EFFECT OF MANNITOL DOSES AND APPLICATION TIME ON YIELD AND YIELD ATTRIBUTES OF AROMATIC RICE (BRRI dhan50)

A THESIS

BY

MD MORSHED ALI

Student No. 1701238

Session: 2022-2023

Thesis Semester: January - June, 2024

MS OF SCIENCE (M.S.)

IN

AGRONOMY

DEPARTMENT OF AGRONOMY

HAJEE MOHAMMAD DANESH SCIENC AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DEDICATED

TO MY

BELOVED PARENTS

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June 2024 *The Author*

EFFECT OF MANNITOL DOSES AND APPLICATION TIME ON YIELD AND YIELD ATTRIBUTES OF AROMATIC RICE (BRRI dhan50)

ABSTRACT

The field experiment was conducted at the Agronomy Research Field, Department of Agronomy, Hajee Mohammad Danesh Science and Technology University, Dinajpur during *Boro* season to evaluate the effect of dose and application time of Mannitol on the growth and yield of BRRI dhan50. The experiment was comprised of two factor viz. A. Dose (0 mM, 75 mM, 150 mM, 225 mM, 300 mM, 375 mM of Mannitol) and B. Application time of Mannitol (50 DAT and 75 DAT). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The data on crop growth characters viz. plant height (cm), number of total tillers hill^{-1} were recorded at 30 DAT, 60 DAT, 90 DAT and during harvest and yield as well as yield attributing characters viz. number of effective and non-effective tillers hill^{-1}, panicle length (cm), number of filled grain panicle^{-1}, number of unfilled grain panicle^{-1}, 1000-grains weight (g), grain yield (t ha^{-1}), straw yield (t ha^{-1}), biological yield (t ha^{-1}) and harvest index (%) were recorded at harvest. D_4 (225 mM of Mannitol) showed the highest number of total tillers hill^{-1} (26.67), effective tillers hill^{-1} (24.60), the minimum number of non-effective tillers hill^{-1} (1.20), panicle length 24.10 (cm), total grain panicle^{-1} (189.45), number of filled grains panicle^{-1} (185.08), the minimum number of unfilled grains panicle^{-1} (4.36), 1000-grain weight 19.82(g), grain yield 6.24 (t ha^{-1}), harvest index 45.73% and D_1 (0 mM of Mannitol) showed the lowest number of total tillers hill^{-1} (20.53), effective tillers hill^{-1} (15.67), the highest number of non-effective tillers hill^{-1} (4.87), total grain panicle^{-1} (168.65), panicle length (19.96 cm), number of filled grains panicle^{-1} (146.47), the highest number of unfilled grains panicle^{-1} (22.18), thousand-grain weight (17.65 gm), and lowest grain yield (4.53t ha^{-1}).In the case of time of Mannitol application T_1 (50DAT) showed the highest number of total tillers hill^{-1} (24.43), effective tillers hill^{-1} (21.64), the minimum number of non-effective tillers hill^{-1} (2.79), total grain panicle^{-1} (189.45), panicle length 22.41 (cm), number of filled grains panicle^{-1} (168.98), a minimum number of unfilled grains panicle^{-1} (12.07), 1000-grain weight 19.24 (g) and highest grain yield (5.67t ha^{-1}), biological yield (13.18 t ha^{-1}). T_1D_4 treatment combination showed the highest yield 6.47 (t ha^{-1}). However, BRRI dhan50 showed a higher yield in the treatment combination of T_1D_4 (50 DAT and 225 mM of Mannitol) in the experiment.

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ABBREVIATION

AEZ	= Agro Ecological Zone
ANOVA	= Analysis of Variance
BADC	= Bangladesh Agricultural Development Corporation
BARI	= Bangladesh Agricultural Research Institute
BBS	= Bangladesh Bureau of Statistics
BINA	= Bangladesh Institute of Nuclear Agriculture
Biotech.	= Biotechnology
CGR	= Crop Growth Rate
CV	= Co-efficient of Variance
DAT	= Days after transplanting
DAS	= Days after Sowing
Environ.	= Environmental
Exp.	= Experiment
FAO	= Food and Agricultural Organization
FYM	= Farm Yard Manure
Hort.	= Horticulture
J.	= Journal
Kg	= Kilogram
LAI	= Leaf Area Index
LS	= Level of Significance
LSD	= Least Significant Difference
mg	= Milligram
mM	= Milli Molar
Physiol.	= Physiological
PCR	= Polymerase Chain Reaction
ppm	= Parts Per Million
RCBD	= Randomized Complete Block Design
Res.	= Research

CHAPTER I

INTRODUCTION

Bangladesh has an agrarian economy in which rice is the dominant crop. It is the 3rd largest country in the world based on rice cultivation (FAO, 2022). Rice is the staple food for about 156 million people in Bangladesh (Israt *et al*., 2016). In Bangladesh, about 9.91% of the total cropped land is covered by Aus rice, 48.91% of the total cropped land is covered by Aman rice, and 41.17% of the total cropped land is covered by Aman rice (BBS, 2024). Food security is the challenge in the 21st century and most of the developing countries are facing this problem. According to the International Rice Research Institute (IRRI) report, in 2025 the demand of rice globally will be 880 million ton which is 70% more than the present production. A total of 507.24 million metric tons of rice was produced worldwide in 2020-2021, which increased to 515.53 million metric tons of rice from 2023-2024 (USDA, 2024).

Besides staple rice, aromatic rice production in Bangladesh is growing exponentially due to the favorable environmental conditions. Genotypes of aromatic rice *(Oryza sativa* L.) are recognized by their distinctive aroma when processed and cooked. This kind of rice is excellent; it is prepared for special occasions and is thought to be of the highest quality (Kader *et al*., 2018). The exquisite aroma and unique cooking properties make aromatic rice a premium agricultural commodity. With the demand for high-quality rice, the demand for fragrant rice in domestic and foreign markets is increasing. Compared to other non-aromatic rice varieties, aromatic rice cultivars are more expensive on the market. Due to the high demand for this type of rice for both domestic consumption and export, Bangladesh has seen a rise in the popularity of its cultivation in recent years. Even though for better output, the agro-climate must be mostly favorable less than 2% of Bangladesh's total rice-growing land is used to grow aromatic rice (Ashrafuzzaman *et al*., 2009). It was planned for more than 4,000 landrace varieties of rice to be favored in various regions of Bangladesh. Only a few of these have distinctive quality characteristics, such as fineness, aroma, taste, and protein content. In general most of the premium quality rice cultivars are low yielding, there are few locally adapted premium quality rice varieties namely Chiniatop, Kalizira and Kataribhog are available (Ahmed *et al*., 2005). A few high-quality modern rice varieties, including BR5, BRRI dhan34, BRRI dhan37, BRRI dhan38, BRRI dhan50, and BRRI dhan70, have been released by the Bangladesh Rice Research Institute (BRRI). After meeting the needs of the local market, these cultivars are also exportable. It was expected that 12.5% of the total transplant Aman rice production area will be occupied by aromatic rice cultivars (BBS, 2005). Due to its high costs and potential for export, aromatic rice production is becoming more widespread in Bangladesh (Dutta *et al*., 2002). Regardless of their cost, it is currently preferred by more clients. Commercially speaking, the introduction of contemporary high-yielding rice varieties resulted in a 23% increase in farmers' overall income (Shrestha *et al*., 2002). BRRI's research has shown that at one time about 57 types of aromatic varieties were cultivated in Bangladesh; now more or less 32 types of aromatic varieties were cultivated.

Among the several aromatic rice varieties BRRI dhan50 which is also known as Banglamati has a significant effect on the economy of Bangladesh. Aromatic rice varieties are sought after for their distinct fragrance and flavor, making them popular choices in various cuisines worldwide. Brridhan50, being an aromatic variety, sets it apart from other rice varieties, making it desirable in markets where consumers appreciate quality and distinctiveness in their culinary experiences. Some corporate companies in Bangladesh are exporting packaged fragrant rice to 137 countries. The amount of aromatic rice exported through various private companies in Bangladesh is about 5,998 tonnes per year (FAO, 2017).

At present, the yield of fragrant rice is generally lower than that of non-fragrant rice. Its yield is comparatively lower because of severe insect infestation, drought, salinity, etc. Drought stress is one of the abiotic stresses that severely limits the production of many field crops. It is considered to be the most destructive stress factor that reduces productivity in agricultural production compared to other environmental stresses (Lambers *et al*., 2008). Under drought conditions, plants give a series of physiological and biochemical responses to maximize water utilization in the environment and retain the water in their cells. The relationship between drought stress and relative water content or osmotic potential was examined in some plant species (Zhang *et al*., 2010). Therefore, the question of how to improve the yield of aromatic rice is the focus of current research.

Mannitol have a positive impact on plant growth, development, and overall stress tolerance. It is a six-carbon, non-cyclic sugar alcohol having different roles like coenzyme regulation, free-radicals scavenging and osmoregulation (Rahnama *et al*., 2011), thereby imparting abiotic stress tolerance. WHO reported that the relative water content and osmotic potential of plants decrease due to the drought effect. For mitigating different stress damages, in recent decades, the use of exogenous osmoprotectants or osmolytes has been found effective (Hasanuzzaman *et al*., 2014). Mannitol, a crucial osmolyte, is abundantly produced in numerous plant species (Ahmed, 2020). Its functions in these organisms are diverse, encompassing carbon storage, scavenging of free radicals, osmoregulation, and acting as a compatible solute (Prabhavathi and Rajam, 2007). Although mannitol plays an important role in osmotic adjustment, it acts as an antioxidant to scavenge of hydroxyl radicals (OH●) (Srivastava *et al*., 2010).

Nevertheless, pre-treatment using external mannitol (100 mM) effectively countered the adverse impacts of salt, bolstering the activities of antioxidant enzymes like SOD (Superoxidase), POD (Peroxidase), and CAT(Catalase). This was evidenced by the emergence of SOD and POD isozyme activity bands and a reduction in lipid peroxidation (Seckin *et al*., 2009). The induction of drought stress through mannitol finds widespread application across various crop species. Seong *et al*. (1988) observed a decrease in both moisture content and seedling length with increasing concentrations of mannitol. While mannitol serves as a key osmolyte synthesized in numerous plant species, it's notably absent in rice (*Oryza sativa*). Moreover, it constitutes a significant portion, up to 50%, of translocated photo-assimilates (Loester *et al*., 1992). Accumulation of mannitol escalates under conditions of low water potential (Patonnier *et al*., 1999), regulated by the inhibition of competing pathways and reduced mannitol consumption and catabolism (Stoop *et al*., 1996). Mannitol is also a phloem translocated photo assimilate in some vascular plants (Pharr *et al*., 1995). However, plant cells exposed to drought try to regulate their osmotic potential by accumulating certain osmolytes to maintain turgor conditions (Vinod *et al.*, 2012).

Therefore, this study will emphasize the effect of mannitol on BRRI dhan50 production. The research will be conducted based on the following objectives:

i. To find out the suitable dose of mannitol on the growth and yield of aromatic rice.
ii. To determine the optimum time of mannitol application for maximizing the growth and yield of aromatic rice.
iii. To know the interaction effect of dose and time of mannitol on aromatic rice.

CHAPTER II

REVIEW OF LITERATURE

Rice is the staple food crop in Bangladesh. The environmental factors, biotic factors, variety, and cultural practices significantly influence the growth and development of the rice plant. Mannitol plays a notable role regarding growth and development as well as yield contributing characters like the number of effective tillers, length of panicle, number of filled grains panicle^{-1}, weight of 1000 grains etc. of rice plants. Ample numbers of research works have been conducted at home and abroad on the effect of foliar application of Mannitol on crop cultivation.

Mannitol is more or less known to the farmers in producing crops in Bangladesh. The present study deals with the effect of foliar application of Mannitol on rice productivity. For this point, the foliar application of Mannitol was used to evaluate the different roles on morphophysiology yield and yield-attributing characters of BRRIdhan50. In this case, an attempt is made to review the available literature related to the present study. Most of the research report shows a positive effect of the foliar application of Mannitol on rice and other crops. The findings of various authors are cited below:

2.1 Consequence of Mannitol on different parameters of Rice

2.1.1 Enzyme Activity

Studies conducted by Upadhaya *et al.* (2019) revealed that the rice plant has evolved various strategies to cope with drought stress. Drought stress responses can be managed by adopting strategies such as; breeding and marker assistant selection; modulating drought responses through the application of plant hormones like abscisic acid, salicylic acid, auxin, gibberellins, cytokinins, and brassinosterroids; enhancing osmotic adjustment with potassium nutrient, and osmolytes like proline, glycine betaine and other amino acids, polyols like sorbitol, mannitol, myo-inositol, pinitol; improving antioxidant function like ascorbate, glutathione, polyamines and enzymes like ascorbate peroxidase, catalase, superoxide dismutase, glutathione reductase etc., as well as generating transgenics for drought tolerance in rice.

Chutipaijit (2016) examined the morphological, physiological, and biochemical reactions of indica rice *(Oryza sativa* L.) genotypes in both unstressed and stressed seedlings.

Using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt) assays, the effects of NB medium supplemented with a 100 mM mannitol treatment, which induced drought stress conditions, were measured for relative growth rate, cell membrane stability, antioxidant enzyme activity (superoxide dismutase [SOD], catalase [CAT], and peroxidase [POD], and total antioxidant capacity. The morphological and physiological parameters' results revealed two distinct rice genotype groups: drought-tolerant and drought-sensitive. Following drought stress, drought-tolerant genotypes exhibited a lower increased rate of SOD activity compared to drought-sensitive genotypes; nevertheless, drought-tolerant genotypes showed a higher overall antioxidant capacity as well as an increased rate of CAT and POD activity.

2.1.2 Cultivar

Cha-Um *et al.* (2010) investigated the physiological, biochemical, and morphological responses to salt stress and iso-osmotic water shortage of three rice varieties. Three rice cultivars' seedlings were photoautotrophically cultivated on MS media, and then they were subjected to salt stress (NaCl) or water-deficit stress (iso-osmotic mannitol, -0.23 or -0.42 MPa). The osmotically stressed seedlings showed significantly lower levels of chlorophyll a (Chl_a), chlorophyll b (Chl_b), total carotenoids (C_{x+c}), maximum quantum yield of PS_{II} (Fv/Fm), and photon yield of PSII (Φ_{PSII}) compared to the control group (without mannitol or NaCl). This resulted in a positive correlation between growth reduction and net photosynthetic rate (P_n). Furthermore, compared to seedlings under water deficiency stress, the physiological alterations and growth metrics of salt-stressed seedlings were more drastically decreased.

Roychoudhury *et al.* (2009) reported that the effect of salinity stress on the physiological and molecular responses of the common aromatic rice Gobindobhog. The objective of this study was to understand the influence of exogenous ABA on some biochemical parameters in Gobindobhog, and comparison with those from non-aromatic M-1-48 and Nonabokra rice. The highest endogenous hydrogen peroxide content and membrane lipid peroxidation (increased malondialdehyde and lipoxygenase activity) were found in ABA-treated Gobindobhog leaves. While the catalase activity was down regulated the most in ABA-treated Gobindobhog leaves, the guaiacol peroxidase activity was induced maximally, indicating the protective role of peroxidase rather than catalase, during ABA-induced oxidative damages. The antioxidant, anthocyanin, showed the highest level in ABA-treated Nonabokra. Enhanced cysteine, following ABA exposure and the highest levels of reducing sugars, total amino acids, proline, and polyamines (putrescine and spermidine) recorded in Gobindobhog, probably served to shield from ABA-induced stress injuries, whereas the spermine levels were comparable in ABA-treated Nonabokra and Gobindobhog. The aroma content, intensified after ABA treatment, was markedly noted in Gobindobhog. Thus, the systematic examination of ABA-mediated stress revealed the most prominent oxidative damages in Gobindobhog, even higher than M-1-48, with a concomitant enhancement in peroxidase system and particularly osmolyte or polyamine levels to ensure its sustenance.

Nishimura *et al.* (2011) experimented to investigate pigment degradation, chlorophyll fluorescence diminution, photosynthetic ability, and growth reduction in two rice cultivars, in response to either iso-osmotic salt stress or water-deficit stress. Seedlings of rice cultivars RD6 and KDML105 were photo-autotrophically grown in MS media and subsequently exposed to –0.23 (control), –0.40 or –0.67 MPa iso-osmotic NaCl (salt stress) or mannitol (water-deficit stress) for 14 days. The survival percentage of the two rice cultivars reduced dramatically when subjected to –0.67 MPa NaCl treatment. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total carotenoids (C_{x+c}), the maximum quantum yield of PS_{II} (Fv/Fm) and photon yield of PSII (Φ_{PSII}) in the stressed seedlings were significantly lower when compared to seedlings in the control group (without mannitol or NaCl), leading to low net-photosynthetic rate (P_n) and growth reduction. In addition, the growth characteristics of plantlets in salt stress conditions were more sharply reduced, and the physiological changes were greater than those in water-deficit stress conditions. On the other hand, non-photochemical quenching in the leaves of stressed plantlets increased significantly, especially in response to iso-osmotic salt stress. In the present study, the overall growth performance and physiological characteristics of $KDML_{105}$ grown under iso-osmotic stress were better than those of RD_6.

A study was undertaken by Pujini *et al.* (2007) with the aim to enhance abiotic stress tolerance in basmati indica rice by introduction of the *E. coli* mannitol-1-phospho dehydrogenase (mt/D) gene, which is involved in mannitol synthesis in plants. Several putative transgenic rice plants were generated by *Agrobacterium*-mediated transformation. The presence of the transgene in the primary transformants was confirmed by PCR using hygromycin phosphotransferase (hpt) and mt/D gene specific primers. Southern hybridization also revealed the integration of the transgene. Transgenic lines exhibited mannitol accumulation, which was correlated to the increased tolerance of the transgenics against salinity and drought stress. The T_1 transgenic seed germination and seedlings growth showed better performance than that of wild type during abiotic stresses under *in vitro* and *in vivo* growth conditions.

Another study (Lutts *et al.*, 1996) depicts that, mature embryo-derived calli were obtained from three rice (*Oryza sativa* L.) cultivars differing in salinity resistance at the whole plant level [(I Kong Pao (IKP), (salt-sensitive), Aiwu (moderately resistant) and Nona Bokra (salt-resistant)] and exposed to three iso-osmotic concentrations of NaCl, KCl, Na_2SO_4, artificial sea water (ASW) and mannitol. Relative growth rates, ion content and proline accumulation were quantified after 1, 2 and 3 months of stress. Among salt treatments, KCl was the most detrimental to callus growth in all genotypes and induced a strong increase in Cl content. The NaCl-induced inhibition of growth was lower in calli issued from Nona Bokra than in calli obtained from IKP; Na and Cl accumulations as well as internal osmotic potential were lower in Nona Bokra and in Aiwu, thus suggesting a cellular component of salt resistance in these genotypes. No obvious differences in growth were recorded among genotypes upon mannitol treatment, which appeared more detrimental than NaCl. Proline accumulation was higher in the salt-sensitive cultivar IKP, whatever the nature of the stressing agent or the stress intensity, and did not appear to be involved in osmotic adjustment. It was concluded that specific ion toxicities are important aspects of salt stress effects on rice cells and that proline is a symptom of injury in stressed rice calli rather than an indicator of resistance.

2.2 Consequence of Mannitol on other crop

2.2.1 Yield

A study from Nyaupane *et al.* (2024) explained that the drought has emerged as a major abiotic stressor that has a significant impact on global wheat production. Changing rainfall patterns, increased atmospheric CO_2 levels, rising atmospheric temperature, and hot and dry winds are the primary causes of drought stress. It has morphological, physiological, and biochemical consequences such as decreased yield performance, yield attributing parameters, germination and seed vigor, early leaf senescence, early maturity, decreased chlorophyll content, reduced Rubisco activity, decreased photosynthesis, and decreased starch accumulation. Drought produces reactive oxygen species, which cause oxidative damage to plants, resulting in programmed cell death. Wheat plants have developed a variety of tolerance mechanisms, including drought escape.

2.2.2 Cultivar

Dawood *et al.* (2022) conducted two field experiments at the Research and Production Station, National Research Centre, El-Nubaria Province, El-Behira Governorate, Egypt, over two consecutive winter seasons. The objective was to assess the physiological impact of mannitol at concentrations of 0, 10, 20, and 30 mM on the growth, productivity, and nutritional quality of faba bean plants, specifically cultivars Misr 2 and Sakha 3. Results indicated that Misr 2 exhibited superior adaptability to sandy soil conditions compared to Sakha 3, displaying higher vegetative growth parameters, photosynthetic pigments, seed yield, yield components, and nutritional value. Mannitol treatments at 20 and 30 mM significantly enhanced plant fresh and dry weight, total photosynthetic pigments, seed number and weight per plant, seed yield (Kg feddan-1), reducing sugar, and starch, while concurrently decreasing total phenolic content, tannins, and vicine content. Notably, mannitol treatments at all concentrations led to significant increases in seed yield (Kg feddan-1), with increments of 23.73%, 31.88%, and 40.15% at 0, 10, 20, and 30 mM, respectively. Furthermore, the interaction between cultivars and mannitol treatments revealed that 30 mM mannitol boosted seed yield (Kg feddan-1) by 56.37% in Misr 2 and by 22.21% in Sakha 3 compared to their respective controls. Overall, the response of Misr 2 to mannitol treatments surpassed that of Sakha 3, indicating Misr 2's greater suitability for sandy soil conditions and its enhanced responsiveness to mannitol treatments. Mannitol treatments exhibited significant improvements across various parameters in both cultivars, with efficacy increasing with higher mannitol concentrations, notably at 30 mM.

Tran *et al.* (2021) studied about the tomato cultivar TN_{704}, which is popularly grown in Vietnam's Southeast and Vietnam's Mekong Delta was selected. The combination of auxin (IAA, indoleacetic acid) and cytokinin (zeatin) at different concentrations was investigated to determine the effective regeneration media. Then, the drought pretreatment was applied to obtain drought-tolerant shoots. The drought tolerance of regenerated shoots was checked by culture in the drought stress condition after two generations (F_1 and F_2). The physiological and biochemical changes of regenerated shoots in the drought stress condition were analyzed. The MS 1 2 medium supplemented with 0.2 mgL^{-1} IAA and 0.5 mgL^{-1} zeatin was the effective medium for in vitro shoot regeneration from tomato leaves. The drought pretreatment (MS 1 2 with 20 g/L mannitol) increased the number of regenerated shoots which can develop in the drought stress condition. The regenerated shoots in the F_1 and F_2 generations grew strongly under drought conditions. The content of chlorophyll, carotenoid and proline, the intensity of respiration and photosynthesis, and the activity of auxin and cytokinin in leaves of F_1 and F_2 plant were higher than the control. A bacterial mannitol-1-phosphate dehydrogenase gene was targeted to tobacco chloroplasts and the resistance to oxidative stress in transgenic tobacco was improved, evidently as a result of mannitol accumulation (Shen *et al*., 1997a).

Zebarjadi *et al.* (2013) assessed the callus induction response and in vitro drought tolerance of eight safflower genotypes. The experiment followed a completely randomized design with a factorial arrangement and three replications. To evaluate drought tolerance, calli were exposed to drought stress by adding different concentrations of mannitol to the culture medium for one month after two subcultures. Under stress conditions, the genotypes were compared based on proline content, cell viability, relative growth rate, ion content (Na and K), relative water content, and tolerance index. Drought affected all measured biochemical and physiological factors, with significant differences observed between the tested genotypes. Proline content increased in drought-stressed calli, and mannitol, acting as a stress agent, stimulated proline synthesis in all genotypes, particularly at the highest concentration (505 mM). Conversely, ion content, cell viability, relative water content, relative growth rate, and tolerance index exhibited significant decreases under drought stress. The study suggested that these biochemical and physiological traits could serve as predictors of safflower genotype drought tolerance. The results indicated that the cultivars Isfahan and LRV-51-51 demonstrated greater drought tolerance under in vitro conditions compared to the other genotypes.

Tumdam (2011) conducted an experiment to investigate the effect of in vitro drought stress on *Brassica* species, including *Brassica campestris* (RTM-314), *Brassica carinata* (PC-5), *Brassica napus* (GSL-1), *Brassica juncea* (Pusa bold), and *Brassica rapa* (Bhavani). The objective was to study the variation in regeneration ability among *Brassica* genotypes under different levels of in vitro drought stress and to identify drought-tolerant *Brassica* genotypes. Axillary buds from 10-15 day-old seedlings were used as explants, and specific media compositions were employed for shoot and root initiation. Mannitol was used at concentrations of 0, 2, 4, 6, and 10% in the shoot differentiation media to induce stress conditions. The study revealed significant variations in mannitol concentration, Brassica species, and the interaction between mannitol concentration and Brassica species for all studied traits. Regeneration capacity, including traits related to shoot and root differentiation, decreased with increasing mannitol concentration. Complete inhibition of regeneration ability was observed at 10% mannitol concentration. Among the five species studied, *Brassica campestris* exhibited the least sensitivity to high mannitol concentrations, indicating a highly genotype-specific response to stress conditions. *Brassica campestris* also showed the highest accumulation of proline, reaching 78.7 mg and 72.9 mg at 6% and 4% mannitol concentrations, respectively. Based on its regeneration capacity and proline accumulation under in vitro stress conditions, *Brassica campestris* (RTM-314) was identified as the drought-tolerant genotype. These findings highlight the potential for genotype-specific responses to drought stress and the importance of identifying and characterizing drought-tolerant genotypes for breeding and improvement programs.

Umair *et al*. (2011) conducted a study aimed at enhancing the yield and biological nitrogen fixation capability of mungbean through priming techniques. Various priming methods were employed, including traditional soaking (hydropriming) and osmo conditioning with solutions containing potassium dihydrogen phosphate (KH_2PO_4), mannitol ($C_6H_{14}O_6$), polyethylene glycol (PEG6000), sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$), and salicylic acid ($C_7H_6O_3$). Untreated seeds served as the control group. The experiment took place at two different locations with varying climatic conditions. All priming treatments resulted in significant improvements in both dry matter yield (ranging from 4001 to 5262 kg ha^{-1}) and seed yield (from 713 to 948 kg ha^{-1}) compared to the control. Particularly noteworthy was the highest biological nitrogen fixation observed in plants primed with phosphorous (46.39 kg ha^{-1}) compared to the control. In summary, the priming of mungbean seeds with phosphorous (at 0.6%) emerged as an exceptionally effective method for enhancing germination and vigor under field conditions. This approach offers a simple and cost-effective technology beneficial for resource-constrained farmers.

Abu-Romman and Suwwan (2011) examined the growth and physiological responses of cucumber micro-shoots to osmotic stress. Micro-shoots were cultured on Murashige and

Skoog (MS) rooting media supplemented with 1.0 mg/L indole-3-methylbutyric acid (IBA). Osmotic stress was induced by adding various concentrations (0, 25, 50, 75, and 100 mM) of sorbitol to the rooting medium. The study revealed that both vegetative and root growth of cucumber micro-shoots were negatively impacted by osmotic stress induced by sorbitol. The leaf cell sap osmotic potential became more negative with increasing sorbitol concentrations, indicating the osmotic stress effect. Additionally, the total protein content decreased significantly as sorbitol concentration increased, while there was an accumulation of proline, a known osmoprotectant, under osmotic stress conditions. Furthermore, the uptake of essential nutrients such as potassium (K), calcium (Ca), and phosphorus (P) was significantly reduced by osmotic stress. These findings highlight the detrimental effects of osmotic stress on cucumber micro-shoot growth and physiology, including changes in osmotic potential, protein content, proline accumulation, and nutrient uptake.

2.2.3 Enzyme activity:

A study was carried out to assess the efficacy of exogenous applications of osmoprotectants-proline, glycine betaine, trehalose, and mannitol in alleviating the adverse effects of water stress in black gram (Pushpan *et al.*, 2020). The seed material VBN (Bg) 4 was used for the study. Water stress was imposed for 7 days on 25 DAS followed by exogenous application of different osmolytes. On 35 DAS, the samples were analyzed for biochemical attributes – proline, soluble proteins, catalase and peroxidase and physiological attributes – chlorophyll content, chlorophyll stability index and relative water content. Growth attributing parameters and yield attributing parameters were recorded at the time of maturity. Exogenous application of all osmoprotectants contributed to overcoming water stress and stress tolerance. However, specifically, the treatment with proline has a greater impact on enhancing photosynthetic activity; Exogenous application of all osmoprotectants has resulted in an increase in the activity of catalase and peroxidase and treatment with glycine betaine has enhanced catalase activity and treatment with mannitol has enhanced peroxidase activity. Based on yield attributes treatment with proline had an increased number of clusters/ plant and number of pods/plant and mannitol treatment yield a maximum of 1000 seed weight.

Mannitol is a six-carbon, non-cyclic sugar-alcohol having a role in storage of energy, regulation of coenzymes and osmoregulation. It is naturally synthesized in many plant species, while is absent in wheat (Stoop *et al*., 1996). It is known to function as scavenger of reactive oxygen species (ROS); therefore, it overcomes the peroxidation of lipids and consequent cell damage (Stoop *et al*., 1996).

2.2.4 Plant Growth

A pot experiment was performed by Habiba *et al.* (2019) to examine the role of foliar applied mannitol (M) in chromium (Cr) stress alleviation in different maize cultivars. Two maize cultivars, one tolerant (6103) and one sensitive (9108) to chromium stress, were grown in soil treated with three concentrations of Cr (0, 5, and 10 mg kg^{-1}) and three levels of mannitol (0, 50, and 100 mg L^{-1}). Chromium stress decreased the overall growth of plants by reducing the plant height, root/shoot dry weight, chlorophyll contents, and enzymatic activities, while exacerbated the severity of reactive oxygen species in both maize cultivars. Chromium-induced reduction in growth attributes of maize plants was relatively higher in sensitive cultivar than that of tolerant one. Uptake of Cr by the plants and its translocation from roots to shoots increased with increasing concentration in the soil. However, foliar application of mannitol significantly alleviated the Cr stress and improved growth, biomass, and photosynthetic pigments of maize plants. Mannitol also considerably reduced Cr contents in leaves and roots of both cultivars. Hence, it is concluded that mannitol can be helpful for crops grown on heavy metal, especially Cr, contaminated soils for remediation purpose.

Olah *et al.* (2017) investigated the growth and developmental responses of potato to osmotic stress under in vitro conditions. They examined the effect of mannitol on five different potato genotypes in both callus and plantlet cultures. After four weeks of cultivation on media containing up to 0.8 M mannitol, various morphological parameters were measured and statistically analyzed. The optimal concentration of mannitol for in vitro screening of osmotic tolerance varied with the type of culture: 0.4 M was suitable for plantlet tests, while 0.8 M was appropriate for callus tests. In callus cultures, the relative increase in callus mass was an effective parameter for assessing the osmotic tolerance of genotypes at the cellular level. In plantlet cultures, a stress index based on the survival rate of in vitro shoots, the number and length of roots per surviving explant, and the rate of rooted explants was useful for categorizing the genotypes into three levels of tolerance: tolerant, medium tolerant, and sensitive. These in vitro results correlated with the field performance of the genotypes.

In salt-stressed maize (*Zea mays* L. cv., DK 647 F1), the mechanism of growth amelioration by exogenously applied mannitol (M) and thiourea (T) was examined (Kaya *et al.*, 2013). After being planted in perlite-filled pots, maize seedlings were exposed to either 0 or 100 mM NaCl in full strength Hoagland's nutrient solution. Ten days after germination, the leaves of maize seedlings were sprayed with two concentrations of M (15 and 30 mM) or T (3.5 and 7.0 mM). The maize plants' dry biomass, chlorophyll content, and relative water content were all significantly reduced by salinity stress. On the other hand, it raised the levels of hydrogen peroxide (H_2O_2) and electrolyte leakage, as well as the activities of polyphenol oxidase (PPO; EC 1.10.3.1), catalase (CAT; EC 1.11.1.6), and superoxide dismutase (SOD; EC 1.15.1.1).

The improved growth of the mtlD gene-contained transgenic wheat genotype plants can be explained also on the basis of the bacterial, *E. coli*, mtlD gene-accumulating mannitol and soluble sugars (Ramadan *et al.*, 2013).

Akram *et al.* (2011) investigated the negative impacts of moisture stress on the germination and seedling growth of wheat. They used four growth substance ethephone, paclobutrazol, succinic acid, and triademifon in varying concentrations, along with distilled water, as seed treatments in a laboratory experiment. Moisture stress, induced by applying-0.4 M pa mannitol, significantly reduced the germination rate and inhibited the elongation of roots, shoots, and coleoptiles. However, due to the relatively lower adverse effect of water stress on root growth, the root-to-shoot ratio increased. The reductions in germination speed, root elongation, shoot growth, and coleoptile length were 51%, 5.70%, 58%, and 2.85%, respectively. The highest values for germination speed (23.98), root length (10.00 cm), shoot length (24.57 cm), and coleoptile length (5.09 cm) were observed in the control group.

Rajshekar (2010) investigated an in vitro screening method for osmotic tolerance in *Prunus* species. Significant growth differences were observed between two micropropagated Prunus accessions after 14 days in culture when 685 mM mannitol was incorporated into the Quorin and Lepoivre nutrient medium. Over a 28-day culture period, accession K537-067 exhibited an 11% increase in fresh weight, while accession New Jerseyplumcot No. 3 showed a 123% increase. Explants of Marianna 2624 were also exposed to two concentrations of mannitol, 275 mM and 550 mM, in the Quorin and Lepoivre nutrient medium. Three consecutive 28-day trials were performed. Explants were assessed for net growth changes at both 14 and 28 days after the experiment began. The inclusion of mannitol at concentrations of 275 mM and 550 mM reduced the fresh weight of Marianna 2624 explants to 36% and 28% of the control, respectively, at 28 days post-initial culture. Initial fresh weight and changes in fresh weight at day 14 varied significantly between trials. However, no significant differences were observed between trials regarding weight changes at 28 days post-initial culture.

Slama *et al.* (2007) demonstrated that mannitol increases the total carbohydrates (source of energy) and mineral content of *Sesuvium portulacastrum*, a halophyte, so that plant growth parameters especially total leaf area and leaf number also increases

2.2.5 Leaf

Leaf growth is a complex, quantitative trait, controlled by a plethora of regulatory mechanisms. Diverse environmental stimuli inhibit leaf growth to cope with the perceived stress. In plant research, mannitol is often used to impose osmotic stress and study the underlying growth-repressing mechanisms. In growing leaf tissue of plants briefly exposed to mannitol-induced stress, a highly interconnected gene regulatory network is induced (Nikonorova *et al*., 2018).

2.2.6 Photosynthates

In numerous plant species, mannitol, a significant osmolyte, is naturally produced in considerable quantities. It typically constitutes around half of the total translocated photoassimilates. Beyond its role in osmotic adjustment, mannitol serves as an antioxidant, effectively neutralizing hydroxyl radicals (OH●) as demonstrated by Kaya *et al.* (2013).

2.2.7 Chlorophyll Content

Kaya *et al.* (2013) reported that chlorophyll (Chl-a and Chl-b) contents were significantly lower in plants grown under NaCl stress than those in the control plants. However, exogenously applied mannitol or thiourea increased chlorophyll contents in the salt-stressed plants compared to those of the salt-stressed plants which were not supplied with mannitol or thiourea. Mannitol was again more effective than thiourea in increasing the chlorophyll contents of the salt-stressed maize plants. Moreover, Salinity stress significantly increased Na+ concentration and reduced K^+, Ca^{2+} and P concentrations in the leaves of maize plants. Salt-stressed maize plants accumulated significantly lower amount of Na^+ and higher amounts of K^+, Ca^{2+} and P upon foliar applications of mannitol or thiourea as compared to those of the salt-stressed plants that did not receive mannitol or thiourea. The leaf Na: K ratio increased significantly after the exposure of plants to NaCl stress, but exogenous application of mannitol or thiourea to salt stressed plants significantly decreased the Na: K ratio. As compared to thiourea, mannitol was found to be more effective in improving the contents of Ca^{2+} and K^+ in the leaves of salt-stressed maize plants, but it was less effective in reducing leaf Na^+.

2.2.8 Stress Mitigation

Zahir *et al.* (2010) explored in their study that, the impact of substrate-dependent auxin production by *Rhizobium phaseoli* on the growth and yield of *Vigna radiata* L. under salt stress conditions. The researchers isolated *Rhizobium phaseoli* strains from mungbean nodules, focusing on the salt-tolerant and high auxin-producing rhizobial isolate N_{20}. This isolate was then assessed, both with and without the presence of L-tryptophan (L-TRP), in enhancing mungbean growth and yield under saline conditions in a pot experiment. Mungbean seeds were inoculated with a peat-based inoculum, and NP fertilizers were applied at rates of 30-60 kg ha^{-1}. The results indicated that salt stress inhibited mungbean growth and yield. However, the application of L-TRP and *Rhizobium* separately showed some mitigation of the salt stress effects. Interestingly, their combined application demonstrated more substantial effects, resulting in increased plant height (by 28.2%), number of nodules per plant (by 71.4%), plant biomass (by 61.2%), seed yield (by 65.3%), and seed nitrogen concentration (by 22.4%) compared to the untreated control. This enhanced growth effect is attributed to the higher auxin production in the rhizosphere and improved mineral uptake, which collectively alleviated the adverse impacts of salinity.

Sugar alcohols (acyclic polyols or alditols) are extensively distributed in higher plants, with mannitol being the most prevalent, occurring in over 70 plant families (Lewis and Smith, 1967). Celery (*Apium graveolens* L.) has served as a model system for studying mannitol metabolism in higher plants. Pulse-chase experiments in celery have demonstrated that mannitol and sucrose are two primary photosynthetic products, produced in approximately equal amounts (Loescher *et al.,* 2017). In celery, mannitol constitutes 10 to 60% of the carbon exported from mature leaves (Daie, 1986) and becomes the main substrate translocated in the dark when sucrose reserves are depleted.

Gangopadhyay *et al.* (1997) successfully isolated mannitol-tolerant callus lines of *Brassica juncea* (L) czem. Var. RW-85-59 using a plated cell suspension culture technique with 165 mM mannitol. They subsequently developed callus lines adapted to a higher concentration of 329 mM mannitol through a direct adaptation process. During the initial stages, these calluses exhibited significant growth reduction when cultured on mannitol-containing media. However, in later passages, the growth of the adapted calluses recovered and became sustainable. These adapted calluses accumulated a notable amount of free proline in mannitol-containing media compared to calluses grown in stress-free conditions. Furthermore, it was observed that short-term osmotic shock treatment with high concentrations of mannitol allowed only the adapted lines to retain maximal levels of free proline for osmoregulation. This likely enabled the cells to resume normal growth once the stress was alleviated.

Mannitol dehydrogenase (MTD) is involved in the catabolism of mannitol in higher plants converting mannitol to mannose. MTD in higher plants is a 1-oxidoreductase which differs from microbial MTD (2-oxidoreductase) which converts mannitol back to MIP (Zamski *et al.*, 2001). The activity of MTD is known to be suppressed under stress allowing the accumulation of mannitol, which can act as an osmoprotectant, antioxidant, and a source of carbon (Prata *et al.*, 1997).

Mohamed *et al.* (2000) conducted in vitro selection and characterization of a drought-tolerant clone of *Tagetes minuta*. Cotyledons of *Tagetes minuta* were cultured on MS medium supplemented with 3 mg/L IAA and 10 mg/L BA, along with varying concentrations of mannitol (0, 20, 40, 60, 80, or 100 mM). Cotyledons on MS medium containing 60 mM mannitol died. Shoot clumps grown on callus growth medium with 80 mM mannitol for six months and subsequently transferred to callus growth medium also died. Four shoots were regenerated from 72 shoot clumps cultured on shoot growth medium after six months on callus growth medium without mannitol followed by three months on callus growth medium with 60 mM mannitol. Additionally, 12 shoots were regenerated from 72 shoot clumps on shoot growth medium after nine months on callus growth medium. Significant variations in biomass were observed among regenerated clones on mannitol-containing medium. After two months in greenhouse conditions, one clone exhibited significant in vitro tolerance to 90 mM mannitol, a concentration that completely inhibited control plant growth. This clone also demonstrated superior drought tolerance in the greenhouse compared to other regenerated and control plants, exhibiting lower water potential, greater biomass accumulation, and a higher relative growth rate.

Ehsanpour and Amini (2003) investigated the impact of drought stress on acid phosphatase activity in alfalfa explants cultured in vitro. They developed calluses from in vitro grown explants of *Medicago sativa* cultivars Yazdi and Hamedani under sterile conditions on MS medium supplemented with NAA and 2,4-D. The calluses and seedlings were then transferred to the same medium containing 0, 2, 4, 6, 8, and 10% mannitol to induce osmotic stress. After two weeks, both salt and drought stress resulted in increased acid phosphatase activity in both cultivars, though there were genotypic differences. They also found that drought stress tolerance in plants was enhanced by increased root length or the presence of more root branches. According to Matros (2015), the *et al.* biosynthesis and accumulation of mannitol and sugars in plants are linked to their salt stress tolerance. These solutes are thought to act as protectors or stabilizers of enzymes and membrane structures that are vulnerable to dehydration or ion-induced damage.

Mannitol plays a dual role in stress protection by aiding osmotic adjustment and supporting redox control (Rathinasabapathi, 2000). During stress conditions, soluble sugars and sugar-like compounds such as mannitol and sorbitol help in osmotic adjustments and contribute to the stabilization of membranes and proteins (Amiard *et al.*, 2003). The synthesis of mannitol can lead to improved carbon utilization efficiency (Stoop *et al.,* 1996), enhanced tolerance to oxidative stress (Keunen *et al.,* 2013), and increased resistance to salt stress, whereas these parameters were at their lowest under moisture stress without any treatment.

Abdelghany *et al*. (2004) utilized tissue culture techniques to identify drought-tolerant genotypes in bread wheat. The study aimed to investigate the growth and regeneration of calli derived from embryos of various bread wheat varieties under different mannitol concentrations (osmotic potential of 0, 0.6, 0.9, and 1.2M Pa) and to compare these in vitro results with growth and yield performance in field experiments. The researchers observed a highly significant interaction between variety and mannitol concentration for callus survival and regeneration ability. Specifically, the varieties Sakha 8 and Giza 157 exhibited the highest mean values for all callus traits, while Sakha 8 and Sids 1 demonstrated the highest callus survival percentage and regeneration ability at high mannitol concentrations. Additionally, Sakha 8 and Sids 1 produced the highest mean grain yield per plant in field experiments where water was withheld after flowering. Varieties with high or stable mean values for most traits under both irrigation systems included Sakha 8, Giza 157, and Sids 1, indicating the potential for selecting drought-tolerant genotypes under laboratory conditions.

Chiang *et al.* (2005) suggested that fructose, glucose, and sucrose play crucial roles in plant metabolism, enhancing the tolerance of plants with high mannitol content to salt stress. Studies on various transgenic plant species have demonstrated that the accumulation of mannitol and soluble sugars in plant tissues can confer tolerance to different types of abiotic stress, including salinity (Van den Ende and El-Esawe, 2014). These findings suggest that the synthesis and accumulation of mannitol and soluble sugars in transgenic tissues are sufficient to provide osmoprotection through a compatible solute mechanism.

Abu-Shama *et al.* (2005) explored the impact of in vitro-induced water deficit on the vegetative growth and physiology of the tomato landrace "Rohaba" (*Lycopersicon esculentum* Mill.) using sorbitol or mannitol at concentrations of 0.1, 0.2, and 0.3 M as osmotic agents. They found that shoot height was significantly reduced at 0.3 M sorbitol, and shoot fresh and dry weights decreased significantly with increasing sorbitol levels in the medium. However, rooting percentage, root number, and root length remained unaffected by sorbitol levels. Conversely, when mannitol was used, shoot height increased significantly at 0.1 M but decreased significantly at 0.2 M and 0.3 M. Both shoot dry weight and fresh weight decreased with increasing mannitol levels. Rooting percentage and root number were enhanced at 0.1 M mannitol, while root length increased in response to different mannitol levels. Additionally, the osmotic potential of tomato micro-shoots decreased significantly as sorbitol or mannitol levels increased.

Hu *et al*. (2005) pointed out that the transgenic plants were better able than wild-type plants to maintain cell membrane integrity under salt stress, which supports the hypothesis that proline, mannitol and soluble sugars serve as a protective function.

Errabii *et al.* (2006) subjected calli from two sugarcane cultivars, R570 and CP59-73, to varying intensities of osmotic stress followed by a period of stress relief. They evaluated the relative rate of growth (RGR), callus water content, and changes in organic and inorganic solutes at the end of both stress and relief periods. Following the stress period, calli from both cultivars exhibited a decrease in RGR, although to a lesser extent in R570 compared to CP59-73. A similar trend was observed in callus water content under mannitol-induced osmotic stress. Interestingly, the researchers found that inorganic solutes did not contribute significantly to osmotic adjustment in mannitol-stressed calli. The concentrations of potassium (K) and calcium ions (Ca^{2+}) decreased markedly, while those of sodium (Na) and magnesium (Mg) were not affected. However, at the end of the relief period, all the parameters returned to control levels, indicating that the changes induced by drought stress were reversible at the cellular level in sugarcane cultivars.

Tarczynski *et al.* (1993) showed that tobacco plants engineered to accumulate mannitol exhibited greater tolerance to salt stress compared to non-transformed tobacco plants. When grown in hydroponic nutrient culture with 250 mM NaCl, the growth of non-transformed tobacco plants was significantly inhibited compared to that of transformed tobacco plants containing mannitol. These experiments provide clear and unequivocal evidence that mannitol can enhance salt tolerance in higher plants.

Debnath (2008) studied the response of Bocopamonnieri to drought stress in vitro system. Distinct morphological changes on organogenesis and callogenesis were observed on stress application. Mannitol is a nonionicosmoticum in nature. Their result revealed that tissue growth was more impaired due to the effect of an ionic osmotic than a non-ionic one. They also reported an increase in root length and root branches in increasing concentration of mannitol of Bocopa suggesting that this plant can tolerate drought.

Errabii *et al.* (2007) investigated the impact of iso-osmotic mannitol stress on calli induced from sugarcane cultivars R570, CP59-73, and NC0310, focusing on callus growth, water content, and ion and proline concentrations. They found that callus growth and water content decreased under mannitol-induced osmotic stress. The ion concentration was significantly altered following exposure to mannitol. Specifically, potassium (K^+) and calcium ions (Ca^{2+}) concentrations decreased markedly, while sodium (Na^+) and potassium ions (K^+) concentrations remained unchanged. Free proline accumulation was observed under mannitol stress, particularly in the stress-sensitive cultivar. Their results indicated that the physiological mechanisms responding to osmotic-induced stress in sugarcane cultivars differed. Among the cultivars, they concluded that stress resistance was closely linked to maintaining adequate water status and high levels of potassium (K^+) and calcium ions (Ca^{2+}) under stress conditions.

Exogenous application of organic compounds such as mannitol have been found to be beneficial in ameliorating the adverse effects of salt stress on leaf photosynthetic pigments coupled with enhanced biomass production (Ali *et al*., 2007).

Chaum *et al.* (2010) investigated the biochemical, physiological, and morphological traits of rice cultivars under iso-osmotic water deficit and salt stress. Seedlings of three rice cultivars were grown in MS media and exposed to varying levels of osmotic stress (-0.23, -0.42, or -0.63 MPa). They observed a decrease in weight and humidity percentage as the level of osmotic stress increased. The effects of osmotic stress were strongly correlated with survival percentage, rooting percentage, and stem length. Based on their findings, they concluded that survival and rooting percentages alone could be used for evaluating drought tolerance. The study revealed variability in drought tolerance among the rice cultivars, with *P. domesticum* being the most tolerant, followed by *P. graveolens*. In contrast, *P. hortorum* and *P.* peltatum were more sensitive to osmotic stress. The method established by the researchers was efficient and simple, allowing for the evaluation of drought tolerance in a large number of genotypes in a short time. It could be utilized to identify and select tolerant and sensitive genotypes for improvement. Additionally, the method could be used to characterize various morphological, physiological, and molecular responses of plants under osmotic stress conditions

Mitoi *et al*. (2009) reported that foliar applications of mannitol and thiourea effectively reduce the adverse effects of oxidative stress on biological membranes by acting as a scavenger of ROS (reactive oxygen species).

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted from December 2022 to June 2023 in the agronomy research field at the Department of Agronomy, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200 to find out the effect of mannitol doses and application times on yield and yield contributing character of aromatic rice (BRRIdhan-50) through the foliar application of mannitol. Below is a description of this chapter, which covers the tools and methods used in the investigation:

3.1 Description of the experimental site

3.1.1 Geographical location

The experiment was conducted at the agronomy field of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The geographical position of the area is between 25.6980º N; 88.6550º E and 37.50 m above sea level. The Agro-Ecological Zone (AEZ) of the area is the Old Himalayan Piedmont Plain (AEZ-1).

3.1.2 Climatic condition

The climate of the experimental site is subtropical, characterized by heavy rainfall during April to September (Kharif season) and scanty rain during the rest of the year (Rabi season). The total rainfall of the experiment site was 221 mm during the experiment. The average maximum and minimum temperature were 29.45°C respectively during the experimental period Rabi season is characterized by plenty of sunshine. Bangladesh Bureau of Meteorology (climate division) provided the maximum and minimum temperatures, humidity, rainfall, and soil temperature for the study period.

3.1.3 Characteristics of soil

The experimental field is situated within the Old Himalayan Piedmont Plain (AEZ-1). The soil in the experimental plot is sandy loam, known for its effective drainage. The experimental area is at a medium-high altitude, with pH levels ranging from 5.5 to 7.5. Before sowing the soil at the experimental sites was analyzed and documented in Appendix-I. The soil in the experiment is classified as loamy clay, exhibiting pronounced cracking. It contains clays with a moderately alkaline nature and has low permeability.

3.2 Experimental details

3.2.1 Planting material

BRRIdhan50 (Banglamati)

Description of BRRI dhan50 variety:

Developed by	: Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh
Main character	: Plant height 82 cm, long slender, white scented
Method of development	: Hybridization
Year of release	: 2008
Yield	: 6.0 t/ha
Special quality	: Aromatic in nature, Cultivable in Boro season.

3.2.2 Treatments of the Experiment

The experiment was conducted to study aromatic rice (BRRIdhan50) through the application of mannitol in different times. There were two factors viz.

Factor A: Mannitol Doses

D_1 : 0 ppm (control)

D_2: 75 ppm

D_3:150 ppm

D_4: 225 ppm

D_5: 300 ppm

D_6: 375 ppm

Factor B: Application times

$T_{1:}$ 50 DAT

$T_{2:}$ 75 DAT

3.2.3 Design of the Experiment

The experiment was done with a Randomized Complete Block Design (factorial). Each treatment in the experiment was replicated three times. The total number of unit plots was 36. The size of the unit plot was 3m x 2m = 6m^2. The treatments were randomly distributed to each block. The distance between two adjacent replications (block) was 1 m and the row-to-row distance was 0.3 m, respectively. The inter-block and inter-row spaces were used as footpaths and irrigation or drainage channels. The layout of the experiment is shown in Figure 1.

3.3 Growing of Crops

3.3.1 Seed Collection and Sprouting

BRRI dhan50 seeds were procured from Bangladesh Agricultural Development Corporation in Dinajpur. Employing a standardized procedure, healthy seeds were meticulously chosen. Subsequently, they underwent a 24-hour immersion in a water container. Upon completion of this process, the seeds were extracted from the water and securely enclosed within burlap sacks. Within 48 hour post-immersion, signs of germination were evident, rendering them ready for planting within a span of 72 hours.

3.3.2 Raising of seedlings

The nursery bed was diligently prepared through a series of plowing and laddering to ensure optimal soil condition. Adequate irrigation was administered with care, and diligent weed management was maintained throughout, addressing any emerging weed growth promptly. Notably, no fertilizers were applied within the nursery bed during this process.

3.3.3 Seed sowing

The seeds are sown on the nursery bed on December 10, 2022 for raising nursery seedlings.

R_1	**R_2**	**R_3**
T_1D_4	T_2D_2	T_1D_1
T_2D_1	T_1D_2	T_1D_6
T_1D_1	T_2D_5	T_2D_6
T_1D_6	T_2D_3	T_1D_3
T_2D_6	T_1D_5	T_2D_2
T_1D_3	T_2D_4	T_1D_2
T_2D_2	T_1D_4	T_2D_5
T_1D_2	T_2D_1	T_2D_3
T_2D_5	T_1D_1	T_1D_5
T_2D_3	T_1D_6	T_2D_4
T_1D_5	T_2D_6	T_2D_1
T_2D_4	T_1D_3	T_1D_4

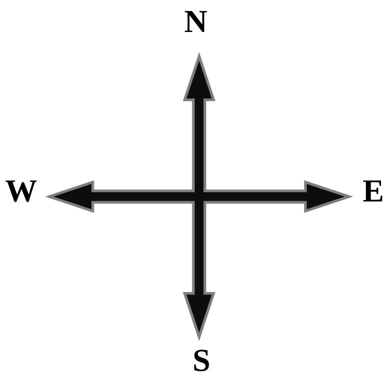

Fig. 1. Layout of the experiment

3.3.4 Preparation of experiment land

The soil of the experiment land was first opened on February 20, 2023, with the help of a tractor-drawn disc plough February 23, 2023, the land was irrigated and prepared by three successive ploughing and cross ploughing. Each plowing was followed by laddering to have a good puddle field. After plowing and laddering, all kinds of uprooted weeds and previous crop residues were removed from the field. After the final land preparation, the field layout was made on February 26, 2023, according to the experimental plan. Individual plots were cleaned and leveled so no water pocket could remain in the puddled field.

3.3.5 Fertilizer dose and methods of application

The experiment unit plots were fertilized with urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum, and zinc sulfate @ 150, 58, 58, 38, and 10 kgha^{1} respectively. The entire amounts of triple super phosphate, muriate of potash, gypsum, and zinc sulfate were applied as basal dose at the time of transplanting of seedlings. No Urea was applied during land preparation. After seedling recovery, urea was applied in three splits. At first, half of the total amount of urea was applied to the soil during the vegetative stage. Half of the remaining urea was applied at the tillering stage and half at 7 days before panicle initiation.

3.3.6 Transplanting of seedlings

The nursery bed was made wet by applying water one day before the uprooting of the seedlings. For transplantation, seedlings were uprooted carefully from the nursery beds on 28 February 2023 without causing much mechanical injury to the roots.

3.3.7 Intercultural operations

After transplanting the seedlings, different intercultural operations were accomplished for better growth and development, which are as follows.

3.3.7.1 Gap filling

After one week of transplantation, a minor gap filling was done where it was necessary to use the seedling from the same source.

3.3.7.2 Weeding

During the early stage of growth establishment, the crop was infested with some weeds. Two hand weeding were done for each treatment; the first weeding at 20 days after transplanting followed by the second weeding at 15 days after the first weeding.

3.3.7.3 Application of irrigation water

Irrigation water was added to each plot as and when required. All the plots were kept irrigated maintaining 3-5 cm stagnant water throughout the entire period up to 15 days before harvesting.

3.3.7.4 Plant Protection Measures

Crop protection measures were taken as and when necessary. No major attack of pest was observed in the field. The crop was protected from birds during the grain-filling stage. For controlling the birds watching was done properly, especially during the morning and afternoon.

3.4 General observation of the experimental field

Observations were regularly made to detect visual differences among the treatment and any kind of infestation by weeds, insects and diseases so that considerable losses by pests could be minimized. The field looked nice with normal green color plants. Incidence of stem borer, green leaf hopper, leaf roller and rice hispa was observed during the tillering stage that was controlled properly. No bacterial and fungal diseases were observed in the field.

3.5 Harvesting and post-harvest operation

When 90% of the grains became golden yellow in color maturity of crop was determined. BRRIdhan50 was harvested on June 08, 2023. Five hills plot^{-1} were preselected randomly from which different growth and yield attributes data were collected and Im^2 areas from middle portion. Each plot was harvested separately, bundled, properly tagged and then brought to the threshing floor for recording grain and straw yield. Threshing was done manually by laborers. The grains were cleaned, and sun-dried at the moisture content of 20% approximately. Straw was also sun-dried properly. Finally, grain and straw yields plot^{-1} were recorded and converted to tha^{-1}.

3.6 Recording of data

The following data were recorded during the experiment.

A. Crop growth characters

Plant height (cm) at harvest

i Number of tillers hill^{-1}

B. Yield and yield components

1. Number of effective tillers hill^{-1}
ii. Number of non-effective tillers hill^{-1}
iii. Length of panicle (cm)
iv. Number of filled grains panicle^{-1} Number of unfilled grains panicle
vi. Number of total grains panicle^{-1}
vii. 1000 grain weight (g)
viii. Grain yield (t ha^{-1})
ix. Straw yield (t ha^{-1})
X. Harvest index (%)

3.7 Detailed procedures of recording data

A. Crop growth characters

i. Effective tillers hill^{-1} (no.)

The height of plant was recorded in centimeter (cm) and measured from the ground level to the tip of the tallest panicle. Plants of 5 hills were measured and averaged for each plot.

ii. Number of tillers hill^{-1}

The number of tillers hill^{-1} was counted at harvest from ten randomly pre-selected hills and averaged as their number hill. Only those tillers having three or more leaves were considered for counting.

B. Yield and other crop characters

i. Effective tillers hill^{-1} (no.)

The total number of effective tillers hill^{-1} was counted as the number of panicles which had at least one grain. The number of effective tillers hill^{-1} was recorded and finally averaged for counting effective tillers number hill^{-1}.

ii. Non-effective tillers hill^{-1} (no.)

The total number of non-effective tillers hill^{-1} was counted as those with no panicle on the head. The number of non-effective tillers hill^{-1} was recorded and finally averaged for counting non-effective tillers number m².

iii. Panicle length (cm)

The length of the panicle was measured from the rachis's basal node to each panicle's apex. Each observation was an average of 5 panicles.

iv. Filled grains panicle^{-1} (no.)

If any kernel was present in the grain, the grain was considered to be filled. The total number of filled grains was recorded on five panicles and finally averaged.

vi. Unfilled grains panicle^{-1} (no.)

Unfilled grains mean the absence of any kernel inside and such grains present on each of five panicles were counted and finally averaged.

vii. Total grains panicle^{-1} (no.)

The total number of grain panicles^{-1} was calculated by summation of filled and unfilled grains panicle^{-1}.

viii. Weight of 1000-grain (g)

One thousand cleaned and dried grains were counted randomly from each sample and weighed by using a digital electric balance at the stage the grain retained about 12% moisture and the mean weight were expressed in gram.

ix. Grain yield (t ha^{-1})

Grain yield determined from the central 1m² areas of each plot were sun dried, cleaned, weighed carefully and adjusted at 12% moisture level. Weight of grains of each plot was converted into t ha^1. Grain moisture content was measured by using a digital moisture tester.

x. Straw yield (t ha^{-1})

Straw yield was determined from the central 1 m^2 areas of each plot. After separating of grains, the sub-samples were oven dried to a constant weight and finally converted to t ha^{-1}.

xi. Biological yield (t ha^{-1})

Grain yield together with straw yield was regarded as biological yield and calculated with the following formula:

Biological yield (t ha^{-1}) = Grain yield (t ha^{-1}) + Straw yield (t ha^{-1})

xii. Harvest Index (%)

Harvest index denotes the ratio of economic yield to biological yield and was calculated with following formula.

Harvest Index = (Grain Yield (t ha^{-1}) / Biological Yield (t ha^{-1})) × 100

3.8 Statistical Analysis

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique and the mean differences were adjudged by LSD test using the statistical computer package program, Statistix-10.

CHAPTER IV

RESULTS AND DICUSSION

This chapter comprises of the presentation and discussion of the results obtained due to the foliar application of Mannitol on the yield performance of BRRI dhan50. The data have been shown in various figures and tables.

4.1 Number of effective tillers hill^{-1}

4.1.1 Effect of Mannitol application time on effective tillers hill^{-1}

Different application times of Mannitol had a significant effect number of effective tilers in hill^{-1} (Table 1). The highest number of effective tillers hill^{-1} (21.64) was found at T_1 (50 DAT) and the lowest number of effective tillers hill^{-1} (19.32) was found at T_2 (75 DAT) treatment combination (Table 1).

Table 1. Effect of Mannitol application time on effective tillers (no.), non-effective tillers (no.), panicle length (cm), grains panicle^{-1} filled grain panicle^{-1}, unfilled grains panicle^{-1}

Time	Effective Tiller	Non Effective Tiller	Panicle Length (cm)	Total grain panicle^{-1}	Filled grain panicle^{-1}
T_1	21.64 a	2.79 b	22.408	181.25	168.98
T_2	19.32 b	4.5 a	21.74	177.11	165.05
CV	7.96	39.99	7.54	4.25	4.38
LS	**	**	NS	NS	NS
$LSD_{0.05}$	1.13	1.01	1.15	5.26	5.05

In a column, figure bearing same, or no letter (s) do not differ significantly at 5% level of significance by Duncan's Multiple Range Test
Legend:
T_1= 50 DAT
T_2= 75 DAT
*= 5% level of significance, **= significance at 1% level of significance
LS= Level of significance; CV= Co-efficient of variance; LSD= Least significant difference

4.1.2 Effect of dose of Mannitol on effective tillers hill^{-1}

The application of different levels of Mannitol had a significant effect on number of effective tillers hill^{-1}. Number of effective tillers increases with the increase of Mannitol concentration level up to a certain limit. The highest number of effective tiller hill^{-1} (24.60) was found in the treatment T_4 due to the application of 225 mM of Mannitol which varied significantly from the control T_1 treatment (0 mM) (Table 2). The lowest number of effective tillers hill^{-1} (15.67) was found in the control T_1 treatment.

Table 2. Effect of doses of Mannitol on effective tillers (no.), non-effective tillers (no.), panicle length (cm), grains panicle^{-1} filled grain panicle^{-1}, unfilled grains panicle^{-1}

Dose	Effective Tiller	Non effective tiller	Panicle Length (cm)	Total grain panicle^{-1}	Filled grain panicle^{-1}
D_1	15.67 d	4.87 a	19.96 d	168.65 d	146.47 e
D_2	18.90 b	3.80 ab	20.65 cd	171.90 cd	156.17 d
D_3	21.88 b	3.23 ab	21.76 bcd	181.88 ab	172.47 bc
D_4	24.60 a	2.07 b	24.10 a	189.45 a	185.08 a
D_5	21.67 b	3.93 a	23.39 ab	183.81 ab	175.90 b
D_6	20.17 bc	3.97 a	22.58 abc	179.37 bc	165.97 c
CV	7.96	39.99	7.54	4.25	4.38
LS	**	*	**	**	**
$LSD_{0.05}$	1.95	1.74	1.99	9.114	8.75

In a column, figure bearing same, or no letter (s) do not differ significantly at 5% level of significance by Duncan's Multiple Range Test

Legend:

D_1 = 0 mM　　D_4= 225 mM

D_2 = 75 mM　　D_5= 300 mM

D_3= 150 mM　　D_6= 375 mM

*= 5% level of significance, **= significance at 1% level of significance

LS= Level of significance, CV= Co-efficient of variance, LSD = Least significant difference

4.1.3 Interaction effect of dose and application time of Mannitol on effective tiller hill^{-1}

The number of effective tillers hill^{-1} significantly varies due to imposition of different combination of dose of Mannitol and their application time at different days after transplanting (DAT). The highest number of effective tillers hill^{-1} (26.13) was observed in T_1D_4 combination and lowest (15.33) was observed in T_2D_1 treatment combination (Table 3)

4.2 Non effective tiller hill^{-1}

4.2.1 Effect of application time of mannitol on number of non effective tillers hill^{-1}

The effect of application time of Mannitol on the number of non effective tillers hill^{-1} varied significantly. The highest number non effective tillers hil^{-1} was produced (4.5) by the T_2 treatment (75 DAT) and lowest was (2.79) prodced by the T_1 treatment (50 DAT) (Table 1).

4.2.2 Effect of dose of Mannitol on number of non effective tillers hill^{-1}

The effect of dose of Mannitol on the number of of non effective tillers hill^{-1} varied significantly. The highest number non effective tillers hill^{-1} was observed (4.87) in D_1 treatment (0 mM) and lowest number non effective tillers hill^{-1} was observed (2.07) in D_4 treatment (225 mM) (Table 2).

4.2.3 Interaction effect of application time and dose of Mannitol on number of non effective tillers hill^{-1}

The number of non-effective tiller hill^{-1} significantly varired due to imposition of different combination of dose of mannitol and time of apllication at different days after transplanting (DAT). The highest nymber of non-effective tillers hill^{-1} (5.67) was observed in T_2D_1 combination and lowest (1.20) was observed T_1D_4 treatment combination (Table 3).

4.3 Panicle length (cm)

4.3.1 Effect of application time of Mannitol on panicle length

The effect of the application time of Mannitol on the panicle length varied significantly. The highest panicle length (22.41 cm) was produced by the T_1 treatment (50 DAT) and the lowest(21.74 cm) was produced by the T_2 treatment (75 DAT) (Table 1).

4.3.2 Effect of dose of Mannitol on panicle length

The effect of dose of Mannitol on the panicle length varied significantly. The highest panicle length (24.10 cm) was observed in the D_4 treatment (225 mM) and the lowest panicle length (19.96 cm) was observed in the D_1 treatment (0 mM) (Table 2).

Table 3. Interaction effect of dose and application time of Mannitol on effective tillers (no.), non-effective tillers (no.), panicle length (cm), filled grains panicle^{-1}, unfilled grains panicle^{-1}

Interaction		Effective tiller no.	Non-effective tiller no.	Panicle Length (cm)	Total grain panicle^{-1}	Filled grain panicle^{-1}
	D_1	16.00 fg	4.07 abc	168.69 e	147.46 f	19.96 d
	D_2	19.33 de	2.67 bcd	170.34 de	156.19 ef	21.12 bcd
	D_3	24.03 ab	1.73 cd	183.52 abc	174.48 bcd	22.06 a-d
T_1	D_4	26.13 a	1.20 d	195.31 a	191.37 a	24.56 a
	D_5	22.67 bc	3.87 abc	187.74 ab	179.50 ab	23.87 ab
	D_6	21.67 bcd	3.20 a-d	181.86 bcd	164.86 de	22.89 abc
	D_1	15.33 g	5.67 a	168.62 e	145.48 f	19.97 d
	D_2	18.47 ef	4.93 ab	173.46 cde	156.15 ef	20.18 cd
T_2	D_3	19.73 de	4.73 ab	180.24 b-e	170.47 bcd	21.47 bcd
	D_4	23.07 bc	2.93 bcd	183.59 abc	178.80 bc	23.65 ab
	D_5	20.67 cde	4.00 abc	179.88 b-e	172.30 bcd	22.92 abc
	D_6	18.67 ef	4.73 ab	176.88 b-e	167.07 cde	22.27 a-d
CV		7.96	39.99	7.54	4.25	4.38
LS		*	*	*	*	*
$LSD_{0.05}$		2.76	2.47	2.82	12.89	12.37

In a column, figure bearing same, or no letter (s) do not differ significantly at 5% level of significance by Duncan's Multiple Range Test

Legend

T_1D_1= At 50 DAT 0 mM of Mannitol (control)
T_1D_2= At 50 DAT 75 mM of Mannitol
T_1D_3 = At 50 DAT 150 mM of Mannitol
T_1D_4= At 50 DAT 225 mM of Mannitol
T_1D_5= At 50 DAT 300 mM of Mannitol
T_1D_6= At 50 DAT 375 mM of Mannitol
T_2D_1 = At 75 DAT 0 mM of Mannitol (control)
T_2D_2 = At 75 DAT 75 mM of Mannitol
T_2D_3 = At 75 DAT 150 mM of Mannitol
T_2D_4 = At 75 DAT 225 mM of Mannitol
T_2D_5 = At 75 DAT 300 mM of Mannitol
T_2D_6 = At 75 DAT 375 mM of Mannitol

*= 5% level of significance, **= significance at 1% level of significance
LS= Level of significance; CV= Co-efficient of variance; LSD= Least significant difference

4.3.3 Interaction effect of application time and dose of Mannitol on panicle length

The length of the panicle significantly varied due to the imposition of different combinations of doses of mannitol and the time of application on different days after transplanting (DAT). The highest panicle length (24.55 cm) was observed in the T_1D_4 treatment combination and the lowest (19.96 cm) was observed T_1D_1 treatment combination (Table 3).

4.4 Number of total grains panicle^{-1}

4.4.1 Effect of application time of Mannitol on the number of total grains panicle^{-1}

The effect of the application time of Mannitol on the number of total grains panicle^{-1} varied significantly. The highest number of total grains panicle^{-1} (181.25) was produced by the T_1 treatment (50 DAT) and the lowest (177.11) was produced by the T_2 treatment (75 DAT) (Table 1).

4.4.2 Effect of dose of Mannitol on the number of total grains panicle^{-1}

The effect of the dose of Mannitol on the number of total grains panicle^{-1} varied significantly. The highest number of total grains panicle^{-1} (189.45) was observed in the D_4 treatment (225 mM) and the lowest number total grains panicle^{-1} (168.65) was observed in the D_1 treatment (0 mM) (Table 2).

4.4.3 Interaction effect of application time and dose of Mannitol on number of total grains panicle^{-1}

The number of total grains panicle^{-1} significantly varied due to the imposition of different combinations of doses of mannitol and the time of application on different days after transplanting (DAT). The highest number of total grains panicle^{-1} (195.31) was observed in the T_1D_4 treatment combination and the lowest (168.62) was observed T_1D_2 treatment combination (Table 3).

4.5 Number of filled grains panicle^{-1}

4.5.1 Effect of application time of Mannitol on number of filled grains panicle^{-1}

The effect of the application time of Mannitol on the number of filled grains panicle^{-1} varied significantly. The highest number of filled grains panicle^{-1} (168.98) was produced by the T_1 treatment (50 DAT) and the lowest (165.05) was produced by the T_2 treatment (75 DAT) (Table 1).

4.5.2 Effect of dose of Mannitol on number of filled grains panicle^{-1}

The effect of the dose of Mannitol on the number of filled grains panicle^{-1} varied significantly. The highest number of filled grains panicle^{-1} (185.08) was observed in the D_4 treatment (225 mM) and the lowest number of filled grains panicle^{-1} (146.47) was observed in the D_1 treatment (0 mM) (Table 2).

4.5.3 Interaction effect of application time and dose of Mannitol on the number of filled grains panicle^{-1}

The number of filled grains panicle^{-1} significantly varied due to the imposition of different combinations of doses of mannitol and the time of application on different days after transplanting (DAT). The highest number of filled grains panicle^{-1} (191.37) was observed in the T_1D_4 treatment combination and the lowest (145.48) was observed T_2D_1 treatment combination (Table 3).

Table 4. Effect application time of Mannitol on 1000 grain weight (g), grain yield (tha^{-1}), straw yield (t ha^{-1}), biological yield (t ha^{-1}) and harvest index (%) of rice

Time	Unfilled grain panicle^{-1}	1000 Grain Weight (g)	Straw Yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest Index
T_1	12.27 a	19.38 a	7.50 a	13.17 a	42.91 a
T_2	12.07 a	17.91 b	7.05 b	12.32 b	42.59 a
CV	35.73	5.21	1.88	3.25	4.78
LS	NS	**	**	**	NS
$LSD_{0.05}$	3.01	0.67	0.097	0.29	1.41

In a column, figure bearing same, or no letter (s) do not differ significantly at 5% level of significance by Duncan's Multiple Range Test
Legend:
T_1= 50 DAT
T_2= 75 DAT
*= 5% level of significance, **= significance at 1% level of significance
LS= Level of significance; CV= Co-efficient of variance; LSD= Least significant difference

4.6 Number of unfilled grain panicles^{-1}

4.6.1 Effect of application time of Mannitol on the number of unfilled grains panicle^{-1}

The effect of the application time of Mannitol on the number of unfilled grains panicle^{-1} varied significantly. The highest number of unfilled grains panicle^{-1} (12.06) was produced by the T_2 treatment (75 DAT) and the lowest (12.27) was produced by the T_1 treatment (50 DAT) (Table 4).

4.6.2 Effect dose of Mannitol on the number of unfilled grains panicle^{-1}

The effect of the dose of Mannitol on the number of unfilled grains panicle^{-1} varied significantly. The highest number of unfilled grains panicle^{-1} (22.18) was observed in the D_1 treatment (0 mM) and the lowest number of unfilled grains panicle^{-1} (4.36) was observed in the D_4 treatment (225 mM) (Table 5).

4.6.3 Interaction effect of application time and dose of Mannitol on the number of filled grains panicle^{-1}

The number of unfilled grains panicle^{-1} significantly varied due to the imposition of different combinations of doses of mannitol and the time of application on different days after transplanting (DAT). The highest number of unfilled grains panicle^{-1} (23.13) was observed in the T_2D_1 treatment combination and the lowest (3.94) was observed T_1D_4 treatment combination (Table 6).

Table 5. Effect of dose of Mannitol on 1000 grain weight (g), grain yield (t ha^{-1}), straw yield (t ha^{-1}), biological yield (t ha^{-1}) and harvest index (%) of rice

Dose	Unfilled grain panicle^{-1}	1000 Grain Weight (g)	Straw Yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest Index
D_1	22.18 a	17.61 c	7.08 b	11.62 c	38.95 b
D_2	15.73 b	18.24 bc	7.25 a	12.10 c	39.91 b
D_3	9.41 cd	18.41 bc	7.31 a	13.13 b	44.26 a
D_4	4.36 d	19.82 a	7.39 a	13.63 a	45.73 a
D_5	7.91 d	18.91 ab	7.28 a	13.08 b	44.35 a
D_6	13.41 bc	18.41 bc	7.32 a	12.91 b	43.30 a
CV	35.73	5.21	1.88	3.25	4.78
LS	**	*	*	**	**
$LSD_{0.05}$	5.20	1.16	0.16	0.49	2.44

In a column, figure bearing same, or no letter (s) do not differ significantly at 5% level of significance by Duncan's Multiple Range Test

Legend:

D_1 = 0 mM　　D_4= 225 mM

D_2 = 75 mM　　D_5= 300 mM

D_3= 150 mM　　D_6= 375 mM

*= 5% level of significance, **= significance at 1% level of significance

LS= Level of significance, CV= Co-efficient of variance, LSD = Least significant difference

4.7 1000 Grain weight

4.7.1 Effect of application time of Mannitol on 1000 grain weight (g)

The effect of the application time of Mannitol on 1000 grain weight varied significantly. The maximum 1000-grain weight (19.24 g) was produced by the T_1 treatment (50 DAT) and the lowest (17.91 g) was made by the T_2 treatment (75 DAT) (Table 4).

4.7.2 Effect of dose of Mannitol on 1000 grain weight (g)

The effect of the dose of Mannitol on 1000-grain weight (g) varied significantly. The highest 1000-grain weight (19.82 g) was observed in the D_4 treatment (225 mM) and the lowest 1000-grain (17.64 g) was observed in the D_1 treatment (0 mM) (Table 5).

Table 6. Interaction effect of dose and application time of Mannitol on 1000 grain weight (g), grain yield (t ha^{-1}), straw yield (t ha^{-1}), biological yield (t ha^{-1}), and harvest index (%) of rice

Interaction		Unfilled grain panicle^{-1}	1000 Grain Weight (g)	Straw Yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest Index
	D_1	21.23 ab	17.33 e	7.39 ab	12.26 de	39.66 bc
	D_2	14.15 bcd	19.05 bcd	7.46 a	12.39 de	39.75 bc
	D_3	9.05 de	19.23 abc	7.53 a	13.40 ab	43.78 a
T_1	D_4	3.94 e	20.83 a	7.58 a	14.06 a	45.97 a
	D_5	8.24 de	19.73 ab	7.46 a	13.48 ab	44.61 a
	D_6	17.00 abc	19.25 abc	7.57 a	13.46 ab	43.69 a
	D_1	23.13 a	17.97 cde	6.78 d	10.98 f	38.25 c
	D_2	17.31 ab	17.43 de	7.06 c	11.80 e	40.08 bc
T_2	D_3	9.77 cde	17.59 de	7.10 c	12.86 bcd	44.75 a
	D_4	4.79 e	18.81 bcde	7.20 bc	13.20 bc	45.48 a
	D_5	7.58 de	18.10 bcde	7.10 c	12.69 cd	44.10 a
	D_6	9.81 cde	17.57 de	7.07 c	12.38 de	42.90 ab
CV		35.73	5.21	1.88	3.25	4.78
LS		*	*	*	*	*
$LSD_{0.05}$		7.36	1.64	0.23	0.70	3.46

In a column, figure bearing same, or no letter (s) do not differ significantly at 5% level of significance by Duncan's Multiple Range Test

Legend

T_1D_1= At 50 DAT 0 mM of Mannitol (control)
T_1D_2= At 50 DAT 75 mM of Mannitol
T_1D_3 = At 50 DAT 150 mM of Mannitol
T_1D_4= At 50 DAT 225 mM of Mannitol
T_1D_5= At 50 DAT 300 mM of Mannitol
T_1D_6= At 50 DAT 375 mM of Mannitol
T_2D_1 = At 75 DAT 0 mM of Mannitol (control)
T_2D_2 = At 75 DAT 75 mM of Mannitol
T_2D_3 = At 75 DAT 150 mM of Mannitol
T_2D_4 = At 75 DAT 225 mM of Mannitol
T_2D_5 = At 75 DAT 300 mM of Mannitol
T_2D_6 = At 75 DAT 375 mM of Mannitol

*= 5% level of significance, **= significance at 1% level of significance

LS= Level of significance; CV= Co-efficient of variance; LSD= Least significant difference

4.7.3 Interaction effect of application time of Mannitol and dose on 1000 grain weight

1000-grain weight significantly varierd due to imposition od different combination of dose of Mannitol and time of application at different days after transplanting (DAT). The highest 1000-grain weight (20.83 g) was observed in T_1D_4 treatment combination and lowest (17.32 g) was observed in T_1D_1 treatment combination.(Table 6).

4.8 Grain Yield (t ha^{-1})

4.8.1 Effect of application time of Mannitol on grain yield

The effect of the application time of Mannitol on grain yield varied significantly. The maximum grain yield (5.67 t ha^{-1}) was produced by the T_1 treatment (50 DAT) and the lowest (5.27 t $^{-1}$) was made by the T_2 treatment (75 DAT) (Fig. 1).

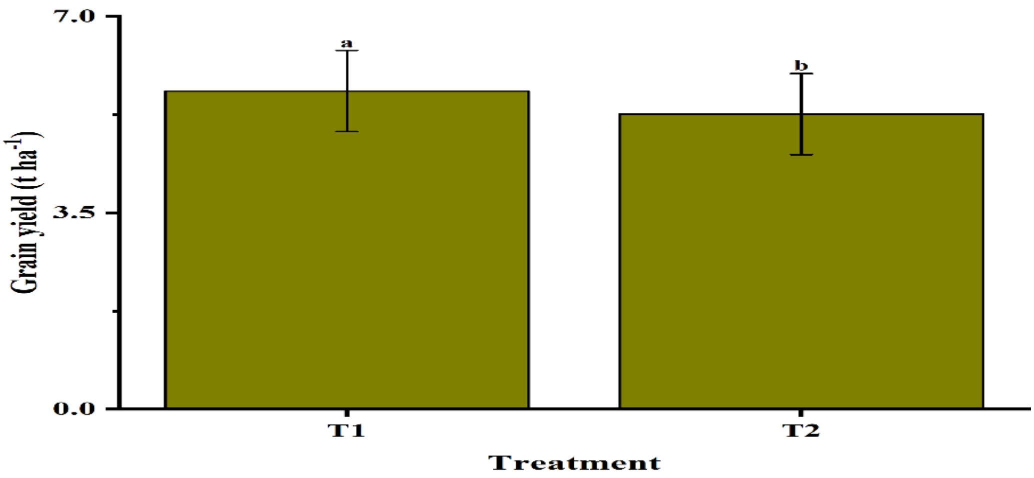

Fig. 2. Effect of application time of Mannitol on grain yield

4.8.2 Effect of dose of Mannitol on grain yield

The influence of Mannitol on grain yield was significantly varied among the treatments Fig. 2 shows the effects of different levels of Mannitol on grain yield of BRRI dhan50. The maximum grain yield (6.24 tha^{-1}) was found in treatment D_4 having 225 mM of Mannitol which differs statically from all other treatments and this result revealed that the grain yield

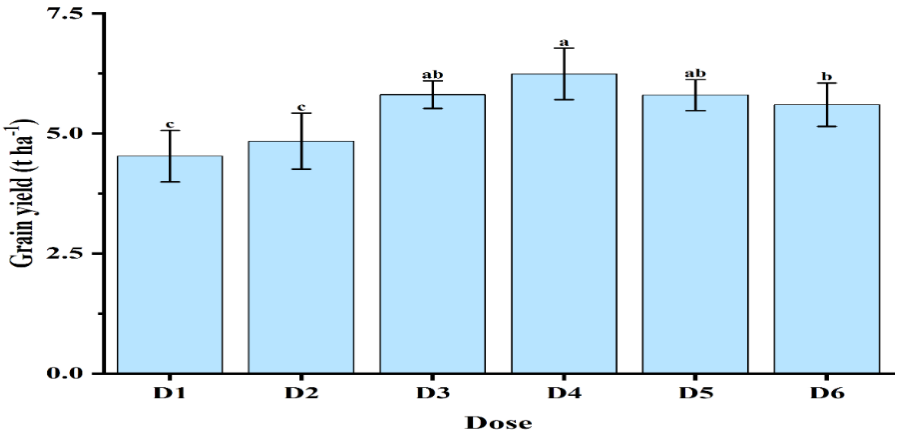

Fig. 3. Effect of dose of Maniitol on Grain Yield

of treatment D_4 had a 27.24 % higher yield over control D_1. Here treatment D_1 shows the lowest yield (4.53 t ha^{-1}). Treatments D_1 and D_2 do not differ significantly. Treatments $D_{3,}$ D_5 and D_6 do not differ significantly but they differ significantly with control (Treatment D_1). The increasing result may cause due to the increase number of tiller $hill^{-1}$,increased filled grain $panicle^{-1}$, increased amount of chlorophyll content which helps the plant to produce more food. Ultimately the grain yield increased. Parveen *et al.* (2020) showed that foliar application of osmolyte can increase the grain yield of rice.

4.8.3 Interaction effect of dose and application time of mannitol on grain yield

Grain yield significantly varied due to the imposition of different combinations of doses of Mannitol and time of application on different days after transplanting (DAT). The highest grain yield (6.47 t ha^{-1}) was observed in the T_1D_4 treatment combination and the lowest (4.19 t ha^{-1}) was observed in T_2D_1 treatment combination (Fig. 3).

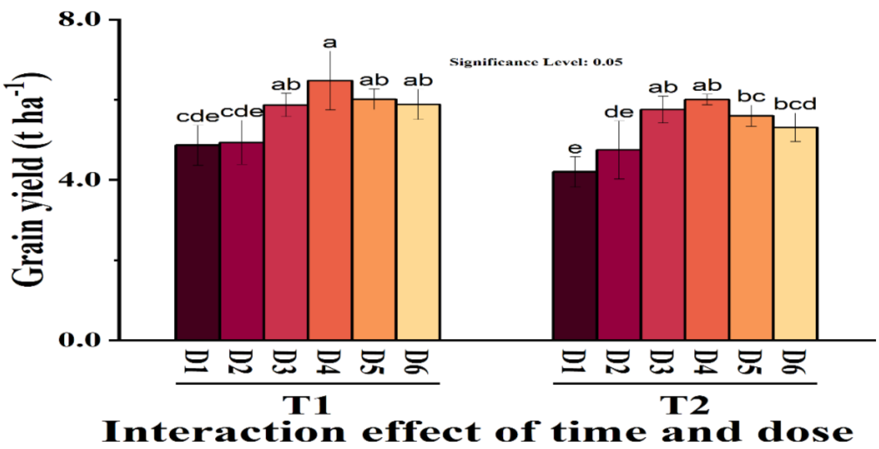

Fig. 4. Interaction effect of dose and application time of Mannitol on grain yield of rice

4.9 Straw Yield (t ha^{-1})

4.9.1 Effect of Application Time of Mannitol on Straw Yield

The foliar application of different levels of Mannitol on straw yield varied significantly. The highest straw yield was (7.50 t ha^{-1}) produced by the T_1 treatment (50 DAT) and lowest (7.05 t ha^{-1}) was produced by the treatment T_2 (75 DAT) (Table 4).

4.9.2 Effect of dose of Mannitol on straw yield

The foliar application of different levels of Mannitol had a significant effect on straw yield. Table 5 shows that the foliar application of mannitol on BRRI dhan50 gave a higher straw yield compared to control (D_1). Here the lowest straw yield (7.08 t ha^{-1}) was found in treatment D_1 (0 mM) mannitol and the highest (7.39 t ha^{-1}) was found in treatment D_4 due to the higher dose of Mannitol (225 mM). The highest straw yield (7.39 t ha^{-1}) was found in treatment D_4 which differs significantly from control D_1. Treatments D_3, $D_{5,}$ and D_6 were statistically identical to D_4 but a higher yield was obtained in D_4. This increased result may caused due to the increased level of chlorophyll content as well as increased dry matter content.

4.9.3 Interaction effect of dose and time of Mannitol application on straw yield

Straw yields significantly varied due to the imposition of a different combination of doses of Mannitol and time of application at different days after transplanting (DAT). The highest straw yield (7.58 t ha^{-1}) was observed in the T_1D_4 treatment combination and the lowest (6.78 t ha^{-1}) was observed in the T_2D_1 treatment combination (Table 6).

4.10 Biological yield

4.10.1 Effect of application time of mannitol on biological yield

The effect of application time of Mannitol on biological yield varied significantly. The highest biological yield (13.17 t ha^{-1}) was produced by the T_1 treatment (50DAT) and the lowest biological yield (12.32 t ha^{-1}) was produced by the T_1 treatment (Table 4).

4.10.2 Effect of dose of Mannitol on biological yield

Significance response was observed in biological yield of BRRI dhan50 due to foliar application of different level of Mannitol (Table 5). The biological yield was varied from 11.62-13.63 t ha^{-1}. The highest was biological yield (13.63 t ha^{-1}) was observed in the D_4 treatment (225 mM) on the other hand , lowest bilogical yield (11.62 t ha^{-1}) was obtained in the D_6 treatment. It was observed that as the rate of foliar application of Mannitol increases biological yield also increases.

4.10.3 Effect of Mannitol doses and application time on biological yield

Biological yield significantly varied due to imposition of different combination of dose of Mannitol and time of application of Mannitol at different days after transplanting (DAT). The highest biological yield (14.06 t ha^{-1}) was observed in T_1D_4 treatment combination and lowest was observed in T_2D_1 treatment combination (Table 6).

4.11 Harvest index

4.11.1 Effect of application time of Mannitol on harvest index

The effect of the application time of mannitol on the harvest index does not vary significantly but the highest harvest index (42.91%) was observed in the T_1 treatment (50 DAT) and the lowest (42.59%) was observed in the T_2 treatment (Table 4).

4.11.2 Effect of dose of Mannitol on harvest index

Harvest index (HI) is the ratio of seed yield to biological yield. Significant response was observed in the harvest index due to the foliar application of different levels of mannitol concentration. A significant difference was not present in treatments $D_{3,}$ D_4, $D_{5,}$ and D_6 but the highest value (45.73%) was observed in D_4. From Table 5 the lowest value (38.95%) was observed in D_1 (control 0 mM) treatment.

4.11.3 Effect of Mannitol application time and dose of Mannitol on harvest index

Harvest index significantly varied due to the imposition of different combinations of dose of Mannitol and application time at different days after transplanting (DAT). The highest harvest index (45.97 %) was observed in the T_1D_4 treatment combination and the lowest (38.25%) was observed in the T_2D_1 treatment combination (Table 6).

CHAPTER V

SUMMARY AND CONCLUSION

The field experiment was conducted at the agronomy research field, Department of Agronomy, Hajee Mohammad Danesh Science and Technology University, Dinajpur, during Boro season to evaluate the effect of dose and application time of Mannitol on the growth and yield of BRRI dhan50 (Banglamoti an aromatic rice). The experiment was comprised with two factor viz. A. Dose (0 mM, 75 mM, 125 mM, 225 mM, 300 mM, 375 mM of Mannitol). B. Application time of Mannitol at 50 DAT and 75 DAT. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

The data on crop growth characters viz. plant height (cm), number of total tillers hill^{-1} and yield and yield contributing characters viz. number of effcetive and non effective tillers hill^{-1}, panicle length(cm), number of filled and unfilled grain panicle^{-1}, 1000 grains-weight(g), grain yield (t ha^{-1}), Straw yield (t ha^{-1}), biological yield (t ha^{-1}) and harvest index (%) were recorded and data analysis was done using the Statistix-10 software.

Results revealed that the effect of Mannitol doses insignificantly influenced rice's growth and yield parameters. D_4 (225 mM of Mannitol) gave the best result for tillers plants^{-1} (26.67); no. of effective tillers plants^{-1} (24.60); minimum no. of ineffective tillers plants^{-1} (2.06); panicle length (24.10cm); total grains panicle^{-1} (189.45) ; filled grains panicle^{-1} (185.08); minimum no of unfilled grain panicle^{-1} (4.36); thousands grain weight (19.82 gm); grain yield (6.24 t ha^{-1}); straw yield (7.39 t ha^{-1}); biological yield (13.63); harvest index (45.73%). On the other hand lowest results were recorded from control 0 mM treatments. D_1 (0 mM of Mannitol) showed the lowest number of total tillers hill^{-1} (20.53), effective tillers hill^{-1} (15.67), the highest number of non-effective tillers hill^{-1} (4.87), total grain panicle^{-1} (168.65), panicle length (19.96 cm), number of filled grains panicle^{-1} (146.47), a highest number of unfilled grains panicle^{-1} (22.18), thousand-grain weight (17.65 gm), and lowest grain yield (4.53 t ha^{-1}).

The effect of different time of application of Mannitol was significant in the most of the collected parameters. Results shows that the highest results was recorded from T_1 50 DAT. The highest no. of tillers plants^{-1} (24.42); no. of effective tillers plants^{-1} (21.64);

minimum no. of ineffective tillers plants^{-1} (2.79); panicle length (22.41cm); total grains panicle^{-1} (181.25) ; filled grains panicle^{-1} (168.98); minimum no of unfilled grain panicle^{-1} (12.06); thousands grain weight (19.24 gm); grain yield (5.67 t ha^{-1}); straw yield (7.50 t ha^{-1}); biological yield (13.17); harvest index (42.91%) were recorded at 50 DAT. At T_2 (75 DAT) gave the lowest result for all parameters. The lowest no. of total tillers plants^{-1} (23.82); no. of effective tillers plants^{-1} (19.32); highest no. of ineffective tillers plants^{-1}(4.5); panicle length (21.74 cm); total grains panicle^{-1} (177.11); filled grains panicle^{-1} (165.05); highest number of unfilled grain panicle^{-1} (12.06); thousands grain weight (17.91 gm); grain yield (5.27 t ha^{-1}); straw yield (7.05 t ha^{-1}); biological yield (12.32); harvest index (42.59%).

Again, The combined effect of dose and time of mannitol on rice was found insignificant for most of the characteristics. The maximum results were recorded from T_1D_4 (225 mM of Mannitol and 50 DAT). T_1D_4 (225 mM of Mannitol and 50 DAT) gave the best result no. of total tillers plants^{-1} (27.33); no. of effective tillers plants^{-1} (26.13); minimum no. of non-effective tillers plants^{-1} (1.20); panicle length (24.56 cm); total grains panicle^{-1} (195.31); filled grains panicle^{-1} (191.37); minimum no of unfilled grain panicle^{-1} (3.94); thousands grain weight (20.83 gm); grain yield (6.47 tha^{-1}); straw yield (7.59 t ha^{-1}); biological yield (14.06); harvest index (45.97%).On the other hand, the minimum results were recorded from T_1D_1 (0 mM of Mannitol and 75 DAT). T_1D_1 (0mM of Mannitol and 75 DAT) showed the lowest number of total tillers hill^{-1} (20.67), panicle length (19.96 cm), thousand-grain weight (17.33 gm), and lowest grain yield (4.53t ha^{-1}) was observed in T_2D_1 (75 DAT and 0 mM of Mannitol).

Conclusion:

The above findings give an in-depth idea about the yield potentiality of BRRI dhan50 by exogenous application of Mannitol. From the above findings it could be concluded that:

1. Mannitol 225 mM can be applied in aromatic rice for maximum yiled,.
2. Mannitol applied at 50 DAT can be effective for maximum yield because a significant decrease occurred for all parameters after 50 DAT.
3. For better growth and yield exogenous application of Mannitol can be used as a combined treatment @ 225mM of Mannitol at 50 DAT (T_1D_4).

Recommendation

Considering the above observations of the present study further investigation in the following areas may be suggested:

1. For regional adaptability, Further study may be needed in Bangladesh's Agroecological Zone (AEZ).
2. More treatments with different doses and times may be selected to study such effects on other aromatic rice.

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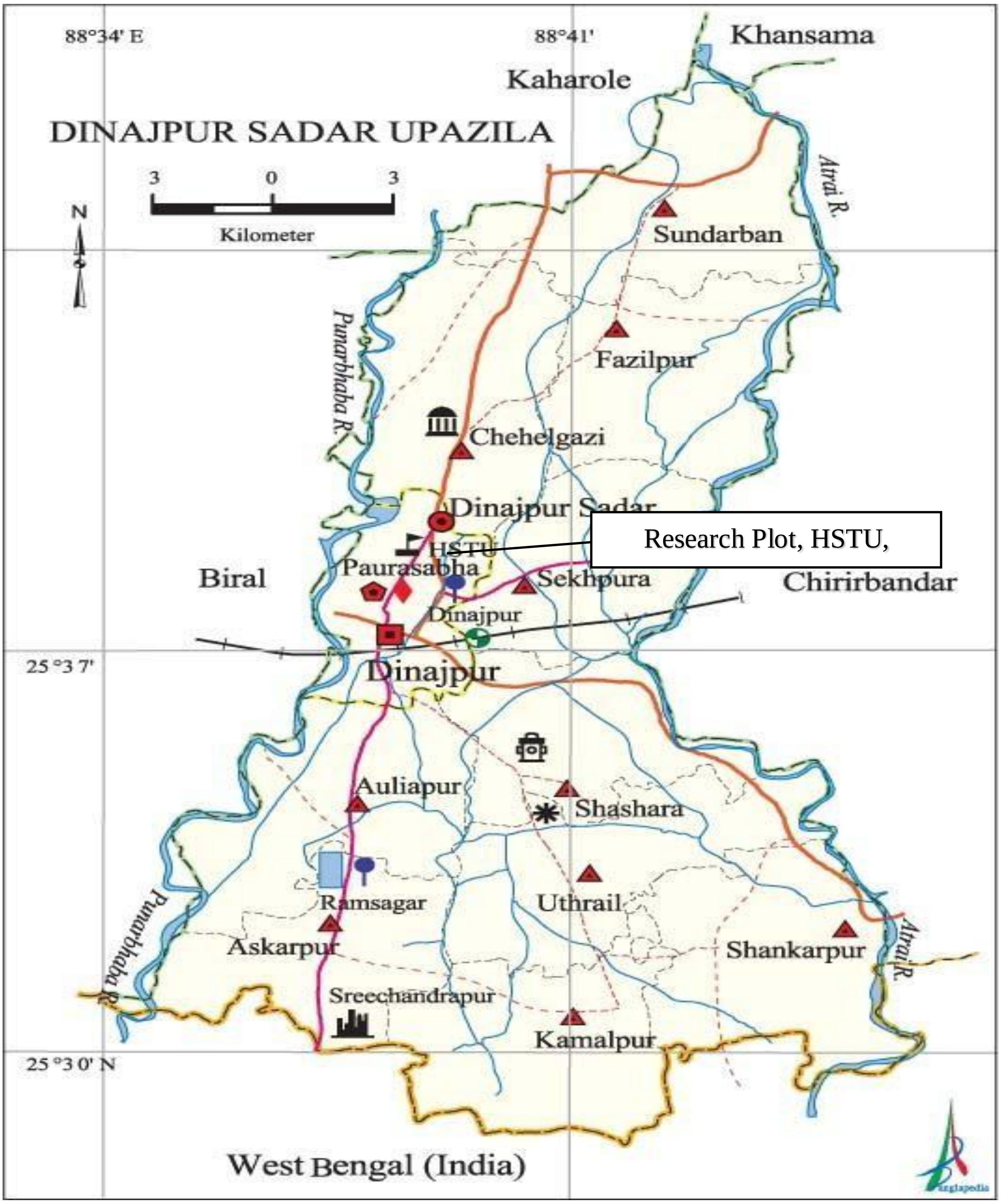
APPENDICES

Appendix I. Location of experimental site (Map of Dinajpur Sadar Upazilla, showing the research plot)



Appendix II: Distribution of monthly temperature, rainfall and relative humidity of the experimental site during the period from December 2023 to June, 2024

Month	Relative humidity (%)	Temperature			Total rainfall (mm)
		Minimum (^{0}F)	Maximum (^{0}F)	Average (^{0}F)	
December	20	51	75	64	0
January	20	50	73	60	0
February	40	55	80	66	0
March	60	64	90	76	10
April	80	72	96	84	65
May	82	78	96	87	0

Appendix III: Morphophysio-chemical properties of soil (collected before sowing of seeds) of the experimental field.

A. Morphological characteristics of the soil

Constituents	**Characteristics**
1. Location	Agronomy net house of Hajee Mohammad Danesh Science and Technology University, Dinajpur
2. Soil Tract	Old Tista alluvial
3. Land type	Medium high land
4. General Soil type	Non-calcareous dark grey floodplain
5. Soil texture	Sandy loam
6. Agro-Ecological Zone	Old Himalayan Piedmont Plain (AEZ-1)
7. Topography	Fairly level
8. Soil Color	Dark grey
9. Flood level	Above flood level
10. Drainage	Well drained
11. Vegetation	Cropped with rice, wheat, jute etc.

Appendix IV: ANOVA for the total tillers, effective tillers, non-effective tillers and panicle length (cm)

Source of variation	Degrees of freedom	Mean sum Square of			
		Total Tillers	Effective tillers	Non-effective tillers	Panicle length (cm)
Replication	2	86.16	39.49	9.20	19.51
Time	1	3.30	48.30	26.35	3.99
Dose	5	29.46	55.34	5.24	15.25
Time * Dose	5	2.92	2.97	1.35	0.20
Error	22	1.57	2.67	2.12	2.77
Total	35				

Appendix V: ANOVA for the total grain panicle^{-1}, filled grain panicle^{-1}, unfilled grain panicle^{-1} and thousand grain weight (gm)

Source of variation	Degrees of freedom	Mean sum Square of			
		Total grain panicle^{-1}	Filled grain panicle^{-1}	Unfilled grain panicle^{-1}	Thousand-grain weight (gm)
Replication	2	1125.62	1224.99	17.087	2.04
Time	1	153.80	139.12	0.368	15.85
Dose	5	357.55	1170.99	241.33	3.23
Time * Dose	5	42.60	42.57	20.01	1.43
Error	22	57.94	53.40	18.89	0,94
Total	35				

Appendix VI: ANOVA for the grain yield (t ha^{-1}), straw yield (t ha^{-1}), biological yield, and harvest index

Source of variation	Degrees of freedom	Mean sum Square of			
		Grain yield (t ha^{-1}),	Straw yield (t ha^{-1})	Biological yield	Harvest index
Replication	2	0.47	1.08	0.48	39.86
Time	1	1.47	1.83	6.59	0.90
Dose	5	2.54	0.06	3.32	43.77
Time * Dose	5	0.067	0.01	0.12	1.07
Error	22	0.17	0.02	0.17	4.17
Total	35				

Appendix VII: Some picture related to my thesis work