ISSN 0258-7122 (Print), 2408-8293 (Online)
Bangladesh J. Agril. Res. 44(1): 1-11, March 2019

PHYSIOLOGICAL RESPONSES OF MUNGBEAN (*Vigna radiata*) VARIETIES TO DROUGHT STRESS

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Abstract

A pot experiment under polyshed condition was carried out at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during the period from 27 March 2017 to 5 May 2017 to study the physiological responses of mungbean varieties to drought stress under varying water regimes. The treatments consisted of four mungbean varieties, namely BARI Mung-5, BARI Mung-6, BUmug 2, BUmug 4 and three water regimes viz., 50 to 60% field capacity (FC), 70 to 80% FC and 90 to100% FC which were considered as severe drought stress, moderate drought stress and non-stress, respectively. The experiment was laid out in a completely randomized design with factorial arrangement having four replications. Results indicated that BARI Mung-6 maintained significantly the highest relative water content, leaf water potential, proline content, shoot dry matter and lower rate of electrolyte leakage at 50 to 60% FC (severe drought stress). BUmug 2 showed the lowest performance in terms of all the water relation and physiological characters which indicates its higher sensitivity to severe drought stress. Variety BARI Mung-6 was relatively water stress tolerant than others in respect of physiological adaptations. So, BARI Mung-6 can be a potential variety for cultivation under drought condition where irrigation facility is limited.

Keywords: Mungbean, water potential, relative water content, proline and drought stress.

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek), a popular pulse crop with good test and important source of plant protein (19.5 to 28.5% proteins), has been widely cultivated throughout the world especially in Asian sub-continent including Bangladesh (Lambrides and Godwin, 2006). In Bangladesh the present area under mungbean cultivation is 101 thousand acres with a total production of 37 thousand ton and an average yield of 351 kg acre^{-1} (BBS, 2016). The crop is cultivated either during early *kharif* or late *rabi* season (March to June). Several biotic and abiotic stresses either singly or collectively caused adverse effect on mungbean plant resulting poor growth and development. Abiotic stresses including drought, have been reported as major constraints to the mungbean

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production projecting more than 50% of yield loss (Gaur *et al.*, 2012). Soil water deficit or drought stress is considered as a severe threat to sustainable agriculture and food security (Foley *et al.,* 2011). The concern is very much alarming to the country like Bangladesh where it is more likely to face the consequences of different anthropogenic activities that might increase the severity of drought stress at near future. It has become a great need for the understanding of drought tolerance mechanisms prior to development of major drought tolerant varieties to achieve sustainable production goal of crop. Plants follow several strategies including morpho-physiological and molecular changes for the acclimation in drought stress. Drought induced several developments of plants seemingly adjust the water crisis either by the alteration of morphological, physiological or both to overcome the soil moisture stress. Physiological adaptation increases the accumulation of osmolytes and adjusted osmotic potential by reducing cellular dehydration (Omprakash *et al.,* 2017). Increased accumulation of proline has been reported in mungbean during drought (Bharadwaj *et al.,* 2018), nevertheless detail understanding of morphological and physiological alteration for screening of mungbean varieties based on the tolerance characteristics mentioned above should be very essential to adjust and mitigate the upcoming challenges. The problem is widespread in the northwestern part of the country where mungbean production is hampered to a great extent by the existing water limiting condition.

Therefore, the present study was carried out to investigate the physiological alterations regarding to the dry matter accumulation in popularly cultivated mungbean varieties during early stages of growth in response to drought stress and thus to identify a suitable variety by observing relative performance of drought tolerance physiological attributes.

Materials and Method

A pot experiment under polyshed condition was conducted at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during the period from 27 March to 5 May 2017. Four mungbean varieties namely, namely BARI Mung-5, BARI Mung-6, BUmug 2, BUmug 4 and three soil moisture levels viz., 50 to 60% FC, 70 to 80% FC and 90 to100% FC were used as treatment variables. The trial was set up in a Completely Randomized Design with factorial arrangement having four replications. The soil of the plastic pot (20 cm internal diameter and 25 cm height) was filled up with mixture of soil and cowdung at a ratio of 4:1. Pot contained 9.5 kg soil. Soil used in the pot was silty clay loam and was fertilized uniformly with urea, triple super phosphate, muriate of potash, gypsum, zinc sulphate and boric acid containing 0.11 g N, 0.08 g P, 0.10 g K, 0.05 g S, 0.002 g Zn, and 0.001 g B, respectively. Total amount of all fertilizers and cowdung were well mixed with the soil before pouring into the pots. Ten seeds of mungbean were sown in each pot on 27 March 2017. Seedlings were emerged after 5 days of sowing and finally six healthy plants pot^{-1} were allowed to grow. Weeding and spraying of insecticides were done as normal management practices.

Drought treatment was imposed at 12 DAS (when the first trifoliate leaf was fully expanded), on the basis of soil moisture status in each treatment. Irrigation water was applied to bring the soil moisture at the higher range of each treatment (60, 80, and 100% FC). Next irrigation was given when the soil moisture came down to the lower levels (50, 70, and 90% FC) of those treatments, respectively. Irrigation water was applied by measuring cylinder. Irrigation requirement was calculated by using the following formula (Giriappa, 1988):

$IR = \{(M_{FC} - M_{BI}) \div 100\} \times A \times D$. Where, IR= irrigation requirement (cm), M_{FC}= Soil moisture (%) at field capacity, M_{BI}= Soil moisture (%) before irrigation monitored by using soil moisture meter, A= Soil bulk density in gcm^{-3}, D= Rooting depth (cm). Soil bulk density and soil moisture (%) at field capacity were computed using the method described by Karim *et al.,* (1988).

Relative water content (RWC) was determined using 30 leaf disks (2.0 mm thick and 4.5 mm wide) from fully expanded uppermost leaves. Fresh weights of the leaf disks were recorded and soaked in 100 ml of distilled water and kept in the dark for 24 hour. The turgid leaves were quickly blotted dry prior to the turgid weight measurement. Dry weights of leaf disks were determined after oven-drying at 70°C for 72 hours. All the weight of the samples was recorded by using analytical balance. Relative water content (RWC) was determined using following formula (Schonfeld *et al.,* 1988):

$RWC (\%) = [(FW - DW) / (TW - DW)] \times 100$. Where, FW = Fresh weight of the leaf disks, DW = Dry weight of the leaf disks and TW = Turgid weight of the leaf disks.

Leaf water potential of a fully expanded leaf was measured using pressure chamber as described by Scholander *et al.,* (1965). The balancing pressure was regarded as the tension originally existing in the xylem sap and approximately equal to water potential of the cells. Sampling was done between 7.00 and 9.00 a.m. to avoid evaporation losses.

Leaf membrane damage was determined by recording of electrolyte leakage (EL) as described by Valentovic *et al.,* (2006) with a few modifications from 30 leaf disks of fully expanded uppermost leaves. The EL was defined as follows:

$EL (\%) = (L_1/L_2) \times 100$. Where, L_1 = Electrical conductivity before autoclave, L_2 = Electrical conductivity after autoclave.

Fully expanded uppermost leaf samples were collected and proline extractions were made using the method outlined by Bates *et al.* (1973). The proline concentration was determined from the standard curve and calculated on a fresh weight basis as follows:

Proline content (µmole g^{-1} fresh wt.) = {µg proline ml^{-1} × vol. of extr. sol. (ml) × toluene used (ml)} / {115.13 µg $mole^{-1}$ × g sample}

Sampling for plant dry weights was done 10 days after appearance of visual symptom (at 40 DAS). Stem and leaf were separated and dried for 72 hours at 70^{o}C in drying oven. Shoot dry weight was calculated by summing up the dry weight of stem and leaf of the plants. The relative performance was calculated using the following formula (Asana and Williams 1965):

Relative performance = Variable measured under stressed condition / Variable measured under normal condition

The data regarding to the physiological factors were recorded at 18 days after imposing of drought treatment (30 DAS), when visual symptom of drought stress appeared. Plant were harvested at 40 days after sowing maintain a drought period of 10 days.

Data thus collected were analyzed with Statisticx 10 program. The differences between the treatment means were compared by Least Significant Difference (LSD) at 5% level of significance.

Results and Discussion

Soil moisture variation: Soil moisture status under three different treatments involving ranges of field capacity (FC) was monitored at 4 days interval from 12 to 37 days after sowing (DAS). Soil moisture was directly influenced by the treatments that is, the treatment maintaining higher ranges of FC exhibited higher soil moisture status throughout the studies (Fig. 1).The treatment subjected to the highest range of FC (90 to 100% FC) maintained the maximum soil moisture (close to FC) throughout the growing season of the crop. The subsequent soil moisture curves followed the declining orders with the decrease of FC range. The bottom curve under the lowest range of FC (50 to 60% FC) showed the minimum soil moisture level (18.40 to 15.34%), sometimes went down to the permanent wilting point condition.

Relative water content (RWC): Relative leaf water content has been considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance (Anjum *et al.*, 2011). Field capacity with different soil water status caused significant differences in leaf RWC of all mungbean varieties (Table 1). RWC ranged from 75.14 to 80.91% at 90 to 100% field capacity, 67.72 to 74.42% at 70 to 80% field capacity and 55.21 to 66.14% at 50 to 60% field capacity. Such results apparently indicates that under decreasing soil moisture conditions, plants became unable to uptake sufficient water through the root system leading to decrease in relative water content. The finding is quite similar to the results supported by Hayatu *et al.* (2014). In the present experiment the treatment with the lowest field capacity

(50 to 60% FC), BARI Mung-6 was found to maintain the maximum RWC (66.14%) while BUmug 2 had the minimum RWC (55.21%). The minimum reduction in RWC recorded in BARI Mung-6 at water deficit condition indicates that the variety was able to uptake water most efficiently among mungbean varieties. Genotypic differences for RWC were also reported by Bharadwaj *et al.*, 2018 in cowpea and other pulses.

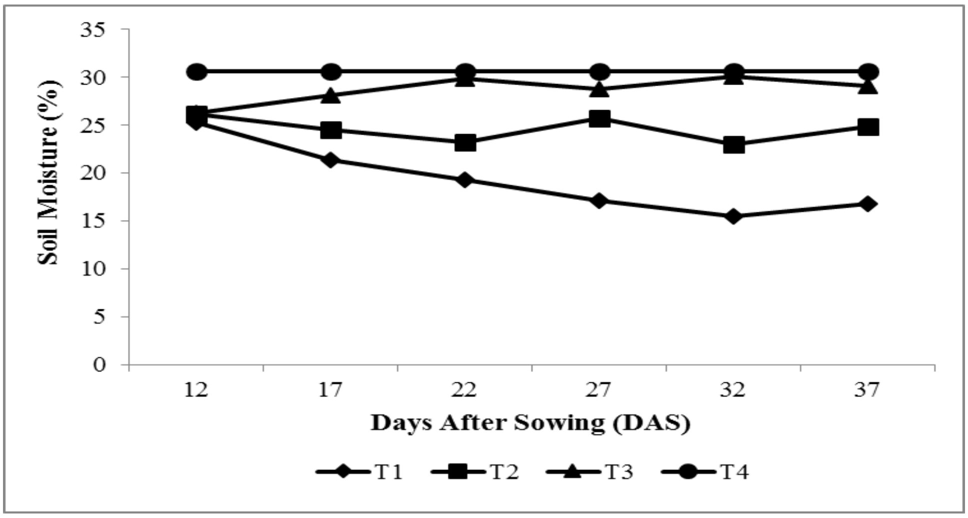

Fig 1: Soil moisture status (%) at regular interval of four days from 12 to 37 DAS. (T_1= 15.34 to 18.40% at 50 to 60%FC, T_2= 21.48 to 24.54% at 70 to 80% FC, T_3= 27.61 to 30.68% at 90 to 100% FC, T_4= Moisture at field capacity = 30.68%.

Table 1. Relative water content (RWC) of four mungbean varieties under three field capacity levels

Variety	Relative Water Content (%)		
	FC of 90 to 100%	FC of 70 to 80%	FC of 50 to 60%
BARI Mung-5	78.74 b	72.92 d	63.80 g
BARI Mung-6	80.91 a	74.42 cd	66.14 f
BUmug 2	75.14 c	67.72 f	55.21 i
BUmug 4	77.36 b	70.29 e	60.67 h
CV%	1.91		

Means along both rows and columns followed by the same letter (s) did not differ significantly at 5% level of probability.

Leaf water potential (Ψ_w): As adjustment of leaf water potential is a very important indication of drought stress tolerance of crop plants (Siddique *et al.*, 2000), the comparison of leaf water potential in the mungbean varieties should be necessary for screening suitable one. Leaf water potential of mungbean varieties was significantly affected by water stress treatments (fig. 2). The Ψ_w of all mungbean varieties was reduced significantly due to reducing field capacity levels. The highest Ψ_w ranging from -0.67 to -0.55 MPa was reported at 90 to 100% field capacity and it was gradually decreased at 70 to 80% field capacity (-0.87 to -0.70 MPa) and became the lowest (-1.64 to -1.13 MPa) at 60-50% field capacity. The results reflect the facts that plants exposed to low field capacity endured drought stress by low level of cellular water for their survival. The result is very consistent to that where lower amount of water content during drought stress acclimation of pulses were observed (Shrestha *et al.*, 2006). At low field capacity (50 to 60% FC), BARI Mung-6 was found to maintain the maximum Ψ_w (-1.13 MPa). It could be due to better capacity of the variety BARI Mung-6 to adjust Ψ_w even at very low moisture level of soil. Lentil genotype with varying leaf water potential during drought stress was also reported by Gangwar and Kumar (2018).

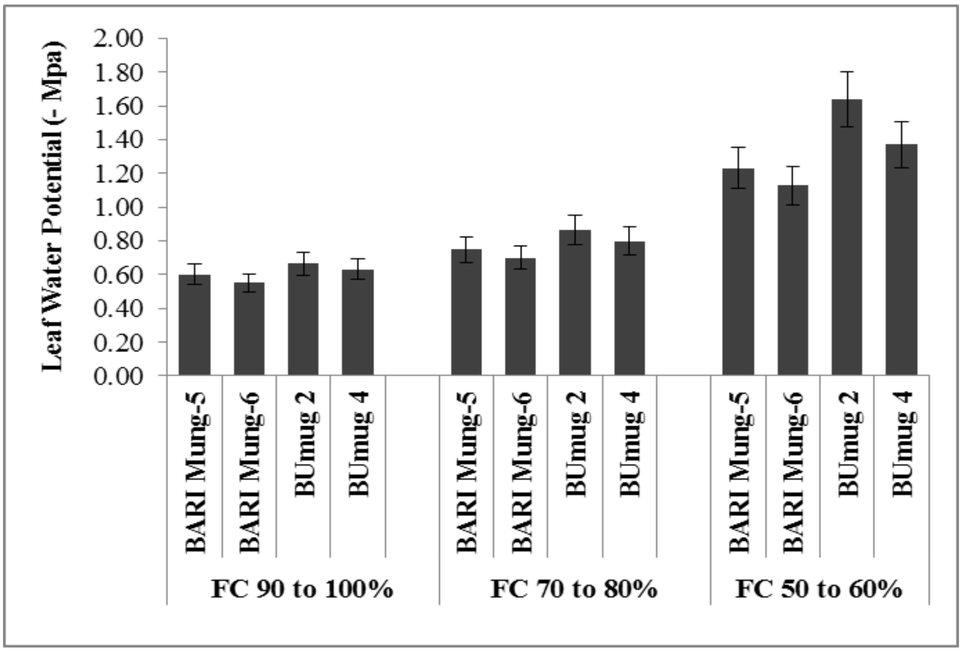

Fig. 2. Leaf water potential (-MPa) of four mungbean varieties at different levels of field capacity (FC). Bars represent mean ±SE.

Electrolyte leakage: Higher electrolyte leakage during drought stress of crop plants indicates higher amount of tissue damage (Tas and Tas, 2007). Significant differences in electrolyte leakage were observed among mungbean varieties under water stress (Table 2). Electrolyte leakage of mungbean leaves was found to be increased with decreasing field capacity of soil in all varieties. It was found maximum at 50 to 60% field capacity and ranged from 64.22 to 75.11%, which at 70 to 80% field capacity, was found to be decreased within a range from 61.39 to 65.25%. Finally electrolyte leakage was recorded lowest (23.91 to 35.15%) at 90 to 100% field capacity. The results indicate that water deficit stress (50% to 60% field capacity) reduced membrane stability by altering the arrangement of phospholipid bilayer and resulted in reduced ability of plants to retain water and solutes causing increased leakage. Increased tissue damaged by enhanced electrolyte leakage was reported by Sairum and Saxena (2000) which is consistent to our results. In the present study, a comparison of electrolyte leakage among mungbean varieties indicates that, BARI Mung-6 had the minimum electrolyte leakage (64.22%) when grown at severe water stress (field capacity 50 to 60%), while BUmug 2 had the maximum electrolyte leakage (75.11%). The results suggest that BARI Mung-6 is able to maintain cell membrane integrity during drought by maintaining higher cell water status. Baroowa and Gogoi (2012) recorded decreased membrane stability in both black gram and green gram when exposed to drought stress.

Table 2. Electrolyte leakage of four mungbean varieties under three field capacity levels

Variety	Electrolyte Leakage (%)		
	FC of 90 to 100%	**FC of 70 to 80%**	**FC of 50 to 60%**
BARI Mung-5	30.46 i	62.17 ef	70.82 b
BARI Mung-6	23.91 j	61.39 f	64.22 cd
BUmug 2	35.15 g	65.25 c	75.11 a
BUmug 4	32.04 h	63.04 de	72.14 b
CV%		1.78	

Means along both rows and columns followed by the same letter (s) did not differ significantly at 5% level of probability.

Proline accumulation: Accumulation of proline is a common phenomenon during drought stress acclimation of crop plants and it was examined and compared in mungbean varieties. A profound effect of soil water crisis was observed on proline content of mungbean varieties (Fig. 3a). The mungbean plants maintained higher proline content (3.38 to 6.12 μmol/g FW) at 50 to 60% field capacity. As field capacity was increased, proline content was found to be decreased to 1.61 to 3.98 μmol/g FW at (70 to 80% FC) and finally proline content was recorded at the lowest in all four mungbean varieties (1.19 to 1.65 μmol/g FW) at 90 to 100% field capacity. This result indicates that water deficit stress triggered the proline accumulation. This is a common physiological response of plants to drought stress (Mafakheri *et al.*, 2010).

Proline accumulation was recorded higher in BARI Mung-6 (6.12 μmol/g FW) at the lowest field capacity (50 to 60% FC), while BUmug 2 had the lower proline content (3.38 μmol/g FW). The relative proline content was also recorded higher in BARI Mung-6 at both 50 to 60% field capacity (3.71) and 70 to 80% field capacity (2.41), while BUmug 2 had the lower relative proline content of 2.84 and 1.35 at 60 to 50% and 70 to 80% field capacity, respectively (Fig. 3b). Thus higher proline content of BARI Mung-6 helped in lowering water potentials and involved in osmoregulation that allowed additional water to be taken up from the soil, thus buffering the immediate effect of water shortages. This drought tolerance mechanisms induced by proline accumulation was reported by Bharadwaj *et al.* (2018). Vendruscolo *et al.*, (2007) also reported that proline is isolated in tolerance mechanisms against oxidative stress and this was the main strategy of plant to avoid detrimental effects of water stress.

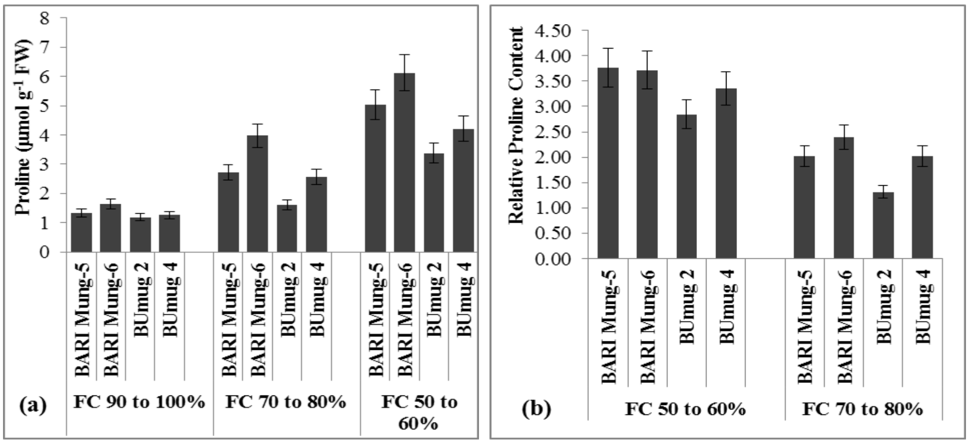

Fig 3. Proline content (μmol/g FW) (a) at three different levels of water regime and relative proline content (b) at 50-60% and 70-80% field capacity compared to 100-90% field capacity of four mungbean varieties. Bars represent mean ±SE.

Shoot dry weight: Field capacity with different soil water status had a profound effect on shoot dry weight (Fig 4a). Shoot weight in all four mungbean varieties was recorded higher at 90 to 100% field capacity with a range from 6.80 to 12.48 g plant^{-1}. Shoot dry weight was decreased to 4.27 to 9.70 g plant^{-1}at 70 to 80% FC and finally at 50 to 60% field capacity, shoot dry weight was lowest (1.93 to 4.88 g plant^{-1}) (Fig 4a). Among the four mungbean varieties BARI Mung-6 recorded with the highest shoot dry weight (4.88 g plant^{-1}) under drought stress condition (50 to 60% FC), while BUmug 2 recorded the lowest (1.93 g/plant) shoot dry weight (Fig 4a). Relative shoot dry weight was also higher in BARI Mung-6 at both 50 to 60% field capacity (0.39) and 70 to 80% field capacity (0.78) compared to that of BUmug 2 which had relative shoot dry weight 0.29 and 0.63 at 50 to 60% FC and 70 to 80% FC, respectively (Fig 4b). This result indicates that BARI Mung-6 is able to gain higher dry matter among all the four mungbean varieties and the results of that is very consistent to the appearance of better physiological attributes such as higher water content and leaf water potential, and increased accumulation of proline content of this variety.

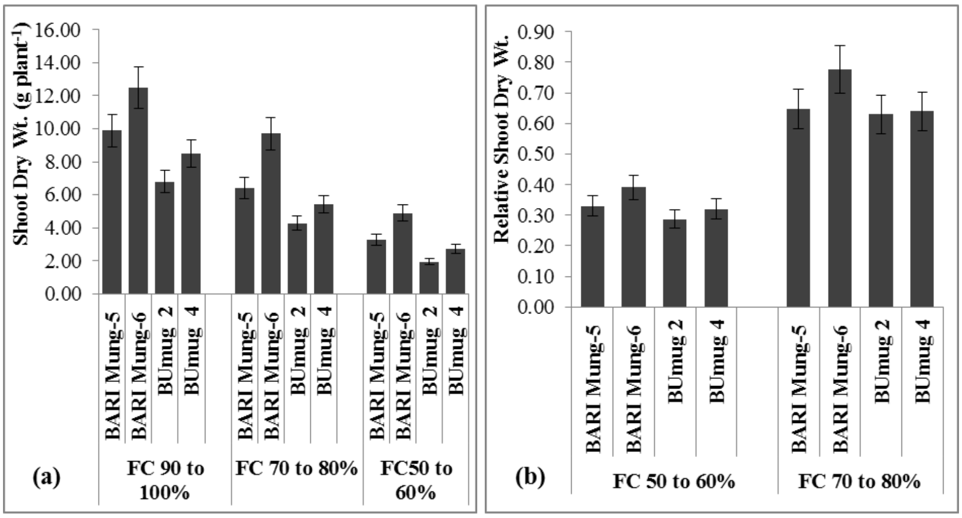

Fig 4. Shoot dry weight (g/plant) (a) at three different levels of water regime and relative shoot dry weight (b) at 50-60% and 70%-80% field capacity compared to 100-90% FC of four mungbean varieties. Bars represent mean ±SE.

Conclusion

Results of the study indicated that the variety BARI Mung-6 is more capable in tolerating water stress. So, BARI Mung-6 is considered as drought tolerant due to its high relative water content, leaf water potential, proline content, shoot dry matter and lower rate of electrolyte leakage.

Acknowledgement

We acknowledge Research Management committee (RMC), Bangabandhu Sheikh Mujibur Rahman Agricultural University and Ministry of Science and Technology for their valuable assistance and economic supports for the successful completion of this research work.

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