to their control condition.

Cai *et al*. (2006) said that the water or drought stress influence the rice grain production which is rely on the various nitrogenous levels. When the rice plants grow under normal nitrogen (N) condition, remarkably decreases the weight of grain and percentage of grain filling due to the effect of water stress resulting the yield of grain reduce significantly. On the other hand, when the availability of nitrogen content is high, this stress enhances the yield of grain production causes for the improvement of grain weight and percentage of grain filling.

2.7 Osmoprotectants

Osmoprotectants or osmolytes are very small, low molecular weight including hydrophilic substances, non-toxic particles, neutral electrical charge in a molar concentration and absolutely liquefiable organic substances like Tre, Pro and GB which play an vital role and support the organisms to alive in the excessive osmotic stress condition (Nahar *et al*., 2016a; Hasanuzzaman*et al*., 2019b). The chloroplast and cytosol are the reservoir of these molecules but in other organelles some of these are scattered. Generally, the accumulation of osmoprotectants in plants range from 5 to 50 μmol g^{-1} FW (Rhodes and Hanson, 1993; Bohnert *et al*., 1995). In mature cell the osmoprotectants are confined to the chloroplasts, cytosol and several cytoplasmic chambers located at more or less 20% area and these osmoprotectants perform significantly at various kinds of abiotic stress condition (Ashraf and Foolad, 2007; Farooq *et al*., 2010). These compatible solutes plays an important role forthe regulation osmotic adjustment, membrane and protein stabilization.

The compatible solutes or osmoprotectants are categorized into three distinct characteristics based on the chemical compositionthat is i) α-amino acid like ectoine and proline, ii) quaternary ammonium substances like β-alanine betaine, GB, dimethyl sulfoniopropionate (DMSP), and chloine, iii) polyols, sugar and sugar alcohols viz. sorbitol, manitol and Tre etc. (Nahar *et al*., 2016a). Osmolytes shows more mechanisms that are protection of biological membrane, toxic substances detoxifications like ROS, ionic toxicity mitigation, protection of mitochondrial structure and photosynthetic activity including metabolism (Alam *et al*., 2014). During the harsh environmental condition, osmolytes regulate the turgor pressure of plant cell through osmoregulation, protecting cellular compounds, replacing the inorganic ions and mitigating the ionic toxicity (Zulfiqar *et al*., 2020).

Osmolytes like Pro,Treperformed various kinds of advantageous role on abiotic stress condition where it act as not only scavenging ROS but also in osmotic adjustment, chelating metal, activation of whole detoxification process, regulate the cell redox reaction, energy storing especially for carbon and nitrogen, buffering of cytosolic pH, stabilization of subcellular structures and membranes, photosystem II (PS II) and also signaling compounds (Trovato *et al*., 2008; Verbruggen and Hermans, 2008; Mattioli *et al*., 2009; Szabados and Savoure, 2010; Hayat *et al*., 2012). However, it is designated that the important protective stress mechanisms is play signaling by the osmolytes in crop plants.

Glycinebetaine, Pro and Tre are associated with the scavenging ROS, stabilization of macromolecules such as lipids, proteins, nucleic acids and also several photosynthetic components like RuBisCO and photosystem II, and serve as a store of nitrogen and carbon sources (Chen and Murata, 2011; Giri, 2011; Ahmad *et al*., 2013, 2017b). Different kinds of advantageous effects were found in plant physiological process. Increased of total chlorophyll content, DW, total N content, growth rate and biomass production per plant were found for the supplementation of osmolytes like Tre which was developed the accumulation of Pro, K^+ and also K^+/Na^+ ratio.The membrane structure and biomolecules was stabilized by the exogenous application of Tre (Aghdasi *et al*., 2008; Duman *et al*., 2011; Luo *et al*., 2010). Nonetheless, the thylakoid membranes stabilized by the help of these organic compounds causes for the upregulation of photosynthesis. However, these osmoprotective molecules develop the plants antioxidant defense mechanisms by the protection of main enzymatic antioxidant and also scavenging the toxic ROS (Hasanuzzaman *et al*., 2014).

Polyamines are very little organic molecules which associated with amino groups located at the eukaryotic cells. Polyamines performs a vital role in the plant improvement process like embryogenesis, cell division and cell expansion, root development, stem expansion and floral development. Some important polyamines such as putrescine, spermine and spermidine are found in plants which involved in the cell growth and proliferation, differentiation of morphogenesis and also programmed cell death (Alcazar *et al.*, 2010a). Moreover, these osmoprotectants act as an activation of gene which is defense related under various environmental stress condition (Wani *et al.*, 2018).

2.8 Role of osmoprotectants under water deficit stress

Osmoprotectants are neutral electrical charges, very small, lower molecular weight, non-toxic molecules and high solubility in molar concentration solution (Singh *et al.*, 2015). They assists plants to alive in extreme osmotic environmental condition. These compatible solutes stabilized the membranes, proteins and enzymesand decreased the membranes osmotic potentiality to protect the cell dehydration of plants (Wani *et al.*, 2013). These osmoprotectants regulated the difference of osmotic balance between cytosol and surrounding of cells and also helps plant to adapt the harsh environmental conditions like drought, salinity and high temperature via increased their osmotic pressure in the cell of cytoplasm (Ahn *et al.*, 2011).

Osmoprotectants are classified into three categories that is i) ammonium compounds like glycinebetaine, polyamines ii) sugars and sugars alcohols like Tre, mannitol iii) amino acids like Pro, ectoine. The responsive genes of these osmoprotectantsare the effective way to develop the tolerance mechanism in plants through increasing their production (Reguera *et al.*, 2012). Other tolerance mechanisms of these Osmoprotectants are protection of biological membrane, detoxifying of harmful toxic substances like ROS, mitigation of ionic toxicity, preservation of mitochondrial composition and photosynthetic process including metabolism (Alam *et al.*, 2014).

Compatible solutes or osmoprotectants such as Pro, GB and Tre plays an important role to adjustment of osmotic balance via stabilization of biological structure, photosynthetic apparatus, and macro molecules which is also help in ROS scavenging. (Bhuiyan *et al.*, 2019). The defensive mechanism of antioxidant protect the plants from harmful effect of ROS through the accumulation of osmolytes. However, the signaling system of osmoprotectants considered as an important protective mechanism against abiotic stress condition.

Semida *et al*. (2020) stated that in response to water deficit stress, the accumulation of Pro in the cell of plants, served as an osmolyte for effectively adjusted the osmotic balance, stabilized the cellular membrane, decreased the lipid oxidation process or photo prohibition, ROS scavenging and buffering redox process leading to improvement the water stress effects. Several studies showed that the foliar application of osmoprotectants proficiently supporting the water stress tolerance (Hossain *et al.*, 2019). Several compatible solutes serve as plant antioxidant defense. Moreover, they tolerates the stress condition through the controlling of gene transcription and regulation (Hayat *et al.*, 2012).

Osmoprotectants detoxifying the adverse environmental stress through two ways. One is ameliorates the plant antioxidant defensive system and another is ion homeostasis sustainability (Singh *et al.*, 2015). In addition to the antioxidant process compatible solutes such as GB, Pro, Tre, polyamines and sugar alcohols regulated the enzymatic antioxidant process with concomitantly enhanced the antioxidant of non-enzymatic activities and also mitigates the harmful oxidative effects (Noreen *et al.*, 2018). Several enzymatic antioxidant such as peroxidase, superoxide dismutase, ascorbate peroxidase and catalase and other non-enzymatic plant antioxidants are ascorbate, glutathione and carotenoids. Both enzymatic and non-enzymatic defensive antioxidants having effective ability to protect the plants through decreasing the toxicity of ROS.

The osmoprotectants protects the cellular components against the harmful effects of water stress such as dehydration and they don't intervene to the active metabolic process at the plant cellular level. Several useful attributes observe for physiological parameters such as enhance relative growth, N contents, chlorophyll contents, biomass plant^{-1} and DW with Tre and Pro supplementation. By the foliar application of Tre ameliorates the accumulation level of Pro, K^+ and also K^+/Na^+ ratio (Duman *et al.*, 2011).

2.9 Proline as an osmoprotectants under water deficit stress

The common compatible imino acid is proline which is high soluble and stability confirmation. Proline is an essential elements of metabolic and cellular cases and also liable for osmotic balance in plant cells (Yancey, 2005).The stabilization of protein formation and defend the cellular molecules by the exogenous application of Pro including ROS scavenged under water stress condition. It may act as source of organic carbon, nitrogen including energy during the rescue from environmental stress (Tyagi and Sairam, 2004). Under dehydrational state the osmotic adjustment is regulated by the application of this osmolyte. In the plant cell higher amount of Pro is also regulates the $NADP^+$/NADPH ratio under the water deficit conditions (Saxena *et al.*, 2013).

Under water stress condition Pro is served as a treating material of seed before sowing resulting the development of root, shoot fresh and dry weight, shoot length including the grain yield under stress and non-stress environmental conditions (Kamran *et al.*, 2009). Gerdakaneh *et al.* (2011) concluded that the foliar application of Pro in the medium of callus culture causes for the growth rate was increased and also their internal Pro level; however, the highest growth rate was recorded for 10 mM Pro level. The internal level of Plants was increased by the application of Pro (50 mM) and minimized the water deficit stress damages through the enhancement of some enzymatic antioxidants such as SOD, CAT, POD and non-enzymatic antioxidants particularly the ascorbate glutathione cycle (Aggarwal *et al.*, 2011).

Besides the osmoregulation, Pro serves as a proficient molecular chaperone and stabilize the subcellular molecules which associated with the photosystem II (PS II), activated protein, enzymes and membranes (Banu *et al.*, 2009). De Carvalho *et al.* (2013) stated that the Pro act as a defensive antioxidant molecules which proficiently ROS scavenged, lessen photo inhibition, and deterioration of photosynthetic apparatus especially minimization of oxidative injury via stabilization of enzymatic antioxidant. Besides, this osmolyte act as an energy reservoir, protein harbinger and also a source of cellular N or carbon (Nahar *et al.*, 2016a). It also assuages the harmful effects of different stresses like water deficit or salt stress.

Caronia *et al.* (2010) stated that exogenous application of Pro reduced the adverse environmental effects of water stress of different kinds of plants in terms of imbibition of N, gained fruit weight and also estimated that the total fruit solid contents increased by 400 mg L^{-1} foliar application of these osmolytes.

Ali *et al.* (2013) reported that thechemical formation of maize seed was significantly injured by the adverse water stress but these adverse situation was minimized by the application of osmolytes like Proand which was ameliorated the chemical composition of maize seeds and also the sugar contents, protein, ash, fiber, oil, seed moisture, linoleic and oleic acid.The antioxidant defensive components like carotenoids, tocopherols, phenolics and flavinoids increased the scavenging action of oily DPPH (1,1-diphenyl-2- picrylhydrazyl) free radicles.

Sofo *et al.* (2004) stated that the compatible solutes prevents the denaturation of protein and cellular membrane damages during the abiotic stress like water stress, high temperature and replenishes the supply of $NADP^+$ during the changeable redox potentials and also serve as an electron receiver and ameliorated the injury of photosystems owing to their photo prohibition by the activating of oxygen molecules. Pro improved the defensive antioxidant system by the decreasing of H_2O_2 and MDA contents and also increasing GR, APX, GSH, CAT and activity of Gly II, ratio of GSH and GSSG which control the ROS actions and enhanced the tolerance levels of oxidative stress (Hossain *et al.*, 2014).

Molla *et al.* (2014) reported that the performance of Pro and GB under water stress in lentil plants. By the foliar spray of 15 mM Pro or GB with the abiotic environmental stress condition such as water stress resulted the GSH elements is increased, modulation higher activities of Gly I and GST which is compared to untreated control where concurrently decreased the H_2O_2 and GSSG contents and also protected the plant cells from the harmful effects of ROS detoxification process.These findings conclude that in case of both osmolytes which gives better protective role under stress by minimizing the H_2O_2 levels with enhancing the defensive antioxidant systems where Pro shows the better performance.

2.10 Trehalose in mitigating water deficit stress

Trehaloseis a very important non-reducing disaccharides which act as analleviating agent of different environmental abiotic stresses such as water stress, high temperature etc. (Ahmed *et al*., 2013a; Alam *et al*., 2014). Under stress condition, Tre stabilized the macromolecules and serve as a compatible solutes. Shafiq *et al.* (2015) recently reported that the exogenous spraying of Tre effectively ameliorated the enzymatic antioxidants like POD, CAT, SOD and non-enzymatic antioxidants like AsA, phenolics defensive process in the plants under water stress condition. Naturally, Tre accumulates in different plants under stress condition and there are two process involves in the production of sugar which operates through two enzymes such as one is trehalose 6-phosphate phosphatase and another is trehalose 6-phosphate synthase.

Redillas *et al.* (2012) stated that the rice plants showed more tolerance to Tre than the other type of dicot crop plants where the visible variation observed in rice plants. In rice the enhancement of Tre accumulation efficiently connected with the highest levels of carbohydrate solubility and improved the photosynthetic capacity under the non-stressed and stress environmental conditions. The tolerance capability of Tre to the plants water stress which is interlinked to the signaling of sugar and act as an osmoprotectants since the concentration is very less. It is thought that the sugar signaling showed the better responses to the environmental abiotic stress like water stress, salinity, high temperature. This study showed that the accumulation of trehalose-6-phosphate (T-6-P) effectively prevents through the more accumulation of Tre causes for the water and salt stress tolerance capacity of the plants was significantly increased. Nonetheless, the massive amounts of osmoprotective Tre generation without the accumulation of T-6-P plays a vital role for soluble sugar metabolism, signaling and allocation in way that finally, mitigate the water stress to the plants.

Akram *et al.* (2016) concluded that the metabolic regulation was the important activity of Tre in plants including the controlled of total energy metabolism and maintenance of sucrose mobilization to various pathway which was significant for structural, metabolism and storage operations in the cell of plants. Ilhan *et al.* (2014) stated that the study of caper (*Capparis ovata*) where Tre and the biosynthetic process of Tre play a vital role that substantially showed the water stress toleranceand the accumulation of Tre significantly enhanced in caper up to 385.25 µg/L after 14 days exposer to water deficit stress.

Llorente *et al.* (2007) stated that the application of 1 mM Tre resulting the proliferation rate was increased and reduced the development of root including the anatomical features of root. At the time of desiccation, application of Tre efficiently stabilized the cellular proteins, lipids and enzymes with concomitantly protecting the biological as well as physical formation from damages.

Ali and Ashraf (2011) studied that the exogenous application of Tre on maize seedlings which was affected by the water stress. These stress minimized the photosynthetic rate, production of plant biomass and plant water relations.The plant oxidative process is produced by the water deficit stress which is changed the enzymatic antioxidant system and non-enzymatic activities. Exogenously 30 mM Tre application efficiently increased the total biomass production, developed the photosynthetic process and the relations of plant water attributes such as solute and water potentiality, turgor, and also RWC. The plant alleviates from oxidative stress by the application of Tre and increases several important enzymatic antioxidants such as POD, CAT and non-enzymatic components like phenolics, tocopherols etc.

The seed oil contents decreased due to water stress resulted the linolenic and oleic acid of oil significantly increased and concurrently the linoleic acid of oil was decreased which ultimately enhanced the maize cultivar ratio of oleic acid and linoleic acid.Under stress and non-stress environmental conditions foliar applications of Tre positively influences the chemical composition of maize seeds. Moreover, the antioxidant mechanism process of oil was increased owing to the application of Tre and scavenged the free radicles actions including enhancement of flavonoids, tocopherols and phenolics content (Ali *et al*., 2012).

Shafiq *et al*. (2015) investigated that the non-enzymatic and enzymatic antioxidative process of plant roots was altered due to the spraying of Tre in water stress of radish (*Raphanus sativus* L.).The application of Tre efficiently decreased the MDA contents where ameliorated the FW of roots, phenolic compounds, AsA contents, GB, TSP and tocopherols as well as the different activities of POD, SOD and CAT.

Aldesuquy and Ghanem (2015) studied about two wheat cultivars that was water stress sensitive (Gemmieza-7) and water stress tolerant (Sahel-1) under water deficient condition by the foliar application of Tre. Under water deficit condition the activities of POD, AAO, PAL was significantly enhanced but in well water conditions the PPO performance was decreased in case of both cultivars of flag leaf at the period of grain filling. Between these two cultivars, the highest enzymatic activity was observed for tolerant cultivars and the sensitive cultivars showed the lowest performance. However, Tre application was efficient to mitigate the negative effects of water deficit stress and better response observed for sahel-1 than the Gemmieza-7.

Chapter III

MATERIALS AND METHODS

This chapter represents a concise statement about the materials and methods of experimental site, climatic condition, soil, preparation of land, transplanting, intercultural operations, irrigation, drainage, fertilizer application, various planting materials including data collection and analysis. Description of materials and methods given below:

3.1 Experimental site

To conduct this experiment in the experimental plot of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, at the period from July 2018 to November 2018. The experimentalarea waslocated between 23°41' N latitude and 90°22' E longitude at an elevation of 8.6 meter from the sea level.

3.2 Soil status

The land of soil is belonged to the Modhupur tract (AEZ No. 28). The soil was dark gray non-calcareous and medium high land. Its pH value ranging from 5.47-5.63.

3.3 Climate

The area of this experiment was situated in the subtropical climatic zone which also characterized by thehigh humidity, excessive precipitation and heavy temperature including unexpected winds at the time of April to September and nominal rainfall was observed at the time of October to March. The explicit meteorological data such as excessive rainfall, humidity andtemperature associated with sunshine hour was observed via meteorology center, Dhaka.

3.4 Plant materials

BRRI dhan72 (*Oryza sativa* L.) seed was used as a good plant materials in this conducted experiment. The seeds were collected from Bangladesh Rice Research Institute, Joydebpur, Gazipur, Bangladesh.

3.5 Stress treatment

Drought stress was imposed at panicle stage by withholding water for 5, 10 and 15 days.

3.6 Osmolytes treatment

Osmolytes such as Pro and Tre used as a protectants with 10 mM concentration, respectively. All chemicals were purchased from Wako, Japan.

3.7 Treatments of the experiment

Factor A: water stress (4)
1. D_0= Well-Irrigated
2. D_1= Water deficit at panicle stage, 5days
3. D_2= Water deficit at panicle stage, 10days
4. D_3= Water deficit at panicle stage, 15days

Factor B: Protectants (2)
1. Water spray (Water)
2. 10 mM Proline spray (Pro)
3. 10 mM trehalose spray (Tre)

3.8 Growing of crops

3.8.1 Seed collection

Seeds of the BRRI dhan72 test crops were collected from Bangladesh Rice Research Institute (BRRI) Joydebpur, Gazipur, Bangladesh.

3.8.2 Seedling raising

The rice plant seedlings raised in wet seedbed at SAU farm. By effectivesoaking of this seeds being sprouted after 72 hours.Uniformly this sprouted seeds sown in the seedbed in 25th July, 2018. Adequate take care was done in the seedbed to grow the seedlings. Appropriate irrigation was provided but no fertilizer and manure application. Properly the unwanted vegetation were picked up from the nursery bed.

3.8.3 Preparation of the main field

To conduct this experiment the selected plot was opened in 17th September, 2018 with the help of power tiller and exposed to the direct sun for a week and this land harrowed properly, cross ploughed and ploughed was done in many times by laddering to achieve well tilth. Unwanted stubbles and weeds were properly dispelled, and ultimately observed a well tilth soil for transplanting of rice seedlings.

3.8.4 Application of fertilizers

The following optimum doses of fertilizers and manure were used.

Fertilizers	Dose (kg ha^{-1})
Cow dung	5000
Urea	220
Triple super phosphate (TSP)	165
Murate of Potash (MP)	180
Gypsum	70
Zinc sulphate	10

One third portion of urea with full amount of cow dung, MP, TSP, zinc sulphate and gypsum were applied in the field during final land preparation following broadcasting method. Rest amount of urea was applied in the field at 20 DAT and 45 DAT, respectively.

3.8.5 Uprooting of seedlings

When the nursery bed was fully prepared and become moist through the application of water before one day of seedlings uprooting. The seedlings were uprooted carefully this seedlingson 27th August, 2018 without any kinds of mechanical damages of the roots.

3.8.6 Seedlings transplanting

The transplanting of seedlings was done on 27thAugust, 2018 and each line of the seedlings was maintained properly where distance of plant to plant was 15 cm and line to line was 20cm in the plot.

3.8.7 Intercultural operations

Various kinds of intercultural operations was done at the period of experimentation which given below:

3.8.8 Irrigation and drainage

Adequate amount of water was provided in the field to maintain the proper growth of the plants. Always, the proper drainage system was balanced causes for the removal of top dress urea including the excess amount of rain water.

3.8.9 Gap filling

Gap filling was done all the plots after the transplanting of 7 to 10 days with the similar aged seedlings.

3.8.10 Weeding

Weeding was performed at 10, 25 and 45 day, respectively after the transplanting and the plants were keep free from weeds.

3.8.11 Plant protection

Rice plants seriously infested by the several insects such as rice hispa, rice bug, leaf roller and rice stem borer which were controlled by applying some insecticides like Ripcord and Diazinon @ 10 ml/10liter water and also applied in the 5 decimal lands in cases of both pots and plots.

3.8.12 General observation of the experimental plots

Each plot was observed carefully that the plants remain normal green in color. At any kinds stage of the plants no lodging was observed. Uniformly not occurred the highest tillering, initiation of panicles and flowering.

3.9 Detecting maximum tillering and panicle initiation stages

The highest tillering and initiation of panicle phases was carefully observed in the field inspection. If the tillering number per hill was high that was indicated the maximum tillering phase. When the little amount of enhancement was observed at the superior nodes of main stem which look like a dome and revealed that the starting of panicle initiation. This stage was varied with the drought treatments.

3.10 Harvesting, threshing and cleaning

Crop harvesting totally rely on the period of grains maturity and this operation done in every plots manually. The maturation period of the grains was evaluated when the grains become golden yellow in color nearly about 80 to 90%. Each plot crops were separately harvested, carefully bundled, tagged properly after that transferred to the threshed floor. Carefully these seeds were harvested, threshed and cleaned. Properly taken the fresh weight of straw including grains. After that the straw materials was dried properly with the help of sunlight and the total grain production and materials of straw per plots were recorded.

3.11 Data collection

Data were collected on the following parameters:

3.11.1 Crop growth parameters

a) Plant height

3.11.2 Physiological parameters

a) Relative water content of main leaf

b) Relative water content of flag leaf

c) SPAD value of main leaf

d) SPAD value of flag leaf

3.11.3 Yield contributing parameters

a) Effective tillers number hill^{-1}

b) Non-effective tillers number hill^{-1}

c) Panicle length

d) Panicle number hill^{-1}

e) Filled grain no. panicle^{-1}

f) Unfilled grain no. panicle^{-1}

3.11.4 Yields

a) 1000 grain weight

b) Grain yield plant^{-1}

c) Straw yield plant^{-1}

d) Harvest index (%)

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

3.12.1 Plant height

The plant height was observed in centimeter. The measurement was done from the base to top of the flag leaf and after that it was averaged.

3.13 Procedure of sampling phenological parameters

3.13.1 Chlorophyll content

Randomly chosen the three leaves of the plant each plots.The bottom, middle and top portion of the plants leaves carefully measured. After that this value averaged and measured the SPAD value.

3.13.2 Relative water content

Randomly chosen three leaves of the plant in each plots chopped through scissors.The fresh weight (FW) of leaf laminas was taken and immediately sink into distilled water in fresh petridish at the period of four hours in dark condition. Turgid weight (TW) was taken by the using of tissue paper to remove the additional leaf surface excess water. Dry weight (DW) was taken after drying the materials at 60^0 C for 48 hours. RWC was calculated the following formula:

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

3.14 Procedure of sampling yield contributing parameter

3.14.1 Plant height

Randomly the height of five plant was taken from the bottom level to upper level of leaf in each plots.

3.14.2 Number of effective tillers hill^{-1}

The number of total tillers hill^{-1} were computed from samples and were categorized in non-effective and effective tillers per hill.

3.14.3 Panicle length

This parameter was calculated from the rachis basal nodes to above the panicle.

3.14.4 Number of total grains panicle^{-1}

For each replication 5 panicles were selected randomly from all the grains were carefully counted and finally determined the average number of grains for each panicle.

3.14.5 Grain yield plots^{-1}

When the plants were ready to harvest, grain yield plots^{-1} were isolated through threshing the plants. After completing threshing the grains were sun dried and then weighted the grain yield throughing measuring scale properly.

3.14.6 Straw yield per plots^{-1}

After completing the measurement of grain yield plots^{-1}, straw yield plots^{-1} were taken. Straw effectively isolated via threshing on the basis of plant^{-1} and weighted correctly.

3.14.7 Weight of 1000-grain weight

Thousands grains from five samples per plants were counted, cleaned and sun dried and was properly weighted by an electronic balance.

3.14.8 Harvest index

It indicates the ratio of economic yield and biological yield and calculated by the following formula. The formula was

Harvest index (HI) = Grain yield/Biological yield x 100

3.15 Statistical analysis

The collected data were subjected to analysis statistically by the following of computer based software CoStat v.6.400 and two-way analysis of variance (ANOVA). To determine the replications mean differences. Fisher's least significant difference (LSD) test at the 5% level of significance was applied.

Chapter IV

RESULT AND DISCUSSION

4.1 Crop growth parameters

4.1.1 Plant Height

4.1.1.1 Effect of drought

Sharp reduction occurred for plant height of different drought stress condition (Figure 1A). All the drought condition highest plant height observed at D_0 (6.3%) drought condition compared to D_3 condition and considerable variation observed for other drought stress condition.

4.1.1.2 Effect of osmolytes

Osmolytes caused a significant variation observed for plant height (Figure 1B). Proline (2.29%) and Tre showed (1.5%) the highest plant height contrast to their watered condition.

4.1.1.3 Interaction effect of drought and osmolytes

Remarkable decline was recorded for plant height of different drought condition (Figure 1C). Interaction between drought and protectants level in relation to plant height for considerable variation was observed. The highest plant height was recorded for 1.7%, 1.9%, 2.4% for Pro and 1.6%, 1.4%, 1.3% for Tre respectively, in D_0, D_1 and D_2 drought condition compared to their respective watered condition (Figure 1C). On the other hand, the lowest plant height was recorded for watered (1.2%) condition at D_3 drought stress which also compared to D_0 (5.2%), D_1 (3.3%) and D_2 (2.3%) drought stress condition. In D_2 and D_3 drought stress condition for trehalose showed statistically similar result.

Exogenous application of osmolytes conduced to ameliorate the height of plant and thus the osmolyte plays a positive role for adjusting osmotic balance and which is also help to inhibit the water loss and elevate the height of plant (Bhuiyan *et al*., 2019).

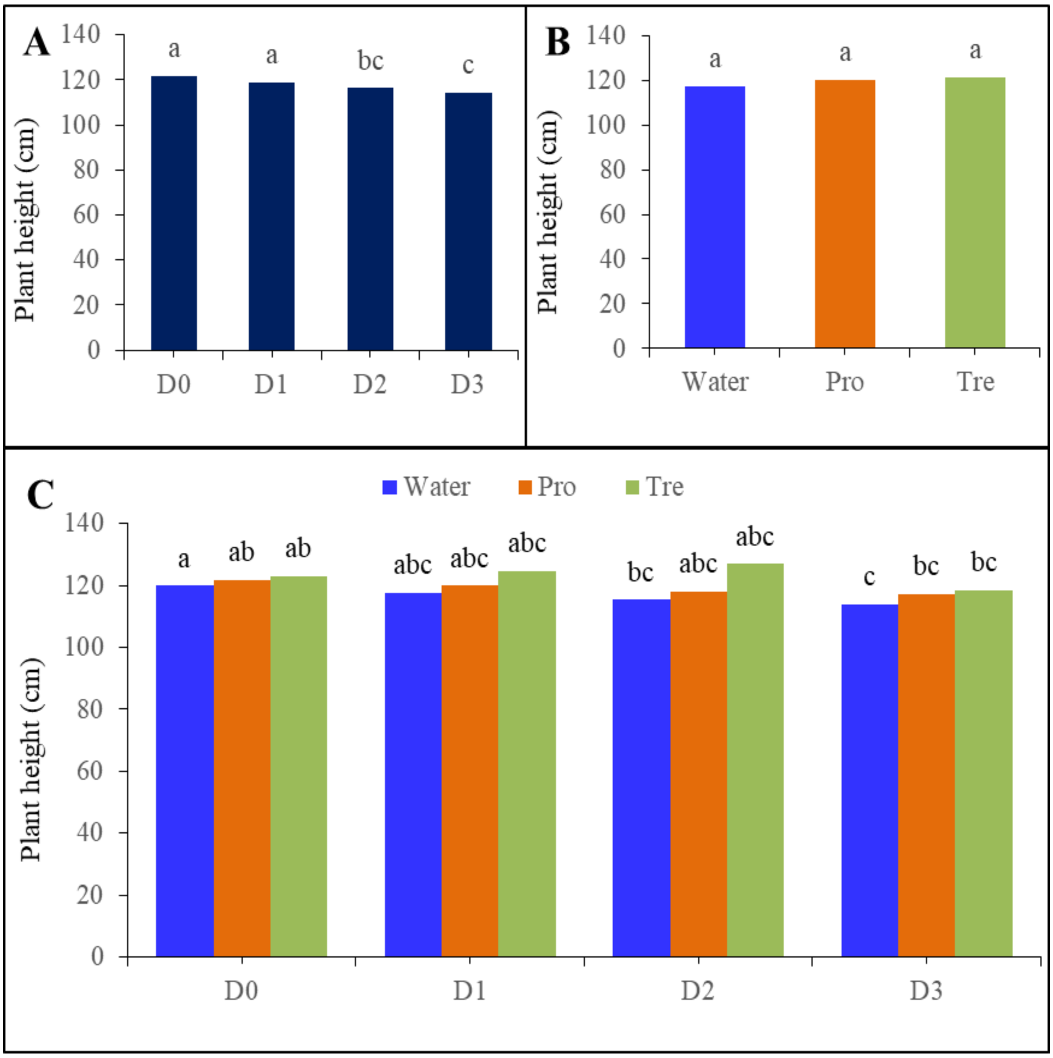

Figure 1. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on plant height (cm) of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Sultana *et al.*, (2014) found that the BRRI dhan47 (66.27 cm at 70 DAT, 95.72 cm at 90 DAT and 89.57cm at harvest) gave the higher plant height at the different drought stress condition than BINA dhan10. Due to the effect of drought stress the plant height was drastically reduced. Similarly, Ahmad *et al*. (2019) stated that the drought stress was directly affected the plant causes for the plant height was decreased due to dehydration of protoplasm including turgor loss and reduction of cell division and cell expansion.Plant molecular and cellular levels responded by the drought stress which ultimately reduced the plant growth and yield (Arzanesh *et al*., 2011).Minimization of water availability and turgor loss owing to imbalanced the osmotic regulation made by the water deficit which is called the primary effects and causes for reduction of plant height. Severe drought stress causes to reduction in plant height including tiller number per plant, biomass production and total grain yield (Tuteja*et al.*, 2013).The plant-water relations is also affected by drought stress where the leaf and plant water content also reduced, cell division and cell expansion significantly inhibited as well as the growth of whole plant (Alam *et al.,* 2014). The drought stress is one of the greatest environmental factors among different kinds of abiotic stresses which ultimately reduces the growth and development including yield of the plants. The plant height significantly enhanced by the exogenous application of osmolytes under drought condition at vegetative and flowering stage because it was developed the tolerance mechanisms. Drought stress have a detrimental effect on plant productivity due to changing the plant growth patterns including physiological and biochemical responses (Hasanuzzaman *et al*., 2018b).

4.2 Physiological parameters

4.2.1 Relative Water Content in main leaf

4.2.1.1 Effect of drought

There was a significant variation observed due to effect of drought condition where the D_0 drought stress produced the highest relative water content which was 16.98% compared to D_3 condition and the other drought stress condition showed statistically similar result (Figure 2A).

4.2.1.2 Effect of osmolytes

Effect of osmolytes caused a considerable variation observed for relative water content in the main leaf (Figure 2B). Trehalose (4.40%) and Pro (3%) recorded the highest relative water content compared to their watered condition.

4.2.1.3 Interaction effect of drought and osmolytes

Upon exposer to the relative water content in main leaf significantly increased for Tre in drought stress condition contrast to their respective watered condition (Figure 2C).

The highest relative water content in main leaf was recorded for Tre 17.3%, 9.2%, 21.2% and Pro 10.32%, 4.2%, 3.8% for D_0, D_1 and D_3 drought condition compared to their watered condition (Figure 2C). On the contrary, the lowest RWC was found for watered (6.8%) at D_3 drought condition. Watered and Pro in D_2 drought stress condition showed the statistically similar result.

Drought stress also hampers the plant water relations, diminish the leaf and plant water content causes for osmotic stress which is resists the cell division and cell expansion as well as the total growth of the plant (Kirkham, 2005; Alam *et al.*, 2013). Osmotic adjustment and RWC is a very important factor for better growth and development of the plant but effect of doughtiness causes for reduction the growth of plant (Khan *et al.*, 2017). It was well to known that, when the transpiration exceeds the uptake of water, turgor of cell decline as RWC and cell volume was reduced (Lawlor and Cornic, 2002) and lower RWC and turgor pressure causes the stomatal conductance and plant growth was decreased. Relative Water Content of main leaf was reduced under different drought stress condition shown in Figure 2A. Lima *et al.*, (2015) and Mostofa *et al.* (2015) reported that due to drought stress the RWC was strongly decreased. After two year studied Doan *et al.* (2019) showed that the various effect of drought stress wasrecovered subsequently due tothe accumulation of proline and trehalose at the growth stage of plant resulting the RWC of main leaf enhanced significantly. Relative water content of main leaf was decreased causes for turgor loss under drought conditionand the process of cell expansion occurred for availability of limited amount of water by the application of osmolytes (Katerji *et al.*, 1997). An experiment was showed that the RWC of main leaf was increased due to the accumulation of proline under effect of drought condition for BRRI dhan-56among the different kinds of rice varieties namely BRRI dhan-56, BRRI dhan-38, BRRI dhan-34, BRRI dhan-32 and BRRI dhan30 (Saha *et al.*, 2019). Osmotic balance was significantly maintained by the use of different kinds of osmoprotectants such as Pro, Tre and also stabilized the proteins andmembranes under severe drought and other stress conditions (Nahar *et al.*, 2016a). Drought stress have a detrimental effects on metabolic process and physiology of the plant (Hasanuzzaman *et al.*, 2014).

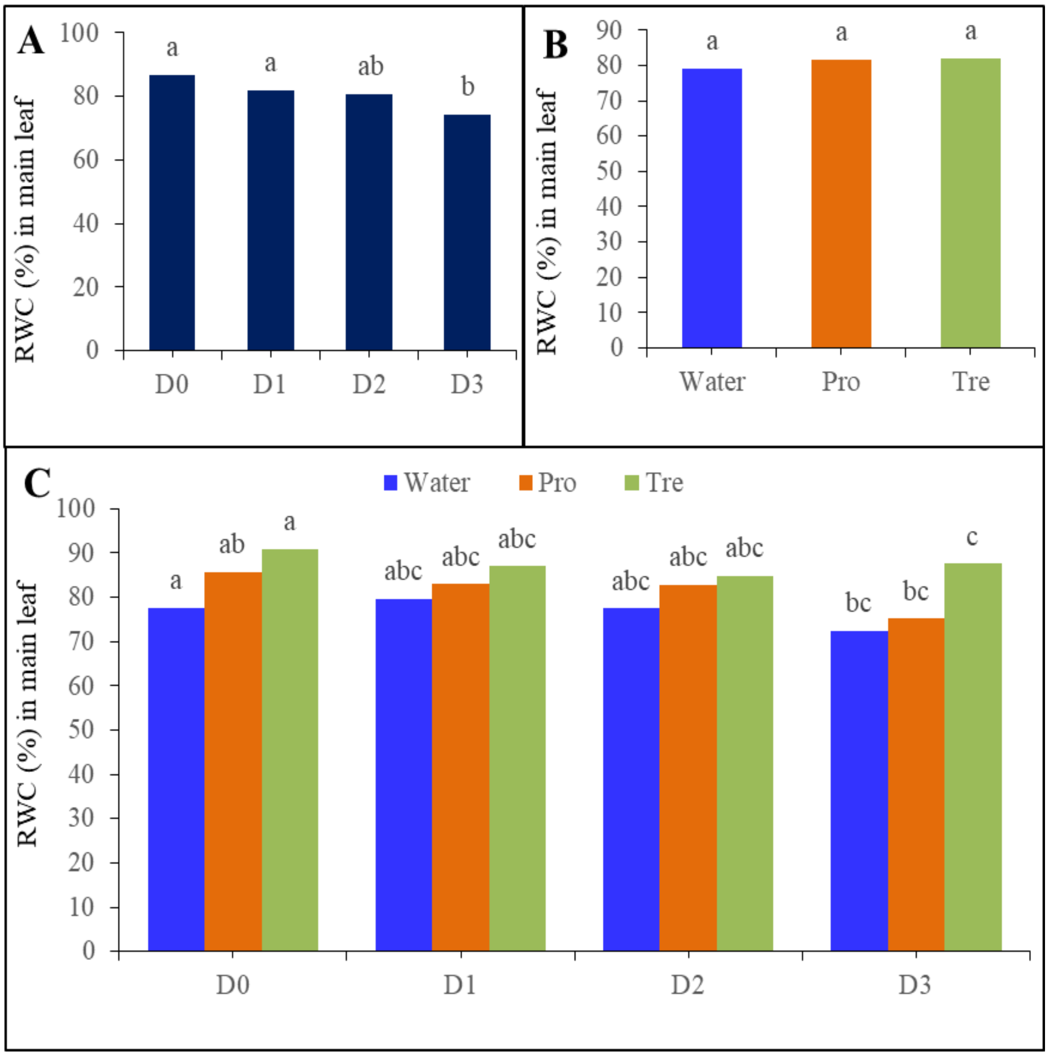

Figure 2. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on relative water content (%) in main leaf of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.2 Relative Water Content in flag leaf

4.2.2.1 Effect of drought

Different kinds of drought stress showed the significant variation for relative water content in flag leaf (Figure 3A). At D_1 (12.5%), D_2 (10.8%) and D_3 (5.9%) drought stress condition recorded the highest result compared to D0 drought condition.

4.2.2.2 Effect of osmolytes

Significant variation observed for relative water content by the application of different kinds of osmolytes (Figure 3B). Trehalose (32.3%) and Pro (12.5%) produced the highest RWC in flag leaf compared to their watered condition.

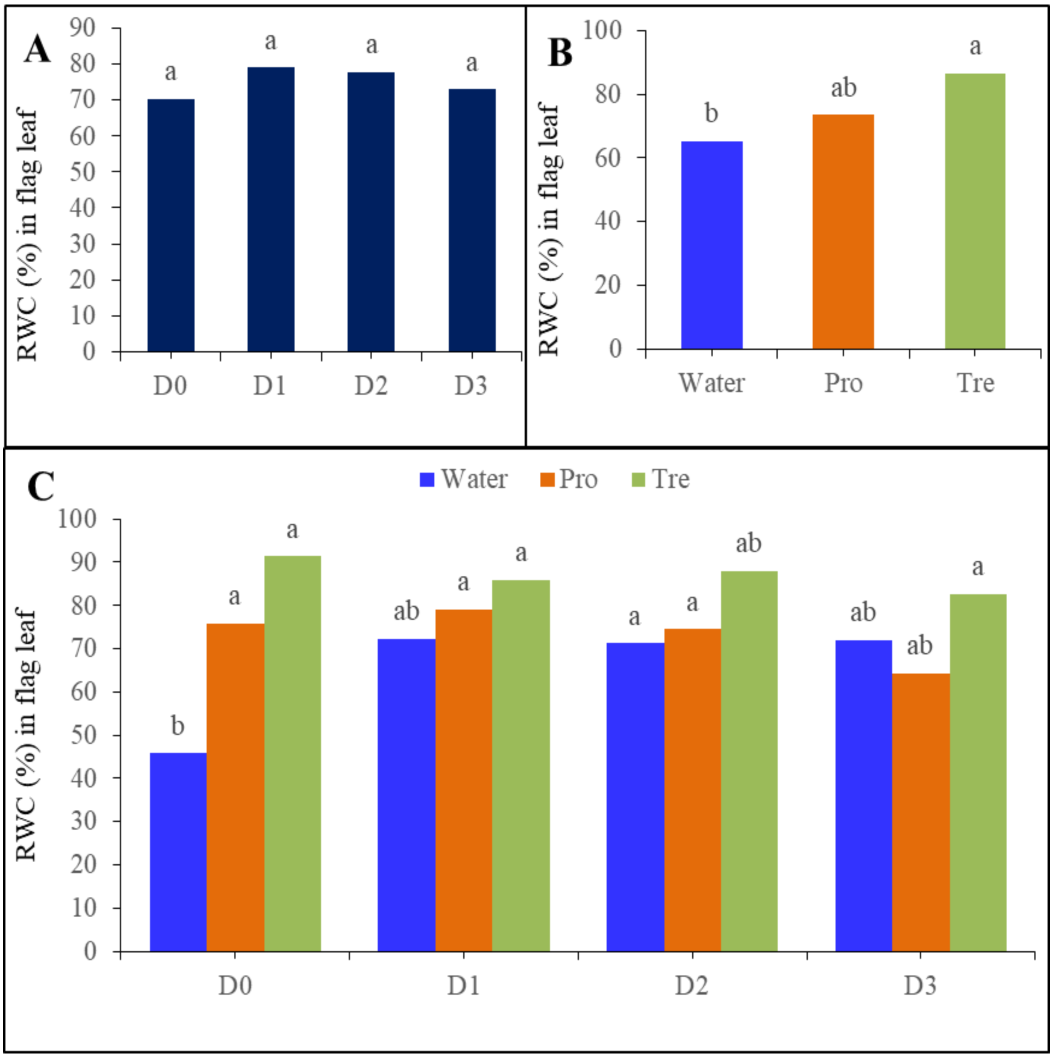

Figure 3. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on relative water content (%) in flag leaf of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.2.3 Interaction effect of drought and osmolytes

The levels of RWC significantly increased at all the drought condition upon exposer to Tre compared to their respective watered condition (Figure 3C). The highest RWC in flag leaf was recorded 99.6%, 18.8%, and 22.2% for Tre and 65.6%, 9.5%, and 6.7% for Pro at D_0, D_1 and D2 drought condition contrast to their untreated watered condition (Figure 3C). On the contrary, at D_3 drought condition for RWC exposer to control (11.9%) showed the highest result than the Pro. At D_0 drought condition water (28.9%) showed the lowest result.

Relative water contentof flag leaf was increased for different rice cultivars by the use of osmolytes under water deficit condition especially in IR20 and PT1 variety (Chaum *et al.,* 2010). During the development of flag leaf water deficit stress distinctly affect the anatomy of plant where the leaf area and thickness also reduced (Biswal and kohli, 2013). Relative water content of flag leaf of two rice cultivars namely BR11 and BRRI dhan28 were observed that the correlation between flag leaf was highly positive and significant (Rahman *et al.*, 2013). Under drought condition two rice varieties namely Super-7 and PR-115 were observed to evaluate that the RWC of flag leaf and osmotic adjustment enhanced; however, Super-7showed the more pronounced reduction (Khan *et al.*, 2017). Relative water content and water potential of flag leaf was decreased due to drought effect progressively also stomatal conductance was reduced leading to decline CO_2 assimilation (Hasanuzzaman *et al*., 2013). Tre and Pro plays a vital role for protection of biological and physiological structure from drought condition and finally, increased the productivity of the plants (Garg *et al*., 2002). In the present study, stressed plant also treated with Tre resulted to increase the Tre endogenous level (Figure 3C), which likely to be indicated that the root easily uptake the Tre and also transported to the plants aerial parts. This result corroborate with previous findings (Luo *et al.*, 2010). The water content and biomass production of plant increased by the exogenous application of osmolytes such as Pro and Tre might have been the active role of these osmolytes in the osmotic adjustment of the plants which is significantly increased the water uptake and development of the growth of plants (Ali 2011; Alam *et al*., 2014; Hossain *et al*., 2014).

4.2.3 SPAD value of main leaf

4.2.3.1 Effect of drought

The SPAD value of main leaf varied significantly due to drought effect (Figure 4A). Sharply reduction occurred by the effect drought. The highest SPAD value observed at D_0 (30.5%) drought condition contrast to D_3 (23.4%) drought condition.

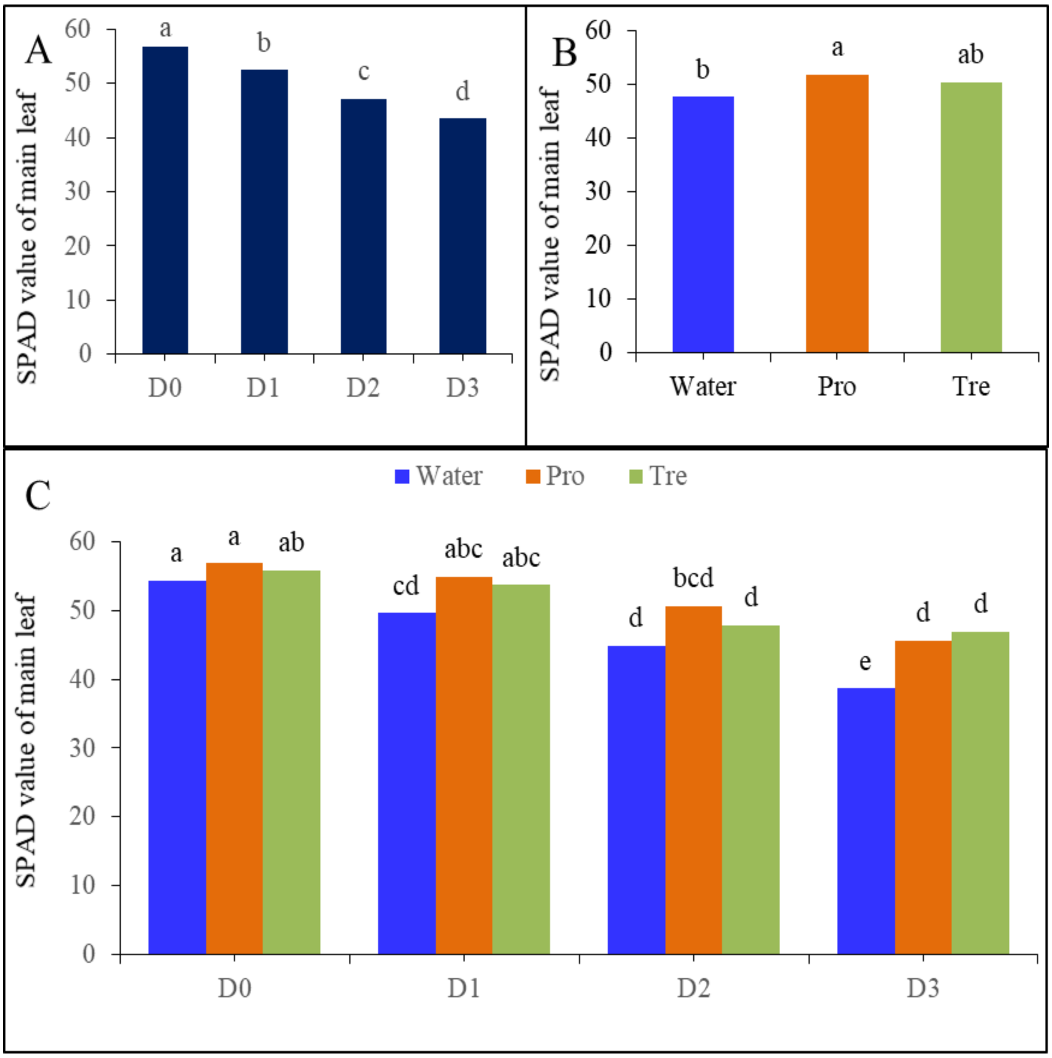

Figure 4. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on SPAD value of main leaf of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.3.2 Effect of osmolytes

Osmolytes caused a significant variation observed for SPAD value of main leaf (Figure 4B).The highest SPAD value of main leaf was recorded for Pro (8.7%) and Tre (5.7%) compared to their watered condition.

4.2.3.3 Interaction effect of drought and osmolytes

Upon exposer to considerable reduction was recorded for SPAD value of main leaf (Figure 4C). The highest SPAD value was observed for Pro (2.7%) at D_0 drought condition compared to their untreated watered. On the other hand, the lowest SPAD value was observed for watered (14.3%) at D_3 drought condition than Pro (18.6%) and Tre (21.5%). Interaction between drought and osmolytes level in relation to SPAD value of main leaf showed the considerable variation which was 10.8%, 13.3%, 18.6% for Pro and 8.3%, 6.7%, 21.5% for Tre at D_1, D_2 and D_3 drought condition compared to their respective watered condition (Figure 4C).

Interaction of Tre and Pro including drought stress ameliorated the levels of photosynthetic pigments and it’s occurred due to significantly biosynthesis of these pigments (Hoque *et al.,* 2007; Alam *et al.,* 2014; Hasanuzzaman *et al.,* 2014). For better crop yield chlorophyll plays a vital role in plant life and which is likely to be a crucial part of photosynthetic pigment (Nahakpam, 2017). The quantity of chl a and b in the main leaf of crop plants has been changed during the effect of drought leading to altering the photosynthetic capacity (Zhang *et al*., 2020). Chlorophyll a, b and total content of chl was largely decreased due to drought stress including the yield of the plants (Mafakheri *et al*., 2010). The highest chlorophyll content was observed for proline (2.7%) at D0 drought condition compared to their untreated watered condition. On the other hand, the lowest chl. content was observed for watered (14.3%) at D3 drought condition than Pro and Tre. Similarly, Timung and Bharali (2020) concluded that thedrought stress caused the chl. content significantly differed at the heading and maximum tillering stage where the Inglongkiri (32.46) recorded the highest chl. content than the Sovak (30.75) and the lowest chl. content was recorded for Balam (26.753) than Sok langlu (28.723). Due to the drought stress chl. content was severely decreased resulting directly injured the chloroplast due to the active oxygen species. By the effect of drought stress resulting the fixation of CO_2 and photosynthetic rate was decreased causes for minimum amount of assimilates generation for crop growth, development and yield of plants. Under drought stress the chl content was decreased and it considered the important symptom causes for the chl. degradation and pigment of photo oxidation (Anjum *et al.*, 2011). Plant photosynthetic process is very important to regulate the growth and development of plants, and this process is very sensitive to drought stress of the higher plants. Kadhimi *et al.* (2016) concluded that the chl content of rice was strongly decreased due to drought and salinity where these stress also minimized by the exogenous application of osmolytes such as Pro, Tre, and GB. Exogenous application of Pro and Tre significantly enhanced the chl pigment under drought stress condition where the productivity of plants also increased (Hanif *et al.,* 2020). Total reduction of photosynthesis, inhibition of transpiration, stomatal conductance, index of membrane stability, also PS II activity and relation of plant water along with the growth and yield also reduced under drought stress of rice plants (Hasanuzzaman *et al.*, 2018b).

4.2.4 SPAD value of flag leaf

4.2.4.1 Effect of drought

Considerable enhancement was observed for SPAD value of flag leaf due to drought stress shown in figure 5A. The highest drought stress was recorded at D_3 (3.8%) drought condition contrast to D_0 condition where there was no significant difference between D_1 and D_2 drought condition.

4.2.4.2 Effect of osmolytes

There was a significant variation observed for SPAD value of flag leaf by the effect of osmolytes (Figure 5B). The highest value was found for Pro (6.2%) and Tre (2.5%) compared to their watered condition.

4.2.4.3 Interaction effect of drought and osmolytes

The SPAD value of flag leaf was increased sharply by the interaction of drought and osmolytes (Figure 5C). The highest SPAD value of flag leaf was observed at D_3 (8.4%) drought condition for pro compared to their watered condition. On the contrary, the lowest value was observed at D_0 (7.5%) drought condition. The highest value was recorded 3.8%, 6.7%, 8.4% for Pro and 1.4%, 2.3% and 2.7% for Tre respectively, at D_1, D_2 and D_3 drought condition compared to their untreated watered conditon. At D_1

and D_3 condition for Tre and D_2 and D_3 condition for control showed significantly similar result.

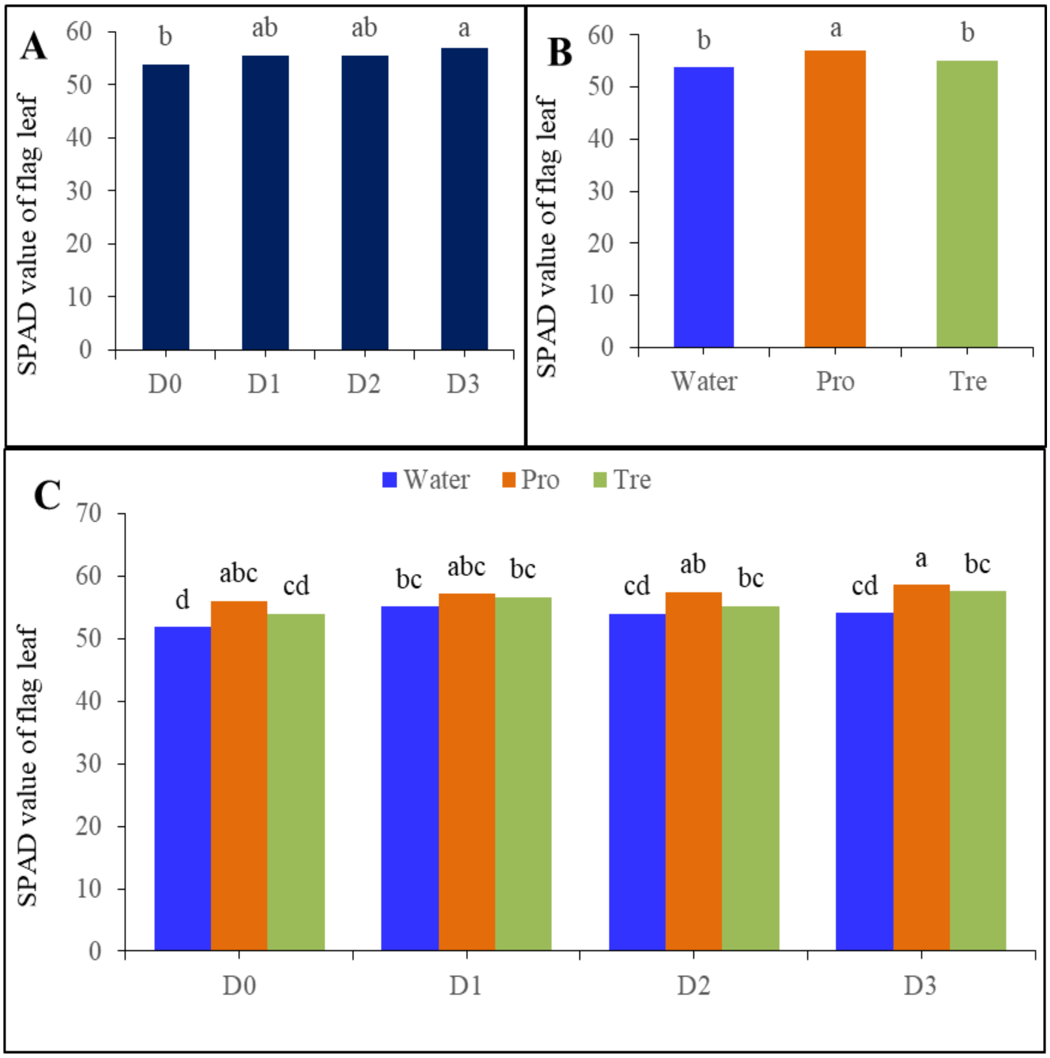

Figure 5. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on SPAD value of flag leaf of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray.Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Flag leaf sheath, culm and internodes increasingly play an important role as a source of carbohydrate and photo assimilates during grain filling (Biswal and Kohli, 2013). Drought stress have a detrimental effect on crop growth and development by the influencing of various biochemical and physiological functions like the chl. synthesis, photosynthesis, translocation and uptake of ion, respiration, nutrient and carbohydrate metabolism (Hussain *et al.*, 2018). The chl. content of flag leaf was declined by the interaction of different kinds of drought and protectants shown in figure 5C. Similarly, Saha and Gupta (1993) observed that the chl content of flag leaf was decreased due to drought stress also might be associated with amino acid and soluble protein levels likely to be decreased. Moreover, the CO_2 uptake through stomatal regulation or internal resistance to diffusion of CO_2 leading to reduction of photosynthesis and enhance of photorespiration favoring to the oxygenase activity under drought condition. Gill *et al.* (2013) showed that the drought stress significantly altered the metabolism of chloroplast by inhibiting the biosynthesis process of chlorophyll. Drought stress caused for reducing the compounds of photosynthetic pigments such as carotenoids, chl, anthocyanin etc. in different kinds of plants and it's occurred for the oxidation of pigments, injured the pigments of biosynthesis and so on (Garcia-Plazaola *et al.*, 2003; Anjum *et al.*, 2011). Ali and Ashraf (2011) found that thefoliar application of Tre significantly enhanced the photosynthetic activity and improved the plant biomass production and relation between plant-water including solute potential, water potential and turgor potential causes for the application of Tre also alleviate the plants from oxidative damage by increasing some enzymatic (CAT and POD) and non- enzymatic (Phenolic and Tocopherols) antioxidant compounds. Photosynthetic rate was increased for drought stress plants by the exogenous application of Pro which was strongly connected with stomatal conductance (gs) and sub-stomatal CO_2 (Ci) (Ali *et al.*, 2007). Osmolyte plays the other mechanisms that the protection of biological membrane, assuage the ionic toxicity, significantly detoxification if toxic compounds like ROS, redemption from photosynthetic and mitochondrial structure (Alam *et al.*, 2014).

4.3 Yield contributing characters

4.3.1 Effective tiller no.hill^{-1}

4.3.1.1 Effect of drought

There was a considerable variation observed for effective tillers hill^{-1} due to drought effect shown in figure6A. It was observed that D_0 drought condition produced the highest effective tiller (28.22%) compared to D_3 drought condition which was produced the lowest effective tiller (22%) where D_1 and D_2 drought condition showed statistically similar result.

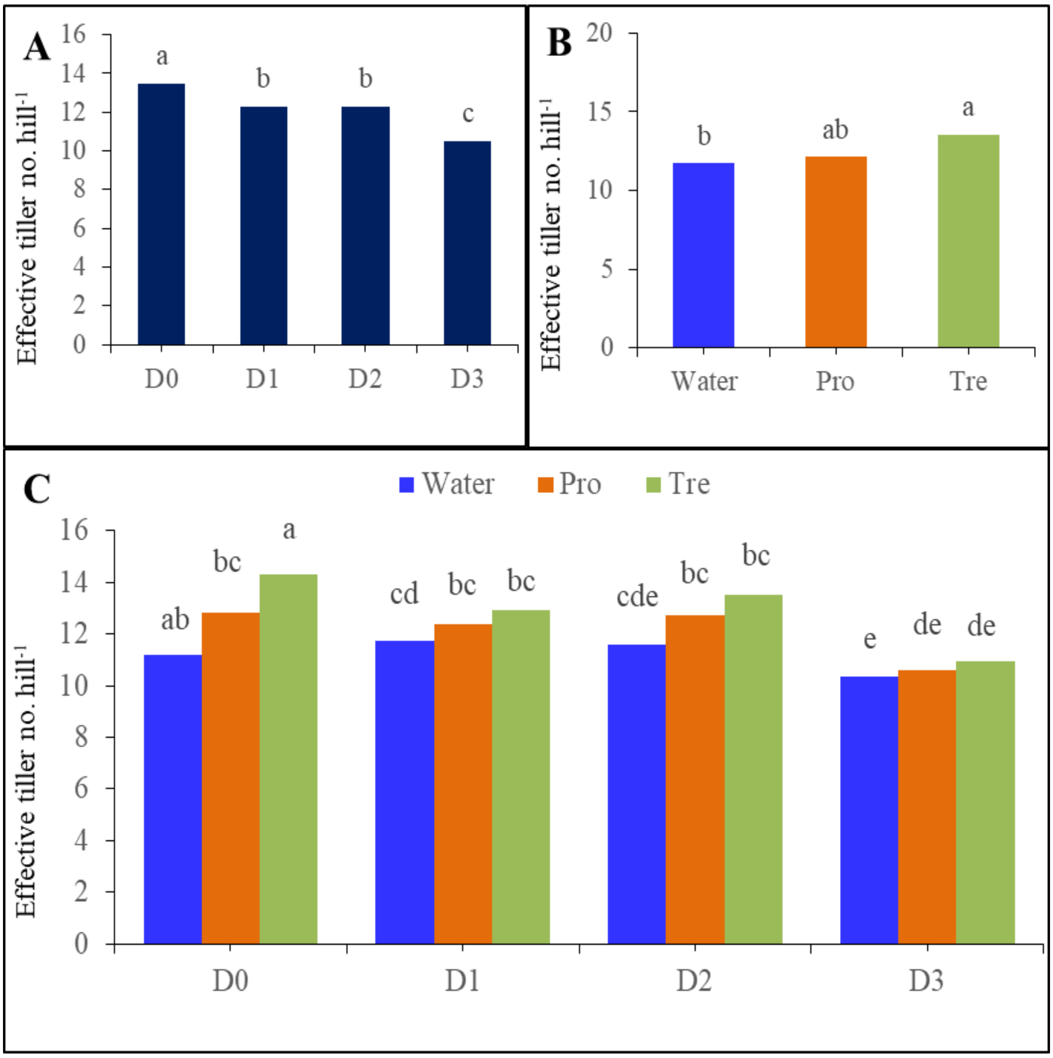

Figure 6. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolyteson effective tiller no. hill^{-1} of rice. Here, D$_0$= Well-Irrigated, D$_1$= Water deficit at panicle stage, 5days, D$_2$= Water deficit at panicle stage, 10days, D$_3$= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.1.2 Effect of osmolytes

Osmolytes caused a significant variation occurred for effective tiller number hill^{-1} compared to watered condition (Figure 6B). The highest effective tiller observed for Pro and Tre which was 3.56% and 15.48% above contrast to watered condition.

4.3.1.3 Interaction effect of drought and osmolytes

Exposer to drought stress resulted in significant variation observed for effective tiller number hill^{-1} (Figure 6C). Effective tillers showed the highest result 14.27%, 5.71%, 9.74% for Pro and 27.83%, 10.23%, 5.80% for Tre at D_0, D_1 and D_2 drought stress condition contrast to their untreated watered (Figure 6C). In all the cases the highest effective tiller found for Tre (27.83%) at D_0 drought stress condition compared to wateredcondition. In D_3 drought stress condition there was no significant difference between them.

The tillering capacity is the major characteristics of the crop plants and it also plays a significant contribution to the evaluation of grain yield because it hampers the per unit area panicle number. The plant survival and tillering ability partially rely on the various environmental factors like soil water relations, nutritional status, temperature, radiation and also varietal traits. Effective tiller numbers also increased with the application of osmolytes than the control condition shown in figure 6C. Likewise, Herath *et al.* (2014) stated that exogenous application of osmoprotectants increased tillers number furthermore with increasing the drought or water deficit stress resulting the effective tiller number was decreased significantly. Effective tiller is an important parameter that reveal the total plant area and crop leaf area index (LAI) which is greatly damage the grain yield under drought condition (Ibrahim *et al.,* 2010). Drought or water deficit stress affects the expansion of crop growth, obstruct the cell elongation and cell division, impedes the germination of rice seedlings, reduced effective tiller number and also plant height (Sahebi *et al*., 2018). Khatun *et al.* (1995) observed that drought stress delayed the flowering, diminished the number of effective tillers and the number of productive florets per panicle. There are different kinds of literature showed that the drought and salinity stress diminished the leaf area, plant height, dry mass and effective tiller in rice (Ashfaq *et al.,* 2012; Ishak *et al*., 2015), wheat (Wang *et al.,* 2010), barly (Ahmed *et al*., 2013a) and maize (Khan *et al.,* 2015).Water deficit stress was more deleterious stage at grain filling condition followed by the stage of panicle initiation of rice plant regarding the total spikelet per panicle, filled grain per panicle, 1000 grain weight per panicle and effective tiller number per hill and these causes for the photosynthetic activity was diminished significantly resulting the total yield of plant was greatly reduced (Sharifunnessa and Islam, 2017).

4.3.2 Non-effective tiller no. hill^{-1}

4.3.2.1 Effect of drought

Significant variation observed for non-effective tiller hill^{-1}at different drought stage (Figure 7A). Drought stress condition D_3 (76.88%) produced the highest effective tiller compared to D_0 (43.47%) drought stress condition and the D_1, D_2 drought condition was showed statistically similar result.

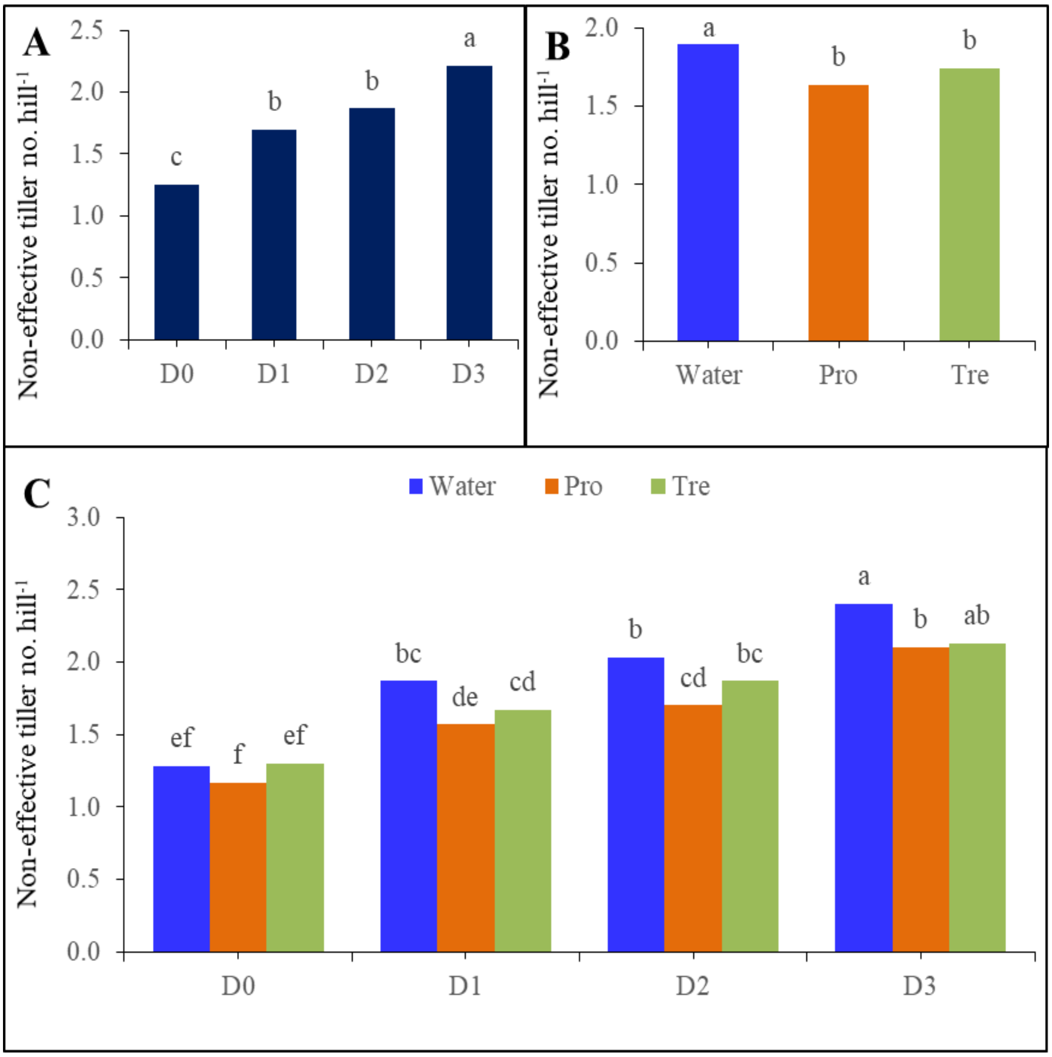

Figure 7. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on non-effective tiller no. hill^{-1} of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.2.2 Effect of osmolytes

Remarkable decline was recorded for non-effective tiller number hill^{-1} by the application of different kinds of osmolytes compared to their watered condition (Figure 7B). The lowest non-effective tiller observed for Pro and Tre which was 13.84%, 8.13% contrasted to their untreated watered condition.

4.3.2.3 Interaction effect of drought andosmolytes

The non-effective tiller number was amplified at different drought stage (Figure 7C). The highest effective-tiller was found on control at D_3 drought stress condition which was 12.7% more than the trehalose. On the contrary, the lowest effective tiller observed on proline at D_0 drought stress condition which was 8.6% compared to the watered condition where the watered and Tre showed statistically similar result. Upon exposer to the drought stress condition for non-effective tiller was decreased by 16%, 16.3%, 12.5% for Pro and 10.7%, 7.9 %, 11.3% for Tre at D_1, D_2 and D_3 drought condition compared to their respective control (Figure 7C).

Highest number of non-effective tiller was produced for control than the osmolytes shown in figure 7C. Similarly, Chouhan *et al.* (2017) showed that the highest non-effective tiller number was observed for Binadhan-16, Binadhan-7 under control condition than the BRRI dhan71. The exogenous application of osmolytes reduced the non-effective tiller number of rice. If the unproductive tillers are diminished or eliminated, it’s not clear that the yield potential of rice could be more increased where tillering capacity of rice is one of the most important characters (Ao *et al*., 2010). The rate of grain yield production of rice plants also influenced by the panicle bearing tillers where the over tillering leads the abortion of tillers, poor grain yield, reduced panicle size also (Badshah *et al.*, 2014). Kandil *et al.* (2010) stated that the drought stress causes for delayed flowering, diminished the number of effective tillers causes for increased the unproductive tillers and also reduced the total number of productive florets per panicle and so on. Generally, more than 40% unproductive tillers have been produced where these kinds of tiller don’t be showed any performance to the ultimate grain yield of the crops, the mineral nutrients also consumed, assimilates and generated by the crop plants in the middle growth stage, thereby the availability of grain filling resources was drastically reduced.

4.3.3 Panicle length

4.3.3.1 Effect of drought

Due to drought stress the panicle length was drastically reduced (Figure 8A).The highest panicle length was found for D_0 (31.6%) drought condition compared to D_3 (24.2%) condition and others drought condition showed the considerable variation.

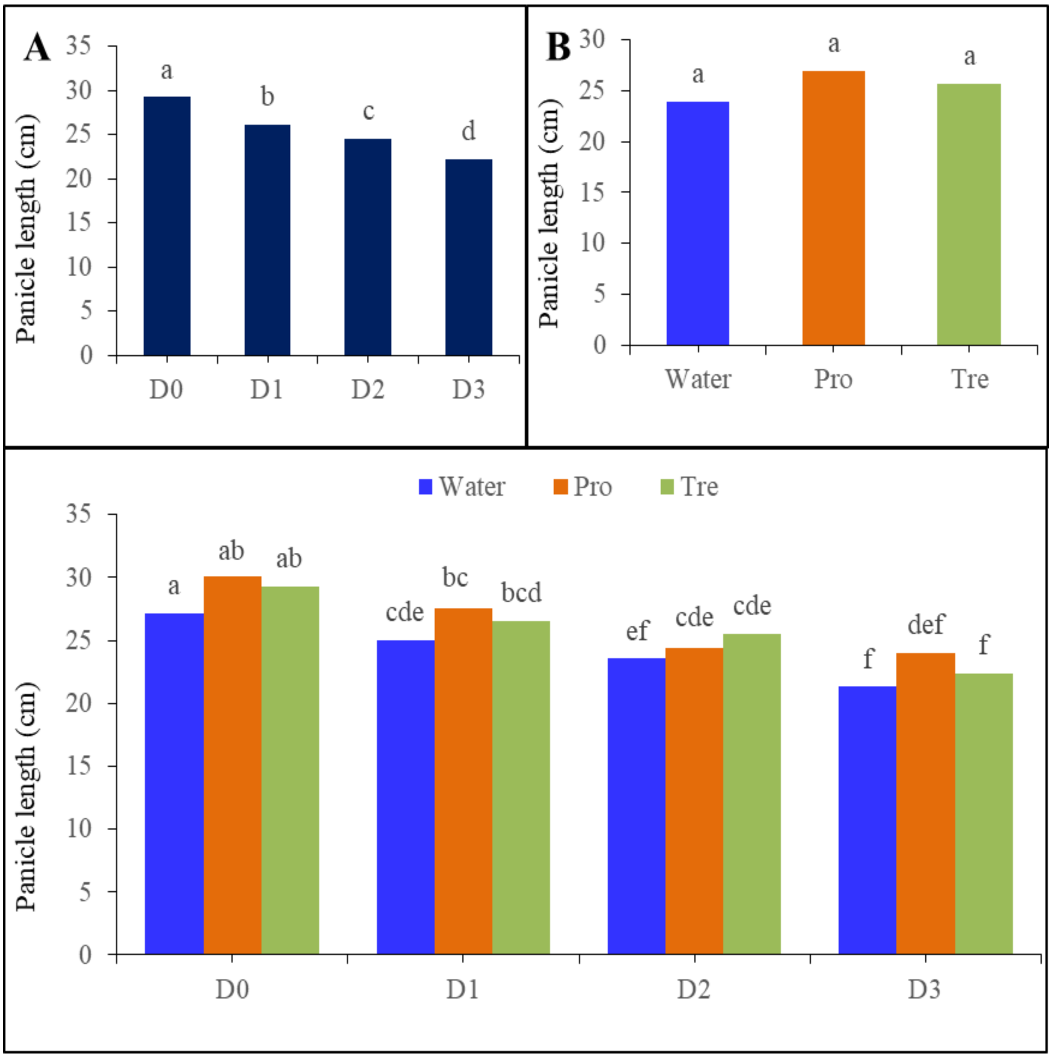

Figure 8. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on panicle length (cm) of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray.Values in a column with Values in a column withdifferentletters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.3.2 Effect of osmolytes

Different protectants treatment showed the considerable variation for panicle length (Figure 8B).The highest panicle length observed for Pro (4.5%) and Tre (3.2%) contrast to their watered condition.

4.3.3.3 Interaction effect of drought and osmolytes

Sharp reduction was observed for panicle length due to drought effect for panicle length (Figure 8C). The highest panicle length was recorded 10.9%, 10.2%, 3.7%, for Pro and 7.9%, 6.7%, 8.5% for Tre at D_0, D_1 and D_2 drought condition as compared to their watered condition. On the contrary, the lowest panicle length was observed for water (4.8%) compared to tre at D_3 drought condition where water and tre showed considerable variation. Moreover, in D_2 drought condition there was no significant variation observed for Pro and Tre.

Drought or water deficit stress caused the panicle development was seriously affected at the reproductive stage of the plant. Water deficit stress was more deleterious at anthesis to maturity (grain filling) stage followed by the panicle initiation, total spikelets panicle^{-1}, filled grain panicle^{-1} and also total grain yield owing to the diminishing of photosynthetic activity consequences the assimilates generation greatly minimized for the panicles growth and grain filled up, finally total rice production was greatly reduced (Sharifunnessa and Islam, 2017). During the rice cultivation various kinds of yield parameters like panicle length, grain weight panicle^{-1}, higher rate of seed setting and panicles bearing primary or secondary branches are drastically diminished by the effect of water deficit stress. Under drought condition diminishing the leaf cell enhancement would reduce the potentiality of vegetative growth and also mitigate the competition along with panicle expansion for assimilates (Ji *et al.*, 2012). Panicle initiation was delayed under drought stress condition and stunted the growth length of panicle and also hastening the flowering rate of crop plants (Craufurd *et al.*, 1993). Spikelets or panicles improvement are the major factor of grain formation at the reproductive stage of crop plants and little changes to the improving of panicle length can severely hampers the yield parameters under drought condition (Sikuku *et al.*, 2012). Effect of osmolytes causes the panicle length gradually enhanced than the control condition of the plant shown in figure 8C. Similarly, both Tre and Pro under water deficit stress condition maintain the tolerance mechanism of plants when the osmolytes also accumulated at more amounts and over expression of different gene such as betaine aldehyde dehydrogenase 1(BADH1), Δ1-pyrroline-5-carboxylate synthetase (P5CS) respectively (Paul and Roy Choudhury, 2019; Kumar *et al.,* 2010; Kim and Nam, 2013; Chen and Murata, 2011). Wei *et al.* (2017) stated that under water deficit condition various kinds of contrasting rice cultivars are separated via characters such as panicle size and shape; grain size, shape and color where the larger panicle and spikelet observed for drought tolerant cultivars than the drought sensitive cultivars owing to take more time for heading and flowering by the impeding effect of water deficit stress in tolerant cultivars than the sensitive cultivars.

4.3.4 Panicle number hill^{-1}

4.3.4.1 Effect of drought

Drought effect caused sharply reduction observed for panicle number hill^{-1} (Figure 9A). The highest panicle number found in D_0 (34.6%) drought condition contrast to D_3 (25.7%) condition where D_1 and D_2 showed statistically significant result.

4.3.4.2 Effect of osmolytes

There was a significant variation observed due to protectants effect for panicle number hill^{-1} (Figure 9B). The highest panicle number observed for Pro (12.4%) and Tre (7.9%) compared to their watered condition.

4.3.4.3 Interaction effect of drought andosmolytes

Number of panicle was decreased in same way which was 6.9%, 16.4%, 17.2% for Pro and 8.2%, 17.4%, 3.5% for Tre at D_0, D_1 and D_2 drought condition compared to their respective control (Figure 9C). The highest (8.2%) number of panicle was observed at D_0 drought condition for Tre contrast to their watered condition where Pro and Tre showed significantly similar result. On the other hand, the lowest (5.2%) panicle number was observed at D_3 drought condition for water where water and Tre showed similar result. In D_2 drought condition, there was no significant variation observed between water and Tre where Pro showed better result.

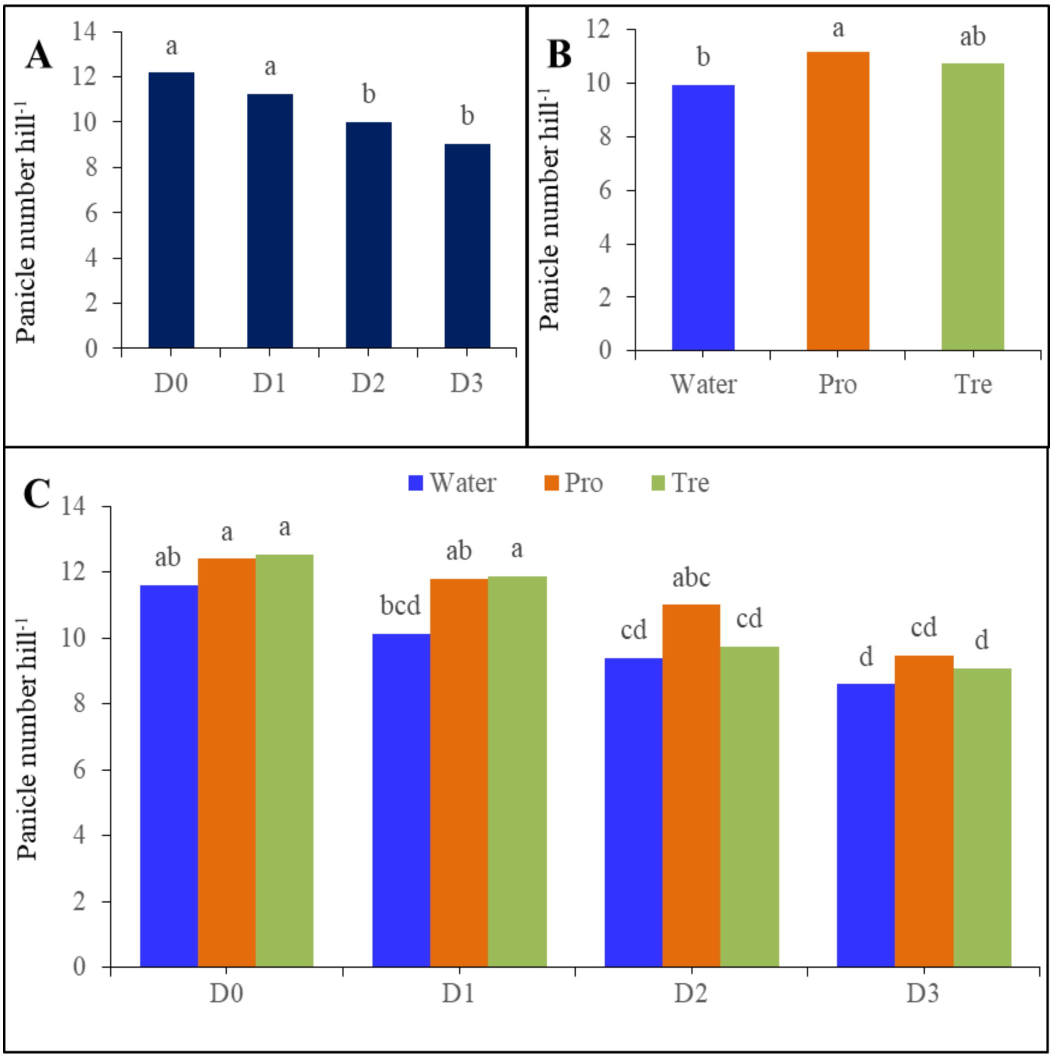

Figure 9. (A) Effect of drought, (B) effect of protectants and (C) interaction effect of drought and protectants on panicle number hill^{-1} of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Water deficit stress during the period of vegetative, flower initiation, and terminal period of crops cultivation could be interrupted the panicle initiation, and grain filling, respectively. Drought stress susceptibility was enhanced with the development of panicle before heading but it severely reduced the production rate (Tsuda *et al*., 1994). If the drought stress affected the plants at flowering stage resulting the panicle number per hill was drastically reduced. There was a significant rapports between the capabilities of panicle production hill^{-1} and panicle size and also delayed the flowering period under water deficit stress condition. Water deficit stress have a negative impact on plant improvement, reduction of the photosynthetic activity, abate panicle numbers per hill, peduncle elongation and ultimately, lower quality of pollen was generated (Mukamuhirwa *et al.*, 2019). Due to the effect of drought stress the panicle number was significantly reduced shown in figure 9C. Similarly, Boonjung and Fukai (1996) concluded that the crop yield decreased up to 30% owing to diminishes the panicle number per hill. Krishnan *et al.* (2011) stated that the number of panicles hill^{-1} was closely interlinked with grain production but there was a negative relation between the spikelets per panicles and panicle numbers per unit area.Total spikelets numbers per unit area was the outcome of panicle numbers hill^{-1} which was totally dependent on effective tillers hill. The panicle initiation of growing stage was the most important stage which was sensitive to drought stress and also expressed the detrimental effect on the plant physiology and agronomic characters (Akram *et al.*, 2013). Among different kinds of crops, rice was the most sensitive plant to water deficit stress particularly at the period of critical growth stage like anthesis, initiation of panicle and grain development (Tao *et al.*, 2006, Yang *et al.*, 2008). Drought stress have a direct effect on panicle exertion, spikelet opening and desiccation causes for sterility of spikelet. The correlation analysis of yield characters and phonological attributes with grain production was expressed the panicle numbers hill^{-1} and tiller numbers which were highly correlated with grain productionboth under water deficit stress condition and well watered condition (Zaman *et al.*, 2018).

4.3.5 Filled grain no. panicle^{-1}

4.3.5.1 Effect of drought

Sharply reduction was found for filled grain panicle^{-1} due to drought effect (Figure 10A). The highest filled grain was observed at D_0 (25.4%) drought condition compared to D_3 condition where there was a considerable variation was found between D_2 and D_3 drought condition.

4.3.5.2 Effect of osmolytes

Osmolytes caused a considerable variation was observed for filled grain panicle^{-1} of rice (Figure 10B). The highest osmolyte effect was recorded for Pro (3.9%) and Tre (4.5%) compared to their water condition.

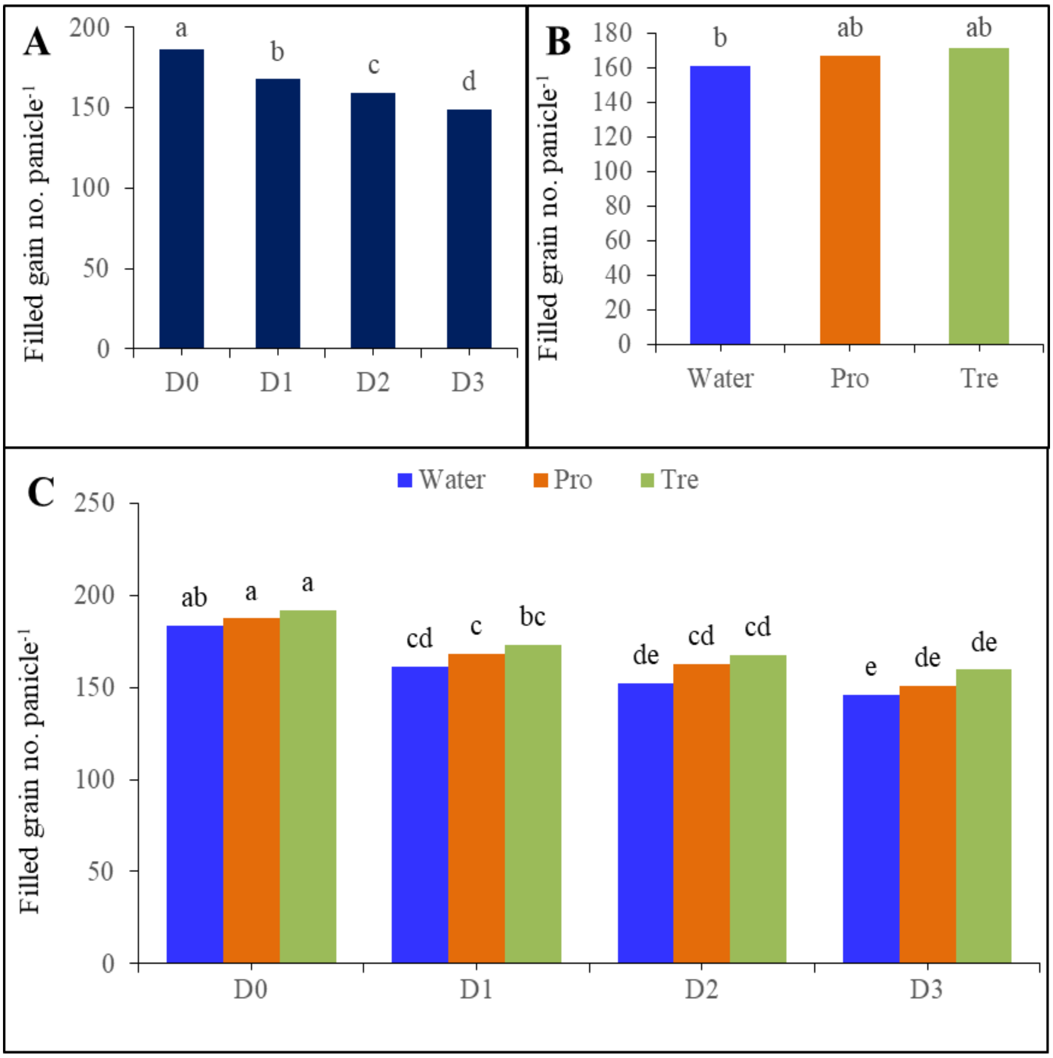

Figure 10. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on filled grain no. panicle^{-1} of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \le 0.05$ applying LSD test.

4.3.5.3 Interaction effect of drought and osmolytes

Interaction caused a considerable variation was observed for filled grain panicle^{-1} due to interaction effect (Figure 10C). Exogenous application of Tre increased the filled grain panicle^{-1} number of rice at all the drought condition compared to their water condition. All the drought condition showed the highest result 2.2%, 4.2%, 3.4% for Pro and 4.8%, 7.2%, and 9.4% for Tre at D_0, D_1 and D_3 drought condition contrast to their untreated water condition. On the contrary, the lowest result was observed for control (3.2%) at D3 drought stress condition. In D_1 and D_2 drought condition Tre showed the similar result.

Several alteration happened in crop plants due to the effect of water deficit stress such as physiological, morphological, biochemical and molecular constituents of photosynthetic activity which also related to grain panicle^{-1} and total yield of the crops. During the development of reproductive stage the grains per panicle sterility, peduncle elongation, sterilized pollen and ovule abortion was very sensitive to drought stress in rice crop cultivation (Yooyongwech *et al*., 2013).Total number of filled grain was decreased when less amount of assimilates transferred to the grains of the plants. Total grain yield was drastically reduced by the effect of water deficit stress on flowering stagewhere the total filled grain panicle^{-1} was reduced without the substantial mitigation of filled grain panicle^{-1} (Nahar *et al.*, 2018). Total number of filled spikelets was enhanced due to the performance of carbohydrate from photosynthesis which was transferred to the grain and also contribution to the grain yield. If the tillering stage was affected by the drought stress resulting the reproductive tiller and filled grain per panicle was drastically reduced (Wopereis *et al*., 1996). If the grain filling process was affected by drought stress causes for the filled grains, individual mass grain reduced up to 40 percent and 20 percent, respectively (Boonjung and Fukai, 1996). The grain panicle^{-1} sterility was increased due to the effects of drought stress including the fizzle of panicle to wholeexert from flag leaf sheath. Casartelli *et al*. (2018) showed that there are two drought tolerant rice varieties like N22 and Dular did not give any momentous reduction for filled spikelets and grain yield contrast to control plants whereas the drought intolerant varieties gives serious reduction but minimize these problems by the exogenous application of osmolytes. Likewise, the filled grain per panicle was remarkably reduced under drought condition shown in figure 10A and the foliar application of osmolytes causes for the spikelets per panicle was enhanced compared to their control condition shown in figure 10C. The period of panicle initiation was delayed due to drought or water deficit stress and also retarded the development of panicle at any kinds of stage up to flowering which ultimately affected the grain per panicle causes for reduction of total yield of the crop plants (Prasad *et al*., 2008). Studies exposed that various yield parameters like primary or secondary branches panicle^{-1}, length of panicle, grain weight panicle^{-1}, grains panicle^{-1} are significantly diminished by the effect of drought in *Oryza sativa* L. (Upadhyaya and Panda, 2019).

4.3.6 Unfilled grain no. panicle^{-1}

4.3.6.1 Effect of drought

Drought stress showed the considerable variation in different kinds of drought stage for unfilled grain panicle^{-1} (Figure 11A). The highest result found at D_3 (23.6%) condition compared to D_0 drought condition and the other drought stage showed similar result.

4.3.6.2 Effect of osmolytes

Different osmolyte caused a significant result observed contrast to water condition for unfilled grain (Figure 11B). The highest result found for water (8.7%) compared to Pro where Pro and Tre showed statistically similar result.

4.3.6.3 Interaction effect of drought and osmolytes

There was a considerable variation was observed at all the drought condition except D_0 condition (Figure 11C). In all the drought condition control showed the highest result compared to Pro and Tre. The highest result was recorded 19.2%, 18.1%, and 6.9% for untreated water condition contrast to their Tre condition at D_1, D_2 and D_3 drought condition where Pro showed better result than the Tre except D_3 condition. At D_0 drought condition Pro showed the lowest result than the Tre.

Hemmati and Soleymani, (2014) stated that the water deficit stress diminished the grain production, 1000-grain weight, harvest index but also increased the unfilled grain panicle^{-1}. Drought stress or before panicle starting decrease the number of spike potentiality and assimilates translocation to the grains resulting lower the grain weight and enhance the number of empty grains or unfilled grains (Zubaer *et al.*, 2007). During agree with Hossain (2001) and Begum (1990). By the exogenous application of osmolytes like Pro, Tre causes for the unfilled grain per panicle was significantly decreased shown in figure 11C.

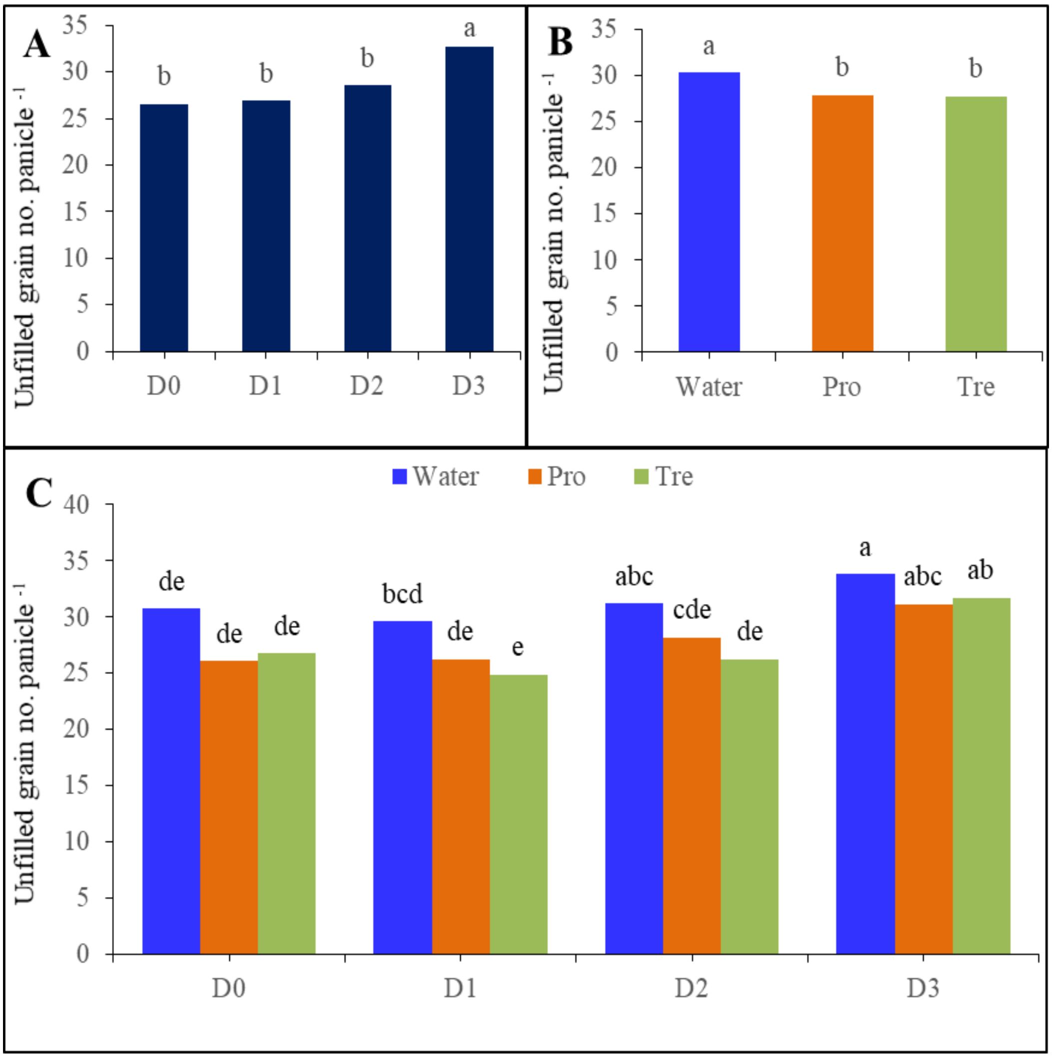

Figure 11. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on unfilled grain no. panicle^{-1} of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

The period of grain filling presumably decreased the supply of photosynthesis under drought stress condition in the plants resulting the unfilled or empty grain percentage was significantly enhanced. Developmental percentage of unfilled grain per panicle was transparent when the crop plants were subjected to water deficit stress at 25% moisture content belonging to the field capacity (FC). Unfilled grain per panicle was increased under water deficit stress owing to dryness of inactive pollen grain, partial pollen tube advancement, incomplete assimilates generation and its allocation to grains. The similar result also concluded by Iqbal (2018) that diversified chemical nature was observed for osmolytes and differ from contributing to regulate the osmotic balance and also act in opposition to oxidative stress as the scavengers of ROS; thus, the unfilled or empty grain was decreased by the exogenous application of osmolytes.

4.3.7 Weight of 1000 grains

4.3.7.1 Effect of drought

Significant reduction was observed at different drought stress condition for 1000 grain weight (Figure 12A). Highest grain weight was found at D_0 (8.7%) drought condition compared to D_3 condition where D_2 and D_3 showed statistically similar result.

4.3.7.2 Effect of osmolytes

Weight of 1000 grains showed significant variation by the application of different kinds of osmolytes (Figure 12B). The highest grain weight was found for Tre (5.2%) and Pro (2.9%) compared to water stress condition.

4.3.7.3 Interaction effect of drought and osmolytes

Remarkable decline was recorded for 1000 grain weight of rice (Figure 12C). The highest result was recorded of 1000 grain weight 2.9%, 4.2%, 3.7% for Pro and 2.2%, 8.1% and 5.7% for Tre at D_0, D_1 and D_3 drought condition compared to their respective water condition. On the other hand, the lowest result was found for water at D_3 (2.7%) drought condition. However, in D_2 and D_3 drought condition Pro showed statistically similar result above water cndition. Water condition was markedly decline at all the drought condition.

It is the most important yield contributing characters and seriously diminished the production of crops. The drought stress significantly affected the yield components resulting reduced different kinds of yield promoting characters such as tiller number, panicle development, spikelet panicle^{-1}, grain weight, total grain yield and also biological yield. Water deficit stress primarily hampers the kernel improvement causes for its potentialitydecreased and in the linear fill period directly impedes the enzymatic activity or during the development of other stage resulting premature desiccation occurred (Nayyar and Walia, 2004). Due to interaction of drought and protectants 1000 grain weight was significantly reduced shown in figure 12C.

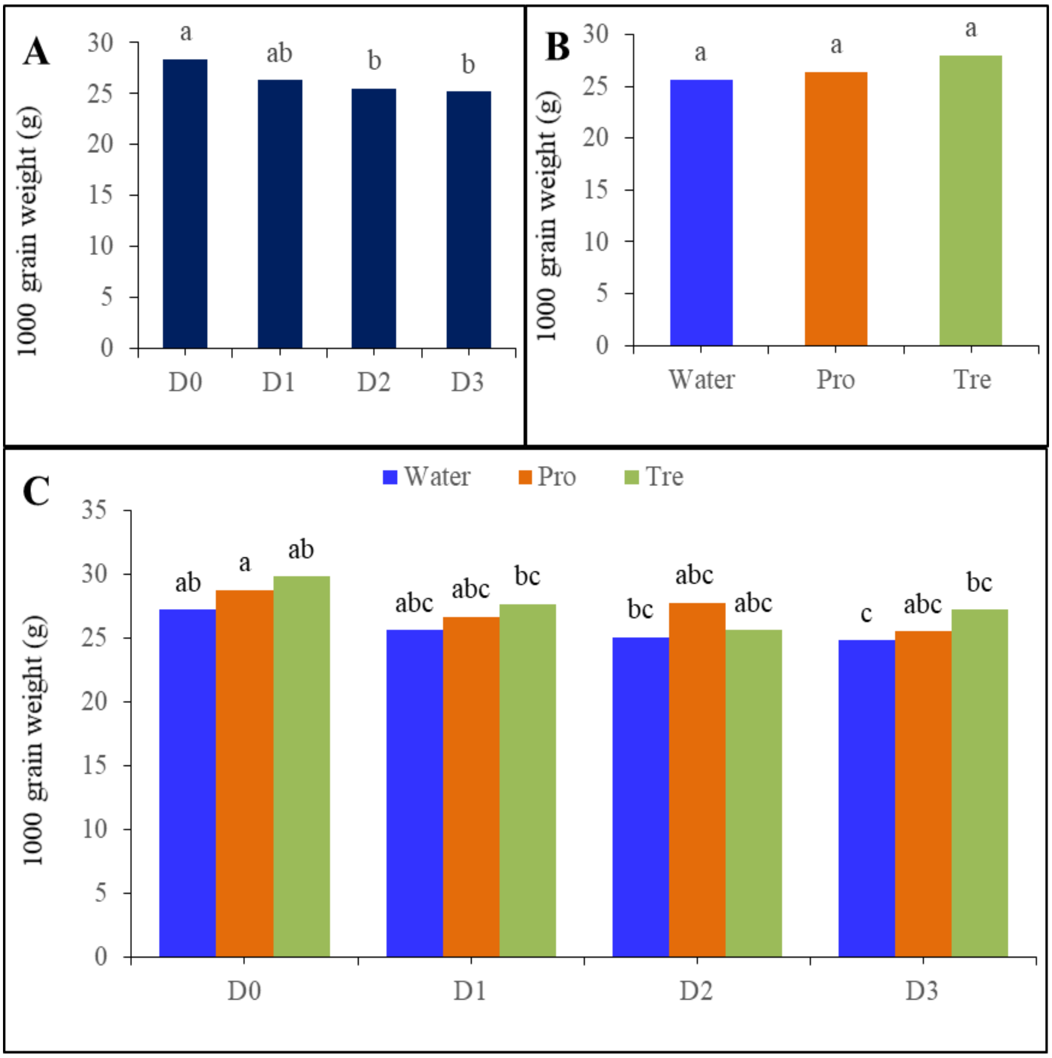

Figure 12. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on 1000 grain weight (g) of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

Likewise, Sinaki *et al*. (2007) showed that under water deficit condition, greatly diminished the 1000-grain weight, straw yield and harvest index. Minimization of photosynthates generation and their transfers to the reproductive organ like grains owing to water deficit stress and ultimately reduction of 1000 grain weight (Asch *et al*., 2005). The osmotic adjustment of cell was regulated by Pro thus mitigate the adverse effects of drought (Kumar *et al*., 2015). Plants accommodate to water deficit stress via various kinds of mechanisms such as morphological, physiological as well as biochemical developmental process where maximum researchers tried to develop drought tolerant varieties and maximum emphasized on the different morphological yield contributing traits like tiller number, panicle length, grain weight, spikelet per panicle and 1000 grain weight (Hossain and Teixeira Da Silva, 2012; Rahman *et al.*, 2009), with minimum importance on physiological and biochemical characters (Raffi and Asaduzzaman, 2015). Foliar application of Pro and Tre has been demonstrated to enhance the number of effective tiller, fertile grain, total biomass production, grain per ear, 1000 grain weight and total grain yield of the crops (Raza *et al.*, 2014). Exogenous application of Pro, GB and Tre reported that germination, seedling growth, chlorophyll content, 1000grain weight, total number of grains per spike and ultimately, the total grain yield was increased under drought condition and also expansion the capacity of drought tolerance of crop plants such as rice (Rehman *et al.*, 2002). The biological process is regulated by osmolytes such as protein interaction, protein folding causes for obstruction in the aggregation of protein, converse of miss-folding (Rabbani and Choi, 2018), the protein structure was stabilized and conserved the enzymatic intracellular operations by them (Ibrahim and Samiullah, 2019).

4.3.8 Grain yield plant^{-1}

4.3.8.1 Effect of drought

Sharply reduction was observed by the effect of drought for grain yield plant^{-1} (Figure 13A). Highest number of grain yield was found at D_0 (35.9%) drought condition compared to D_3 condition where there was no significant difference observed between D_1 and D_2 drought condition.

4.3.8.2 Effect of osmolytes

Osmolytes caused a significant variation that observed for grain yield plant^{-1} (Figure 13B). The highest number of grain yield was found for Pro (31.8%) and Tre (20.4%) contrast to their water condition

4.3.8.3 Interaction effect of drought and osmolytes

Drought stress caused a considerable variation that observed for grain yield plant^{-1} of rice (Figure 13C). Grain yield showed the highest result 8.2%, 8.6%, 12.6% for Pro and 7.2%, 3.5 18.2%, for Tre at D_0, D_1 and D_2 drought condition contrast to their respective watered condition.

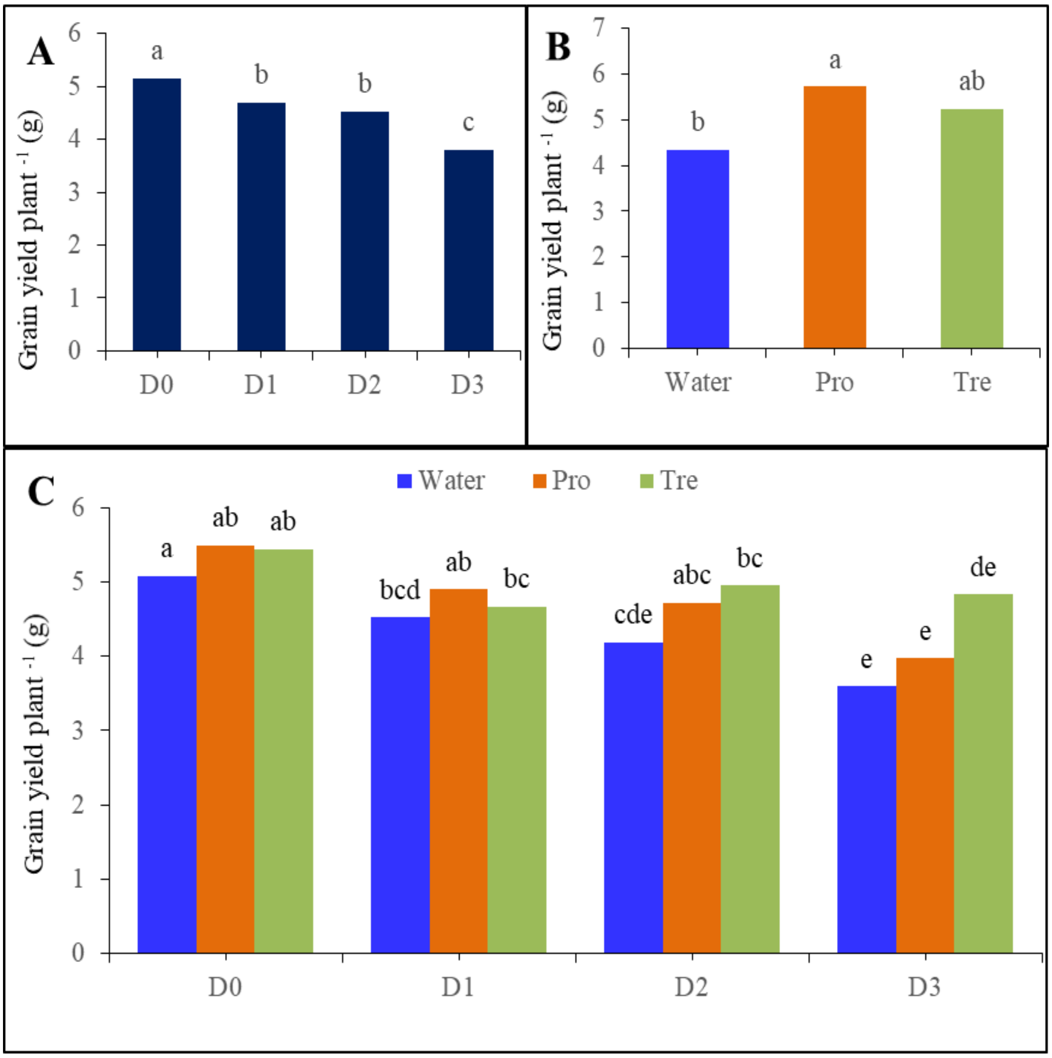

Figure 13. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on grain yield (g) plant^{-1} of rice. Here, D$_0$= Well-Irrigated, D$_1$= Water deficit at panicle stage, 5days, D$_2$= Water deficit at panicle stage, 10days, D$_3$= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

On the other hand, the lowest result was recorded for water (9.6%) at D$_3$ drought condition. Within the D$_1$ and D$_2$ drought condition trehalose showed statistically similar result. Considerable decline was recorded for control at all the drought condition than the osmolytes. At the stage of vegetative growth during rice cultivation flowering and terminal period can impedes the grain filling and floret initiation, respectively which causes the spikelet become sterile (Mostajeran and Rahimi-Eichi, 2009). Usually, drought stress occurred the early senescence at grain filling stage under drought condition and reduces the period of grain filling but also enhances the remobilization of assimilates.If the drought stress was affected the plants at flowering time and caused to the grain yield was significantly reduced than drought stress at other times. Due to the interaction of drought and protectants the grain yield was reduced 7.8%, 11.2% and 9.6% at D_1, D_2 and D_3 drought condition contrast to their respective watered condition shown in figure 13C. Similarly, Sarvestani *et al*. (2008) showed that one studies that grain yield on an average was reduced 21%, 50% and 21% for grain filling, flowering and vegetative stage, respectively under drought condition compared to control. At the early crop cycle drought stress is less devastating; however, 1 to 30 % grain yield is loss at the mild drought stress of plants, whereas the grain yield decrease up to 58 to 92 % of flowering and grain filling period of extended mild drought stress condition (Farooq *et al.*, 2015). The rate of grain filling was reduced owing to decrease of photosynthesis, sink limitations and leaf senescence was accelerated under drought stress condition. Particularly rice crop cultivation is more sensitive to water deficit stress contrast to other crops especially at critical growth stage like anthesis, panicle initiation and also grain filling (Akram*et al.*, 2013). During the period of vegetative growth of crop plants particularly at booting stage (Pantuwan *et al.*, 2002) the terminal period and flowering can impede the initiation of florets due to sterile of spikelets and grain filling resulting the grain weight significantly reduced and finally, the limited amount of yield was obtained (Kamoshita *et al.*, 2004; Botwright *et al.*, 2008). Severely increasing the drought stress causes for the synthesis of photosynthetic pigments, photosynthesis, lipid metabolism and protein synthesis gradually decreased resulting significantly the final grain yield. Drought stress also liable for water balance, mineral nutrients and membrane permeability. Effect of water deficit stress in crop plants leads to the oxidative stress owing to accumulation of ROS in chloroplast (Ashraf, 2009) where the generation of antioxidants by plants such as phenolics, tocopherol and ascorbic acid which also minimized the ROS effect. Moreover, by the exogenous application of osmolytes like Pro, Tre, GB, ascorbic acid which are also protects the plants from drought stress by impeding the ROS (Shafiq *et al.*, 2014) and efficiently increasing the grain yield and biomass production (Ejaz *et al.*, 2012).

4.3.9 Straw yield plant^{-1}

4.3.9.1 Effect of drought

Straw yield was decreased slightly at different drought stress condition (Figure 14A). The highest straw yield was observed at D_0 (22.7%) drought condition compared to the D_3 where slightly difference were observed for other drought stress.

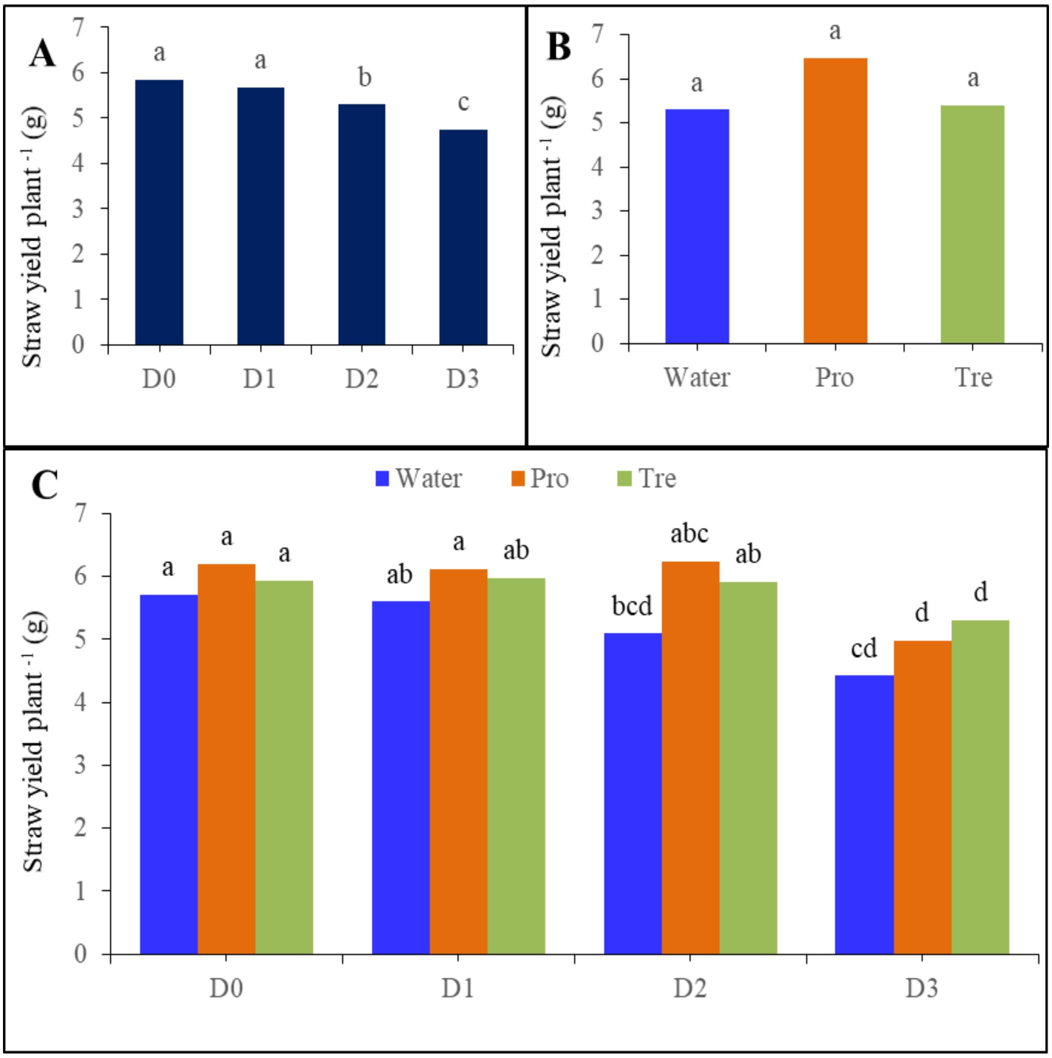

Figure 14. (A) Effect of drought, (B) effect of osmolytes and (C) interaction effect of drought and osmolytes on straw yield plant^{-1} (g) of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.9.2 Effect of osmolytes

Osmolytes caused a significant variation was observed for straw yield shown in figure 14B. The highest straw yield was found for Pro (3.5%) and Tre (2.8%) compared to their water condition.

4.3.9.3 Interaction effect of drought and osmolytes

Exposer to drought stress resulted in the highest straw yield was observed for Pro (22.6%) at D_2 drought condition contrast to untreated watered (Figure 14C). Furthermore, the highest result was recorded 8.6%, 9.3%, 12.7% for Pro and 3.9%, 6.6% and 19.9% for Tre at D_0, D_1 and D_3 drought condition compared to their respective water. On the contrary, the lowest straw yield was found for control (11.2%) at D_3 drought condition. In D_2 and D_3 drought condition, Tre showed the significantly similar result. Sharply reduction result was found for water at different drought stress condition.

As the enhancement of water deficit stress causes for water potential, photosynthesis, plant growth, spikelet number per panicle, 1000 grain weight, grain yield and straw yield was significantly decreased (Samarah *et al*., 2009). The straw yield was greatly interlinked to grain production where the two traits were positively connected to plant height before harvesting of the crops. Katerji *et al*. (2009) concluded that the water deficit stress have a detrimental effect on plant water relations during flowering period and ear formation and it also diminished the grain yield 37% and straw yield 18%. Due to the effect of drought stress the straw yield was decreased shown in figure 14C. Similarly, Emam *et al*. (2014) stated that Under water deficit condition straw yield was significantly reduced and also accompanied by lignin, pectin and cellulose percentage which was concurrent with sharply declined of soluble sugar content in the straw yield. Akram (2011) stated that straw production and harvest index are sensitive to water deficit stress for different cultivars of rice. Generally, different kinds of inorganic and organic solutes was accumulated in the cytosol of plants like Pro which provided tolerance mechanism in opposition to oxidative stress and it was also considered as a major strategy to cope with the pernicious effect of drought stress and also regulated the driving gradients and turgor pressure for sufficient amount of water uptake (Johari-Pireivatlou, 2010).

4.3.10 Harvest index

4.3.10.1 Effect of drought

Harvest index showed significant variation due to drought effect (Figure 15A). The highest harvest index observed at D_0 (5.4%) drought condition compared to D_3 where D_1 and D2 showed 2.8% and 3.6%.

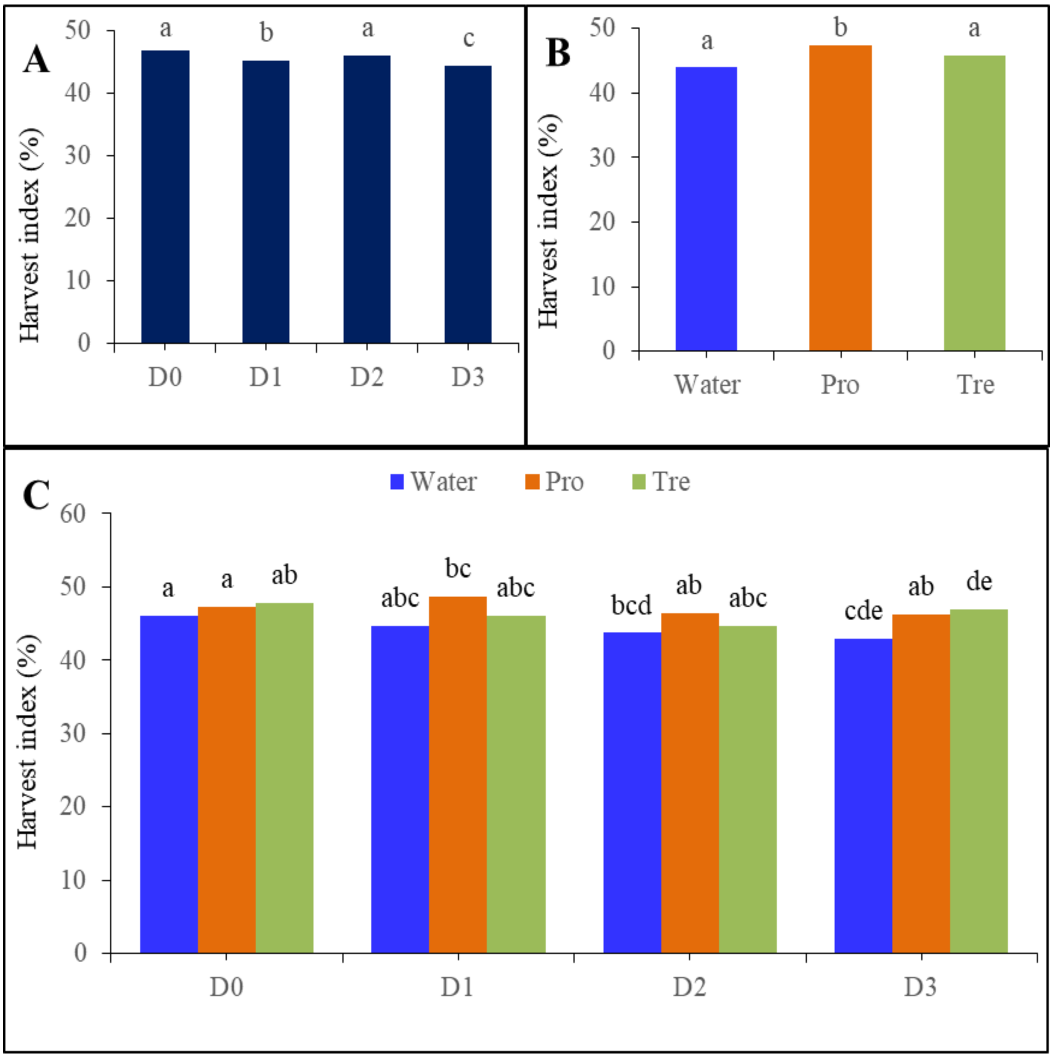

Figure 15. (A) Effect of drought, (B) effect of osmolytesand (C) interaction effect of drought and osmolytes on harvest index of rice. Here, D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5days, D_2= Water deficit at panicle stage, 10days, D_3= Water deficit at panicle stage, 15days, Water= Water spray, Pro= 10 mM Proline spray, Tre= 10 mM trehalose spray. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test.

4.3.10.2 Effect of osmolytes

Different kinds of osmolytes caused a considerable variation was observed (Figure 15B). Proline (2.9%) and Tre (1.8%) showed highest harvest index contrast to water condition.

4.3.10.3 Interaction effect of drought and osmolytes

Sharply reduction was recorded due to interaction effect of drought and osmolytes for harvest index (Figure 15C). Harvest index showed the highest result 1.2%, 3.8%, 5.5% for Pro and 1.7%, 2.3% and 2.7% for Tre at D_0, D_2 and D_3 drought condition compared to their respective water. On the contrary, the lowest result was found for water (2.5%) at D_3 drought condition. However, in D_1 and D_2 drought condition Tre showed significantly similar result above watered. Upon exposer to exogenous application of Pro and Tre showed highest result at all the drought stress condition.

Harvest index (HI) indicates that it's a ratio between two parameters such as grain yield and biological yield. Harvest index determined from the dry matter partitioning between the cereal grains and other parts of the physiology of plants. Water deficit stress have an important effect at pollination on biomass production and at the period of physiological maturity on grain size, grain yield, 1000 grain weight and harvest index (Chimenti *et al*., 2002). Water deficit stress declined harvest index remarkably by Oladir *et al*. (1999) who concluded that drought stress have a detrimental effect on vegetative and reproductive development which significantly abate the 1000-grain weight, straw production and HI of rice plants. As the severity of water deficit stress enhanced causes for the photosynthetic activity, plant height, grain filling, water potential, 1000 grain weight and HI significantly decreased (Samarah *et al*., 2009). Water deficit stress reduced the leaf water content and cell turgor pressure, thereby impeding the expansion of cell division resulting diminished the plant growth, dry mass accumulation, 1000 grain weight, straw yield and HI (Hammad and Ali, 2014). Total grain production was positively linked with straw yield, HI, spikes m^{-2} and kernels spike^{-1} under drought condition during the grain filling stage. In all the rice cultivars, water deficit stress lowered the total dry matter in the straw production and grains. The grain yield was reduced from 14 to 57 % by the effect of water deficit stress, while HI and straw yield were minor invaded by water deficit stress than total grain yield (Kottmann *et al*., 2016).

Chapter V

SUMMARY AND CONCLUSION

A field experiment was conducted to mitigate the water deficit stress in aman rice by the exogenous application ofPro and Tre. The rice variety BRRI dhan72 was used for this experiment which was also done at the experimental field of the Department of Agronomy, Sher-e-Bangla Agricultural University ($90^0$33’ E longitude and $23^0$77’ N latitude) under AEZ-28 (Modhupur Tract), in Dhaka from July 2018 to November 2018. BRRI dhan72 was picked up from Bangladesh Rice Research Institute (BRRI), Joydebpur, and Gazipur.

This field experiment was arranged in randomized completely block design (RCBD) with three replications. There were total 36 plots in the field and replications with given factors. Total plot area was 400 m^2. The experiment was carried out two factors one was water deficit stress viz. D_0= Well-Irrigated, D_1= Water deficit at panicle stage, 5 days; D_2= Water deficit at panicle stage, 10days; D_3= Water deficit at panicle stage, 15 days and another was osmolytes viz. 10 mM Proline spray (Pro) and 10 mM trehalose spray (Tre).

Data of morphological, physiological, yield and yield contributing traits were gathered at different days and harvest. The recorded data were analyzed using CoStat v.6.400 package. It was noticed that the morphological, physiological, yield attributes and yield were increased by the exogenous application of osmolytes compared to control condition. Non-effective tillers per hill and unfilled grain per panicle was decreased with increasing the drought stress levels.

Different water deficit stress had a significant effect on crop growth parameters like plant height. The tallest plant height was recorded for 1.7%, 1.9%, 2.4% for Pro and 1.6%, 1.4%, 1.3% for Tre stress respectively, in D_0, D_1 and D_2 water stress condition compared to their respective water. On the other hand, the lowest plant height was recorded for water (1.2%) condition at D_3 water stress which also compared to D_0 (5.2%), D_1 (3.3%) and D_2 (2.3%) drought stress condition.

Water deficit stress had a momentous effect on physiological parameters such as the highest relative water content in main leaf was recorded for Tre 17.3%, 9.2%, 21.2% and Pro 10.32%, 4.2%, 3.8% at D_0, D_1 and D_3 water stress condition compared to their untreated control. The highest RWC in flag leaf was recorded 99.6%, 18.8%, and 22.2% for Tre and 65.6%, 9.5%, and 6.7% for Pro at D_0, D_1 and D_2 drought condition contrast to their untreated watered. The highest chlorophyll content of main leaf was observed for Pro (2.7%) at D_0 water stress condition compared to their untreated watered. The highest chlorophyll content of flag leaf was observed at D_3 (8.4%) water stress condition for Pro compared to their watered condition.

Water deficitor drought stress treatments had an important influence on various yield contributing traits like panicle elongation, total number of effective tiller hill^{-1}, total number of panicle hill^{-1}, total number of filled grain panicle^{-1}, 1000-grain weight,including straw yield and finally the harvest index. These parameters given the maximum result by the foliar spray of Pro and Tre than the untreated control where the unfilled grains panicle^{-1} andnon-effective tiller number hill^{-1} showed lowest result than the watered condition.

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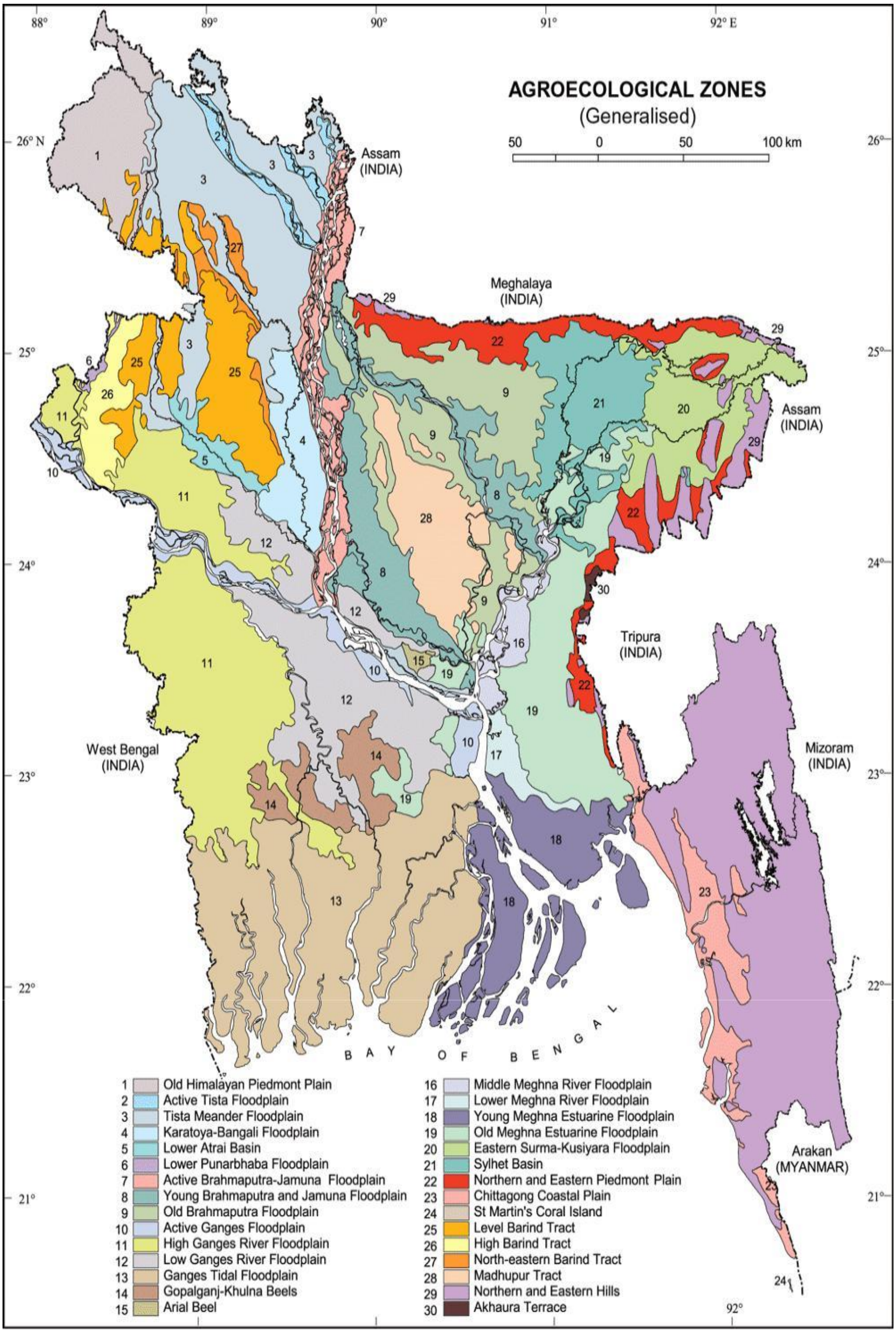
APPENDICES

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



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

Characteristics	Value
Particle size analysis	
%Sand	26
%Silt	41
%Clay	33
Textural class	Silty-clay
pH	5.7
Organic carbon (%)	0.45
Organic matter (%)	0.72
Total N (%)	0.04
Available P (ppm)	18.00
Exchangeable K (me/100 g soil)	0.12
Available S (ppm)	42

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

Appendix III. Mean square values for plant height, relative water content for main and flag leaf of rice

Source of variation	Degrees of freedom	Mean square values		
		Plant height at 70 DAT	Relative water content for main leaf	Relative water content for flag leaf
Drought	3	89.6992	242.888	152.85
Osmolyte	2	1.66609	26.062	1362.12
Drought× Osmolyte	6	2.1496	7.588	252.47
Error	24	17.6003	45.7	262.99

Appendix IV. Mean square values for chlorophyll content for main and flag leaf of rice

Source of variation	Degrees of freedom	Mean square values	
		Chlorophyll content for main leaf	Chlorophyll content for flag leaf
Drought	3	306.075	7.7689
Osmolyte	2	52.677	33.6592
Drought×Osmolyte	6	16.604	2.0539
Error	24	12.101	3.0142

Appendix V. Mean square values for effective tillers and non-effective tillers hill^{-1} of rice

Source of variation	Degrees of freedom	Mean square values	
		Effective tiller number hill^{-1}	Non-effective tiller number hill^{-1}
Drought	3	13.4584	1.43563
Osmolyte	2	1.9931	0.20882
Drought × Osmolyte	6	0.6046	0.01382
Error	24	0.6173	0.02951

Appendix VI. Mean square values for panicle length, panicle number hill^{-1}of rice

Source of variation	Degrees of freedom	Mean square values	
		Panicle length	Panicle number hill^{-1}
Drought	3	78.6294	17.3937
Osmolyte	2	3.8728	4.6744
Drought × Osmolyte	6	3.3	0.7026
Error	24	2.6982	1.0422

Appendix VII. Mean square values for filled and unfilled grains panicle^{-1} of rice

Source of variation	Degrees of freedom	Mean square values		
		filled grains panicle^{-1}	Unfilled grains panicle^{-1}	1000 grain weight
Drought	3	2302.53	73.6307	8.80753
Osmolyte	2	176.59	24.8744	1.72939
Drought × Osmolyte	6	19.52	6.1752	0.14909
Error	24	68.3	6.16	1.79927

Appendix VIII. Mean square values for grain yield plant^{-1}, straw yield plant^{-1} and harvest index of rice

Source of variation	Degrees of freedom	Mean square values		
		Grain yield plant^{-1}	Straw yield plant^{-1}	Harvest index
Drought	3	2.88817	2.15814	9.76451
Osmolyte	2	0.43874	0.10058	5.22601
Drought × Osmolyte	6	0.02908	0.042	1.57127
Error	24	0.14019	0.12827	8.30016