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Effect of different dosage of EMS on germination, survivability and morpho-physiological characteristics of sunflower seedling

https://doi.org/10.1515/helia-2021-0008
Received April 8, 2021; accepted May 12, 2021; published online June 4, 2021

Abstract: To find out the effect of EMS dosage on sunflower variety BARI Surjamukhi-2, different EMS concentrations were compared at the molecular biology laboratory (MBL), Oilseed Research Centre (ORC), Bangladesh Agricultural Research Institute (BARI), Gazipur. A total of 10 seeds were treated with each treatment and % germination, % survival and early seedling growth was investigated. Decreased % germination was found with the increasing dose of EMS for all the treatment compared to the control, except for T_4 (0.6% EMS) treatment where a 100% seed was found to be germinated. With an exception of treatment T_4, a decreasing trend of % survivability with the increasing dose of EMS was observed. The gradual reduction with the increasing dose of EMS were observed for most of the studied characters such as leaf area, number of internodes, internode length, fresh and dry weight of leaf, dry weight of shoot, and fresh and dry weight of root in comparison to non-treated control seedlings. However, stimulatory effect with lower dosage of EMS was observed for shoot length in T_2 (0.2% EMS), root length in T_3 (0.4% EMS), and stem fresh weight in T_2 over control. Based on a quadratic regression analysis, using % survivability data, LD50 was calculated and optimum EMS dose was found 0.5%. Hence, the optimized EMS dose of 0.5% may be used to develop large scale desirable mutant of sunflower variety BARI Surjamukhi-2.

Keywords: ethyl-methane sulphonate; germination; seedling growth; sunflower; survivability.

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Introduction

Creating genetic variability is one of the essential tasks for the plant breeder and geneticist. In higher plants such variability has most often been achieved through hybridization and spontaneous mutation in nature. But the rate and frequency of spontaneous mutation is very low. Therefore, to create genetic variability induced mutation might be a potential alternative through which desired quantitative and qualitative inherited traits may be achieved in crops. Induced mutation techniques are mostly been used to improve yield, oil quality, qualitative traits namely earliness, dwarfism, biotic and abiotic stress resistance in crops (Parry et al. 2009; Schnurbush et al. 2000). Many mutant varieties of more than 100 plant species including a few economically important crop like barley, wheat and cotton, occupy the majority of the cultivated lands in the world (Maluszynski et al. 1995). Successful mutation breeding has also been reported in oilseed crops by many authors (Bacelis 2001; Ferrie et al. 2008; Parry et al. 2009; Spasibionek 2006).

Mutation can be induced by using physical and chemical mutagenic agent for both seed and vegetative propagated crops (Jain 2010). Ethyl-metahne sulphonate (EMS) is one of the most common, powerful and effective chemical mutagenic agents, specially recommended for seed material to be mutagenized. Application of EMS and monitoring of the mutation's outcome are relatively easy (Bahar and Akkaya 2009). EMS induced mutant were reported for diseases resistance in tomato (Yudhvir 1995), increased vitamin C in chili (Alcantara et al. 1996), increased pollen viability and fruit rot resistance in bell pepper (Ashok et al. 1995). Several successful mutation breeding were also reported in various oilseed crops. EMS induced 20–22% higher yield in linseed (Bacelis 2001), changed fatty acid composition in *Brassica napus* (Spasibionek 2006), low saturated fatty acid content in *B. rapa* (Ferrie et al. 2008) were reported. Besides, EMS induced mutant with high oleic and linoleic was reported in sunflower (Cvejić et al. 2016).

EMS causes point mutation on chromosome where random gene mutations are often occurred within the genetic material (Emmanuel Levy 2002). In such a way potential alteration of loci or candidate gene would be happen in which desirable deadly linked alleles can be present which enable plant breeder to obtain useful alleles of interest. Usually any mutagenic treatment either physical or chemical creates deleterious effects on plant which can be visible in M1 and successive generations. Embryo lethality, reduced seed germination, reduced survivability, plant sterility, less vigor is the most reported deleterious effect of mutagenic treatment. Such deleterious effect mainly depends on the dose and mode of action of a particular mutagen for a specific crop, species or cultivars of same species.

Due to mutagenic effect, there is substantial killing of plants during different stage of development, thus considerably reduces the survival of the resulting plants. An overdose of mutagen can kill too many treated population and lower dose can produce fewer mutations. On the other hand, an optimum dose of mutagen will produce high frequency of desirable mutants with minimum killing (Yadav et al. 2016). Dose dependent mutagenic effect were reported for reduced emergence, survival and fertility (Flippetti and Pace 2004), severe reduction in germination, frequency of normal seedlings, plumule and radicle length and physiological injuries in broad bean (Rupinder and Kole 2005), reduced germination, pollen fertility and survivability in mung bean (Khan et al. 2004). Hence, before going to large scale mutation induction it is essential to determine the optimum dose of mutagen in which high frequency desirable mutant could be obtained. Most of the researcher use lethal dose 50 (LD50) to determine the optimum dose of mutagen for a particular trait (Anbarasan et al. 2013; Talebi et al. 2012; Warghat et al. 2011). In mutation breeding to produce high frequency desirable mutant, optimum dose determination is necessary (Arisha et al. 2014; Hohmann et al. 2005) and ignoring it, mutagen dose can either be too high or too low to obtain desirable mutant. So far, our knowledge there is no reports in Bangladesh in this regard. Therefore, this study has been undertaken to determine the effect of different doses of EMS on seed germination, survivability and morpho-physiological characteristics of sunflower seedling. In this experiment an optimum EMS dose will be determined for successful mutagenesis in sunflower variety BARI Surjamukhi-2.

Materials and methods

The seeds of sunflower variety BARI Surjamukhi-2 were used in this study. For mutagenic treatment, chemical mutagenic agent Ethyl-Methane Sulphonate (EMS) solution of six different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2%) was prepared in 0.1 M phosphate buffer at pH 7 to avoid rapid hydrolysis (Bosland 2002). Germination experiment was conducted by following completely randomized design (CRD) with three replications. Each treatment was consisted of 30 seeds. Seeds were pre-soaked in water at room temperature for overnight followed by 3.5 h treated with the above-mentioned concentration of EMS solution with orbital shaking at 120 rpm. Subsequently the seeds were thoroughly washed with running water for 3 h. A total of 30 seeds were also exposed to the same condition without EMS treatment and was considered as non-treated control. All the treated and non-treated seeds were plated separately in 90 mm Petri dish (10 seeds/Petri dishes as a replication) containing moist blotting paper covered with lid to emerge radical and plumule. At day 7, the germination percentage was recorded. The germination percentage was calculated using the following equation (Chowdhury et al. 2018):

$$\text{Germination percentage (GP)} = \frac{\text{No. of germinated seeds}}{\text{Total no. of seeds}} \times 100$$

After seven days of emergence, each germinated and non-germinated seeds were sown separately in poly bag. All the poly bags were placed in greenhouse and maintained for 30 days following standard cultural practices for proper growth. Data was recorded on % survival at 7, 14, 21 and 30 days after sowing. The data on morphological attributes were taken at harvest on 30 DAS from three randomly selected seedlings. Number of leaves and number of internodes were counted as number basis. Length of shoot and root were taken by using a measuring tap in centimeter. Fresh leaf, root, and shoot weight was taken using an electrical balance in gram. At harvest, leaf, root and shoot from the same selected seedlings were kept in oven at 72 °C until they reached a constant weight and weighed using an electrical balance in gram. The leaf area was measured using a leaf area meter (LI COR LI-3100C, USA) from the same selected seedlings.

SPAD value of forth fully expanded leaf was taken using a SPAD meter (Konica Minolta SPAD 502, Japan) from the leaf tip, leaf base and middle part of leaf of the same selected seedling and average value was used for analysis. Internode length of each selected seedling was taken from first, second and third internode from the base and average value was used for analysis. The treatments differences were compared by least significant differences (lsd). All the obtained data were analyzed statistically using R software (R Core Team 2017).

Results and discussion

The effect of different doses of EMS on germination and morpho-physiological characteristics in sunflower seedling were evaluated to determine an optimum dose of EMS for successful mutagenic treatment in sunflower. The mean percentage of germination and survivability at different days for different treatments are presented in Figures 1 and 2 respectively. From the result it was found that % germination decreased with the increasing dose of EMS except for treatment T_4.

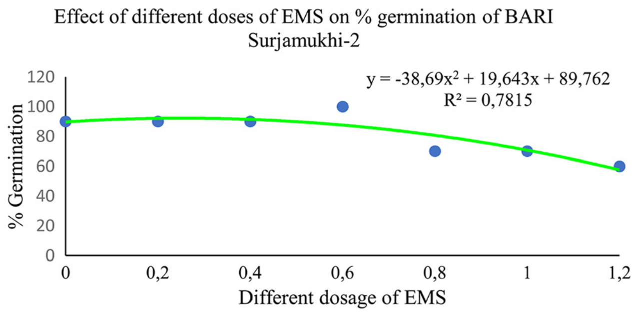

Figure 1: Effect of different doses of EMS on % germination of BARI Surjamukhi-2.

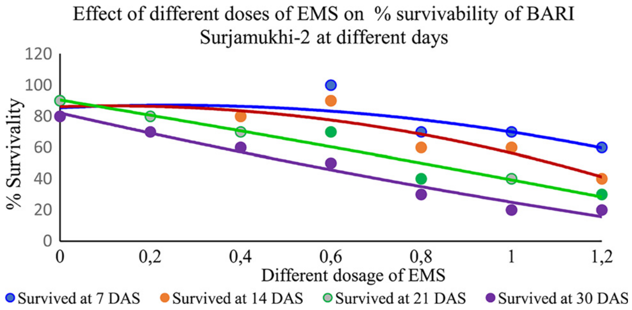

Figure 2: Effect of different doses of EMS on % survivability of BARI Surjamukhi-2 at different days.

The highest % germination was recorded in T_4 treatment (100%), and lowest was recorded in T_7 treatment (60%). In this study the % germination have shown increased at lower dose of EMS with the highest in T_4 treatment (100%). This is might be due to a certain mutagenic concentration have a stronger effect on surface of plant cells which caused the ultimate breakdown of the seed coating allowing the germination to increase (Aynehband and Afsharinafar 2012). Akshatha et al. (2013) used four different dosage of Gy (25, 50, 100 and 200 Gy) and found highest germination at 100 Gy. The stimulatory effect of lower dose of mutagen might be due to the activation of RNA and protein synthesis which occur early stage of germination after treating the seeds (Abdel-Hady et al. 2008).

The % survivability was found decreased with the increasing dose of EMS with time-course (Figure 2). In control treatment T_1, the % survivability decreased from 90 to 80% during 7 DAS to 30 DAS. Though 100% survivability was recorded at 7 DAS for T_4 treatment, it was decreased to 50% at 30 DAS. At 30 DAS the lowest % survivability (20%) was found both in the higher concentration of T_6 and T_7 treatment. The reduction of % germination might be due to alteration of enzyme activation or delay or inhibition of biological and physiological processes (Talebi et al. 2012), damage of cell constituents (Kumar et al. 2013), or might be because of damage of germ cell (Serrat et al. 2014).

Reduced % germination and % survival with increasing dose of EMS was reported in cluster bean (Velu et al. 2007), maize (Gnanamurthy et al. 2011), rice (Talebi et al. 2012), soybean (Satpute and Fultambkar 2012), okra (Jadhav et al. 2012), sesame (Anbarasan et al. 2013), cowpea (Gnanamurthy et al. 2013), pearl millet (Ambli and Mullainathan 2014), pigeon pea (Ariraman et al. 2014). From the present study, it could be said that a 0.6% EMS concentration is effective for obtaining good germination and 50% plant survival. Our results are in line with the findings of Kumar et al. (2013) who also reported 0.6% EMS dose as optimum for sunflower germination and survivability.

The effects of different doses of EMS were easily observed by the morpho-physiological observations. The result of different EMS doses on morpho-physiological characteristics of sunflower seedling at 30 DAS are presented in Table 1. Though significant differences were observed for % germination and % survival, interestingly the number of leaves and chlorophyll indicator which is SPAD value did not differ significantly between different treatments compared to control treatment. This is might be due to the surviving plants recovered at later stage of development and showed similar rates of leaf development (Amanda et al. 2008). However, highly significant differences for leaf area among the treatments were observed in comparison with control seedlings. Gradual decrease in leaf area with the increment of EMS dose was observed in this study (Table 1) which might be due to the smaller size of leaf (Figure 3). The highest leaf area (917.10 cm^2) was recorded in non-treated control seedlings which was the lowest (161.19 cm^2) in seedling treated with the highest dose (T_7) of EMS. Our results were in consistent with the findings of previous research findings. Reduced leaf area with the increasing dose of both gamma ray and EMS were reported in tomato by Naheed (2014). He suggested the reduced leaf area was the cause of decreased cell wall expansion and the structural change in plant cell wall due to the stress and imbalance of cellular pH imposed by the mutagenic increment. Burssens et al. (2000) also reported the slow growth rate caused by reduced cell division and biosynthesis of membranes due to the stress of mutagens. In addition, Pakorn et al.

Table 1: Effect of different doses of EMS on different characters of BARI Surjamukhi-2 at 30 days after sowing (DAS).

Treatment	NL	LA	SPAD value	NI	IL (cm)	SL (cm)	RL (cm)
T_1:0.0	18	917.10 a	13.5	9 a	7.36 a	62 b	15 a
T_2:0.2	19	751.79 b	16.82	8 b	7.11 a	67 a	13 b
T_3:0.4	18	634.36 c	18.93	7 bc	6.63 ab	58 c	15 a
T_4:0.6	16	463.48 d	17.10	7 bcd	6.11 abc	50 d	12 c
T_5:0.8	16	294.44 e	17.53	6 cd	5.44 bc	43 f	9 d
T_6:1.0	16	254.12 e	15.49	6 cd	5.42 bc	40 e	9 d
T_7:1.2	15	161.19 f	14.98	6 d	4.97 c	31 g	7 e
Significance level	ns	*	ns	**	*	**	**
CV	8.536	8.093	16.124	10.132	15.38	25.84	6.305
LSD	2.5	70.38	4.61	1.26	1.64	3.1	1.23

NL, No. of leaf; LA, Leaf area (cm^2); NI, No. of internode; IL, internode length (cm); SL, shoot length (cm), RL, root length (cm). *, ** Significant at 5 and 1% levels of probability, respectively.

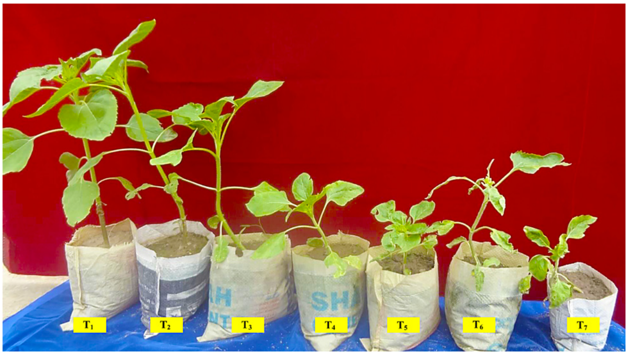

Figure 3: Reduction of seedling height with increment of EMS doses of BARI Surjamukhi-2.

(2009) documented decreased leaf area in *Anubias* with the increasing dose of irradiation due to destruction of genetic materials and reduction of cell division. Behera et al. (2012) reported the induction of EMS mutagenesis in *Asteracantha longifolia* affected the leaf size. A study of Nuananong (2020) showed reduced leaf size due to the disturbance in the structure, spatial arrangement, and division or expansion patterns of leaf due to deleterious effect of EMS.

Significantly decreased number of internode and length of internode was observed with the increasing dose of EMS (Table 1) compared to control treatment in this study. Maximum number of internodes was found in control seedlings (9) and minimum (6) was recorded in the seedlings treated with higher dose of EMS from T_5 to T_7. The longer internode was produced by the non-treated control seedlings (7.36 cm) while, the shorter (4.97 cm) internode was given by the seedlings treated with the highest dose of EMS (T_7). The gradual decreasing trend of lower number of internode as well as shorter internode with increasing EMS dose perhaps an indication of mutagenic lethality in mitotic inhibition, disruption in cell division and cell wall expansion. Decrease in internode length with the increasing dose of EMS was reported in *A. longifolia* (Motilal et al. 2012).

Root length is an important parameter to determine the mutagen dose. A stimulatory effect was observed for root length where, longer root (15 cm) was produced by the lower dose of EMS (T_3) treated seedlings which was statistically similar with that of the non-treated control seedlings (15 cm). Increase of root length under low dose of chemical mutagen also reported in *Jatropha curcas* (Dhakshanamoorthy et al. 2010). However, decreased root length with the subsequent increasing dose of EMS was found in this study (Table 1). Decreased root length with the increment of EMS dose was also reported in sunflower (Jayakumar and Selvaraj 2003), sesame (Birara et al. 2014), and proso millet (Ramesh et al.

2019). Reduction in root length with increasing mutagen might be due to the marked suppression in mitotic division (Datta 1992).

Seedling height is one of the most investigated morphological characters which perhaps might be attained by the reducing number and/or length of internode and short stature mutants is one of the most common products of induced mutations (Christov 1995; Jambhulkar 2002). To determine the biological effect of mutagen, shoot length is considered as an important index (Konzak et al. 1972). From the result (Table 1), longest shoot length (67 cm) was found in the lowest dose of EMS (T_2) over control (62 cm). But further gradual decrease in shoot length with subsequent higher dose of EMS was observed in our study. Increased shoot length in lower mutagenic dose in tomato was reported by Aliyu and Adamu 2007. Reduced shoot length with higher dose of mutagen was reported in onion (Joshi et al. 2011), and in proso millet (Ramesh et al. 2019). Therefore, for obtaining dwarf plant phenotype a higher dose of EMS would be preferable.

Among the different treatment of EMS doses, the stimulatory effect with lower dose was observed on leaf, root and stem fresh weight where all three parameters gave higher fresh weight in lower dose than higher dose. A similar trend was also observed for leaf, stem and root dry weight where higher value was recorded in lower dose of EMS (Table 2). This stimulatory effect reveals the lower dose would enhance the cell division rates and the activation of growth hormone like auxin (Zaka et al. 2004). However, drastic reduction in leaf, stem and root fresh and dry weight between different treatments in comparison to control were observed with

Table 2: Effect of different doses of EMS on different characters of BARI Surjamukhi-2 at 30 days after sowing (DAS).

Treatment	LFW (g)	STEMFW (g)	RFW (g)	LDW (g)	STEMDW (g)	RDW (g)
T_1:0	36 a	25 b	15 a	4.82 a	3.14 a	3.51 a
T_2:0.2	31 b	29 a	12 b	3.30 b	2.05 b	1.66 b
T_3:0.4	25 c	27 ab	10 c	2.74 c	1.72 c	1.18 c
T_4:0.6	17 d	18 c	6 d	1.87 d	1.09 d	0.67 d
T_5:0.8	11 e	12 d	3 e	1.41 de	0.82 e	0.36 e
T_6:1.0	12 e	10 de	3e f	1.10 e	0.70 ef	0.25 e
T_7:1.2	8 f	8 e	2 f	0.92 e	0.51 f	0.24 e
Significance level	**	**	**	**	**	**
CV	7.182	7.277	8.999	12.271	7.754	10.135
LSD	2.50	2.34	1.13	0.49	0.19	0.19

LFW, leaf fresh weight (g), STEMFW, stem fresh weight (g), RFW, root fresh weight (g), LDW, leaf dry weight (g), STEMDW, stem dry weight (g), RDW, root dry weight (g). *, ** Significant at 5 and 1% levels of probability, respectively.

the gradual increment of EMS dose. Though significantly higher stem fresh weight was found in T_2 treated seedlings (25 g) compared to control, gradual reduction with the increasing dose of EMS for leaf, stem and root fresh weight were observed. The highest leaf and root fresh weight of 36 and 15 g respectively, were found in non-treated control seedlings where all three parameters were found the lowest in the highest dose of EMS (T_7) treated seedlings (Table 2). The highest leaf, stem and root dry weight (4.82, 3.14 and 3.51 g, respectively) were found in non-treated control seedlings and the lowest dry weight for leaf, stem and root (0.92, 0.51 and 0.24 g, respectively) were found in the seedlings treated with highest dose of EMS (T_7). From the results it was noticed that the lethality increased with the increasing dose of EMS over control. This perhaps due to the physiological and chromosomal damage (Kumar and Yadav 2010; Mudibu et al. 2012; Nuananong 2020). Reduced shoot and root fresh and dry weight were also reported in wheat (Bahar and Akkaya 2009).

In this study, increased lethality was found over control with the gradual increment of EMS dose with some exception such as stimulation effect of seed germination in T_4, shoot length in T_2, root length in T_3, and stem fresh weight in T_2. Therefore, an optimum dose determination is needed to obtain high frequency desirable mutants with minimum lethality.

The LD50 calculation is considered the best way for determination of optimum mutagenic dose both for physical and chemical mutagen (Anbarasan et al. 2013; Talebi et al. 2012; Warghat et al. 2011) and ignoring it the mutagenic dose would be misleading. In a previous study of Kumar et al. (2013) reported the LD50 of sunflower was 0.6%. Success of any mutagenic treatment depends on the dose of mutagenic agents (Deshmukh et al. 2018; Suthakar et al. 2014), genetic constituents, parentage and ploidy level of a genotype (Yadav et al. 2016). Singh (2000) reported that LD50 varies with the crop species and mutagen used. Wanga et al. (2020) found four different LD50 of EMS dose for four sorghum genotypes. So, it is found that the LD50 of EMS dose used were different in different varieties. Therefore, to induce mutants with desirable frequency, the information on a suitable dose of a particular mutagen for a specific variety is needed. Therefore, in this study effect of different dosage of EMS and a LD50 was determined for the sunflower variety BARI Surjamukhi-2. The most developmental parameters are % survivability which commonly employed to calculate the lethal dose (LD50), at which half of the tested populations are killed. A quadratic regression line was developed using percent survivability data at day 30 (Figure 4) to calculate the EMS dose of LD50 for approximately 50% seedling reduction using the following equation (Amanda et al. 2008):

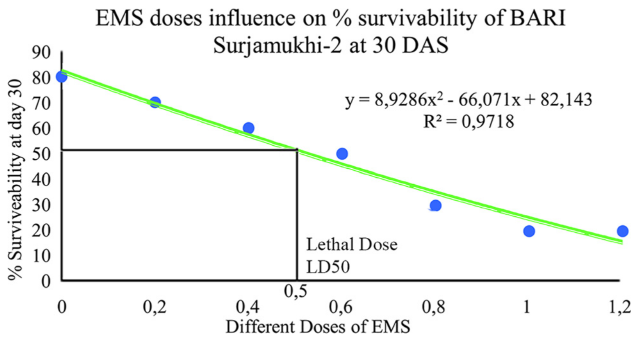

Figure 4: Effect of different doses of EMS on % survivability of BARI Surjamukhi-2 at 30 days after sowing (DAS).

$$y = -66.071x + 8.9286x^2 + 82.143$$

Where, y represents the % survivability and x, the mutagen dosage.

From the regression equation, a 0.5% EMS dose was found optimum for obtaining 50% seedling at day 30. The LD50 also varies depending on particular mutagen, specific crop, species, as well as different varieties of the same species (Singh 2000). Yadav et al. (2016) reported LD50 0.42 and 0.73% of EMS dosage respectively, for two different *Brassica juncea* varieties. While, Emrani et al. (2011) reported EMS dose of 0.8% as LD50 for *Brassica napus*. Talebi et al. (2012) reported 0.5% EMS dose as LD50 for Malaysian rice. Kumar et al. (2013) reported an EMS dose of 0.6% as LD50 for sunflower. Therefore, the optimum EMS dose (0.5%) find in this study could be used to develop large scale desirable mutants for sunflower variety BARI Surjamukhi-2.

Conclusions

The present study was conducted to find out the effect of different dosage of EMS on germination, survivability and morpho-physiological characteristics and to determine an effective and efficient EMS dose for successful mutagenesis in sunflower variety BARI Surjamukhi-2. Stimulatory effect was observed for seed germination, root length and shoot length and stem fresh weight at T_4, T_3 and T_2, treatments, respectively over control. But, increased lethality with increased EMS dosage was found for most of the cases. LD50 determination based on a quadratic regression analysis for most vital plant character % survivability showed that EMS concentration of 0.5% was significant where 50% seedling survivable was obtained. Thus showing a 0.5% EMS dose would be effective and efficient for successful mutagenesis in sunflower variety BARI Surjamukhi-2.

Acknowledgments: The authors thanks to Oilseed Research Centre (ORC), Bangladesh Agricultural Research Institute (BARI) for providing all logistics support for conducting the research.
Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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