

Effect of Elevated Temperature and Ethyl Methane Sulfonate on the Growth and Yield Attributes of Summer Onion (*Allium cepa L.*)

Asif Hasnath Luhan

Department of Environmental Science,
Bangladesh Agricultural University, Mymensingh-2202

Muhammad Aslam Ali

Department of Environmental Science,
Bangladesh Agricultural University, Mymensingh-2202

Md Rafiqul Islam

Bangladesh Institute of Nuclear Agriculture,
Bangladesh Agricultural University Campus, Mymensingh-2202

Shah Tasdika Auyon

Department of Environmental Science,
Bangladesh Agricultural University, Mymensingh-2202

SK Md. Fazlay Rabbi

Department of Environmental Science,
Bangladesh Agricultural University, Mymensingh-2202

Deboki Kundo

Department of Environmental Science,
Bangladesh Agricultural University, Mymensingh-2202

Tazbeen Tabara Nitu

Department of Soil Science,
Bangladesh Agricultural University Campus, Mymensingh-2202

Humayun Kabir

Department of Seed Science,
Bangladesh Agricultural University, Mymensingh-2202

ABSTRACT

Onion cultivation during summer wet season faces problems in seedlings raising and difficulties in management practices in the main field, which is a great concern. Ethyl methane sulphonate (EMS) is a chemical mutagen which helps to develop desirable mutant by point mutation. The present experiment was carried out at the Horticulture field laboratory of BINA, Mymensingh during the period July to October, 2019 to investigate the effect of ethyl methane sulphonate (EMS) for developing desirable mutants of onion under ambient and elevated temperature

conditions. In this experiment five different doses of EMS viz., 0%, 0.5%, 1%, 1.5% and 2% were applied on onion seeds and the treated seedlings were grown under ambient and elevated temperature conditions. The experiments were conducted following Randomized Complete Block Design (RCBD) in field and Completely Randomized Design (CRD) in pot experiment under greenhouse condition. In the field trial, under ambient condition total leaf area, leaf area index, chlorophyll content and total bulb yield were significantly influenced by different concentrations of EMS. At ambient temperature, the highest bulb length (6.52 cm) and diameter (5.04 cm), single bulb weight (38.3 g) and total bulb yield (14.95 t/ha) were recorded with 0.5% EMS application, which decreased with further increasing concentrations of EMS (1.0-2.0%). On the contrary, the above yield parameters and total bulb yield were decreased under elevated temperature condition (+ 2°C higher than ambient) irrespective of EMS concentrations. Above all, the interaction effects of temperature and EMS concentrations on onion seeds germination, seedling growth and yield components were found non-significant. Conclusively, growth and yield attributes of summer onion may be enhanced by applying chemical mutagen EMS at 0.5% under ambient field condition, however elevated temperature (+ 2°C higher than ambient) in the changing environment could badly affect the yield of summer onion at all levels of EMS applied.

Keywords: EMS, elevated temperature, Mutant, bulb yield, summer onion.

INTRODUCTION

Onion (*Allium cepa* L.), being one of the important spices and condiments, belongs to the family Liliaceae. The edible part of onion is green leaves, immature and mature bulbs. Being a biennial species, it takes more time to improve this crop by conventional methods such as hybridization, recombination and selection [1]. Bangladesh largely depends on locally produced winter onion, being recorded 1.8 million metric tons (BBS, 2019), which meets only one fifth of the total requirement of the country [2]. However, the country needs to import onion about 10 lac MT annually from different countries due to storage loss, production shortage and the use of onion bulbs for onion production. Moreover, increasing storage facility is a costly and time-consuming endeavor. Therefore, producing onions in off season is the viable solution.

In Bangladesh, onion is mainly cultivated during October to march. The demand for onions in Bangladesh is growing steadily in conjunction with the population growth and rising per capita income. The country requires about 25 lakh tonnes of onion each year, according to an estimate by the Bangladesh Trade and Tariff Commission, however, a big portion of the vegetables are lost due to lacking of proper storage facilities, resulting in a shortfall of the bulb onion during the lean period. Severe scarcity of onion bulbs is seen from August to March in the vegetable markets which are fulfilled by import mainly from neighbouring countries. Therefore, off season summer onion production may become a major landmark in overall onion production and supply chain, which will reduce annual import cost. Summer onion cultivation can be a new way to increase the onion production in Bangladesh and make it available throughout the year. Bangladesh is mostly vulnerable to climate change, which has been affected overall agricultural production [3]. Although onion may be grown under diverse environmental conditions, however the summer-planting of onion could be difficult due to production of seedlings for the transplant [4].

Increasing temperature and uneven rainfall patterns may affect onion production in Bangladesh. During the summer, high temperature and heavy rainfall coincide, which makes the management difficult to grow onions in open field condition, excessive accumulation of water in the root system, root diseases and weed infestation, thereby the onion plant becomes less competitive. Therefore, the off-season onion production will be the best alternative to substitute the imports and reduce the higher demand of onion bulb during the lean period of production. Unfortunately, very little attention has been given to the improvement of onion either through selection, hybridization and induced mutation using gamma rays or chemical mutagens in Bangladesh. Chemicals induce mainly point mutations, and are thus ideal for producing missense and nonsense mutations, which would provide a series of change-of-function mutations. Ethyl methane sulfonate (EMS) is a mutagenic and carcinogenic organic compound, which is considered to be the best chemical mutagen to create point mutations in genetic material by single nucleotide substitution; particularly through G: C to A: T transitions induced by guanine alkylation in plants [5]. To date there is no research findings available on the combined effect of EMS and temperature on the growth and yield of summer onion in Bangladesh. Therefore, this research experiment was undertaken to investigate the growth and yield components of summer onion with EMS application under the ambient and elevated temperature conditions.

MATERIALS AND METHODS

The experiment was carried out in the field Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during (Kharif II season of 2019 -2020). The experiment was conducted to investigate the growth and yield attributes of *Allium cepa* influenced by different growing conditions like ambient and elevated temperature with different concentrations of Ethyl Methane Sulphonate (EMS).

Experimental Site, Climatic Conditions and Field Preparation

The experiment was located at 24°N Latitude, 94° E Longitude at a mean elevation of 1m above the sea level belonging to the Non-Calcareous Dark Gray Floodplain soil under the Old Brahmaputra Floodplain, AEZ 9. The climate of the experimental area is under the sub-tropical climate which is characterized by high temperature, high humidity and heavy precipitation with gusty wind from (April to September) and scanty rainfall associated with moderately low temperature during (October to March). The land was Inceptisols, medium high land, above flood level and firm in consistence, when dry. The soil lies on the alluvial deposits of Brahmaputra River. The soil of the experimental field belongs to Sonatola Series. The land is moderately well drained with a sandy loam texture and soil pH value 6.7. The initial soil organic carbon, T-N, and available P contents were 1.13%, 0.22% and 70 ppm.

Experimental Materials, Treatments and Design

Seeds of BARI Onion-5 variety were collected from Horticulture Division, BINA, Mymensingh. Dry and uniform seeds were pre-soaked in double distilled water for 9 hours at room temperature (24±2°C). Seeds were manually shaken after every two hours to ensure the uniform soaking. For EMS treatment, pre-soaked seeds were treated with 0 (control), 0.5%, 1.0%, 1.5% and 2.0% v/v freshly prepared aqueous solution in double distilled water for 1.5 hours and kept at 25°C temperature in an incubator shaker. After mutagen treatment, seeds

were washed thoroughly five times for 3 minutes with water to wipe out mutagenic chemical completely. After thorough washing, seeds were put in the petridish having Whatmann filter paper at the base to soak adhering water with seeds. Immediately, seeds were sown in the field after germination.

Field Experiment

Field experiment was laid out in a randomized complete block (RCBD) design with three replications. Seeds of the selective cultivar of onion were treated with different concentrations T1 (Control, 0%), T2 0.5%, T3 1.0%, T4 1.5% and T5 2.0% of Ethyl methane sulfate (EMS) prior to set the experiment. The experimental site was thoroughly ploughed and soil was brought to fine tilth. In total there were 15-unit plots. The area of each plot was 1.5m² (length 1m and width 1.5m). Recommended doses of chemical fertilizers and organic fertilizer (5 ton /ha) were applied as basal during final land preparation (Fertilizer Recommendation Guide, 2018). Seeds were sown in July, 2019. After 30 days of seeds sowing the plants were transplanted into the experimental field. Growth and yield attributes were observed till 120 days of set up. Data were collected at every 15 days interval and finally onion bulb yield as well as yield components were recorded at bulb maturation stage.

Glasshouse Experiment

To observe the growth and yield attributes of onion cultivar under elevated temperature pot experiment was carried out in glasshouse with similar treatments. The experimental design was completely randomized design (CRD) with three replications. Total number of pots were 15.

Growth Parameters of Onion

Germination percentage was counted at the time of seed sowing.

$$\text{Germination percentage (\%)} = \frac{\text{Number of seed germinated}}{\text{Total number of seed}} \times 100$$

Seedling root and shoot lengths were measured after 15 days of sowing for both control and treated seeds. Root length and shoot length were calculated by measuring the length (cm) from root tip to the root-shoot junction and shoot tip to the root-shoot junction. Plant height, number of leaves/plants, Leaf length and diameter, Leaf areas and leaf area index of onion were measured manually with measuring scale from randomly selected 3 plants from each treatment after 45 DAT. Leaf chlorophyll content was measured by SPAD meter (CCm-200 plus, Optiscience) at active tillering stage at 45 DAT.

Yield Components and Total Bulb Yield of Onion

Neck diameter and length of bulb were measured using measuring scale after harvest. Split bulb (%) was calculated following the below equation.

$$\text{Split bulb (\%)} = \frac{\text{Number of Split bulb}}{\text{Total number of bulb after harvest}} \times 100$$

After harvest the weight of single bulb was measured randomly from five plants of each treatment. Finally, total bulb yield (tha^{-1}) was calculated from bulb weight (g) following the equation,

$$\text{Total bulb yield (tha}^{-1}\text{)} = \frac{\text{Total weight of bulb (g)} \times 10000 \times 0.001}{\text{plot size} \times 10000}$$

Harvesting and Data Collection

Onion bulbs were harvested at their proper matured stage. The harvested bulbs of each plot were collected separately and weight was taken properly. Morphological data such as, root length, shoot length, leaf number, leaf area index, chlorophyll content was measured carefully. Plant height were measured every 15 days interval. After harvest yield parameters such as, neck diameter, single bulb weight, split bulb percentage and total yield were recorded properly.

Statistical Analysis

Data were statistically analyzed to ascertain significant differences in main effects and interactions among EMS dose and temperature with growth and yield parameters of summer onion. To test the differences between five different EMS doses, multiple comparisons test was performed using LSD. All statistical analyses were considered significant at $p < 0.05$, unless otherwise mentioned. The statistical analyses were performed with STATISTIX 10.

RESULTS

Seed Germination (%)

Onion seed germination was recorded 87.5% in elevated temperature condition at 29°C ($+2^{\circ}\text{C}$ higher than ambient), which was significantly higher ($p < 0.01$, Fig 1) than ambient temperature condition (mean value 74.9%). In addition, germination percentage of seeds significantly varied ($p < 0.05$, Fig 1) with the concentrations of EMS.

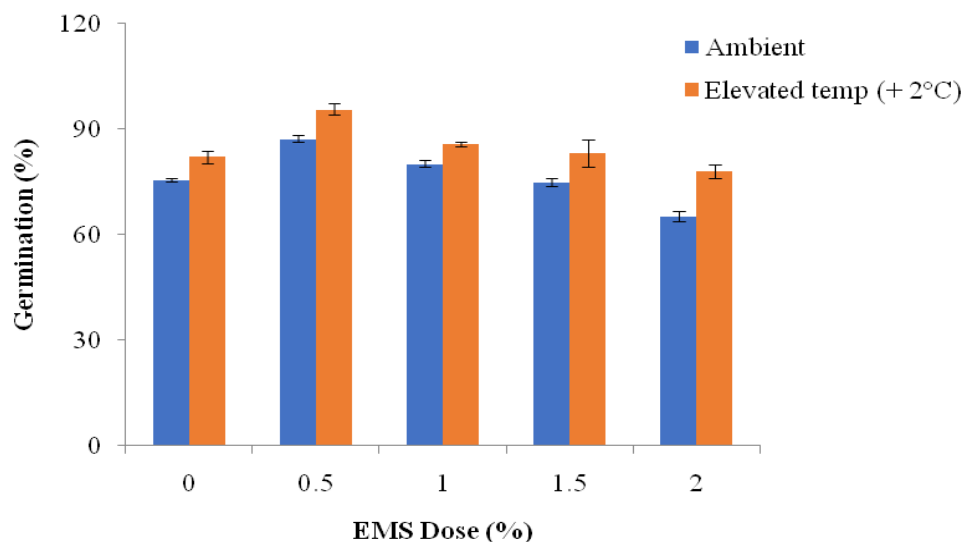


Fig 1: Effect of EMS concentrations and temperature on summer onion seed germination (%)

The germination rate was significantly higher with EMS 0.5% than control, however, the increasing concentrations of EMS (1.0%-2.0%) revealed decreasing trends of germination. The interaction effects of growing temperature and EMS doses on germination percentage of onion was found non-significant.

Root Length (cm)

Root length of onion was significantly ($p < 0.01$, fig 2) higher in elevated condition than that in ambient condition showing the mean value of 2.63 cm in elevated condition and 2.48 cm in ambient condition respectively. Likewise, the root length of onion was significantly varied with the different concentrations of EMS at ($p < 0.05$, fig 2).

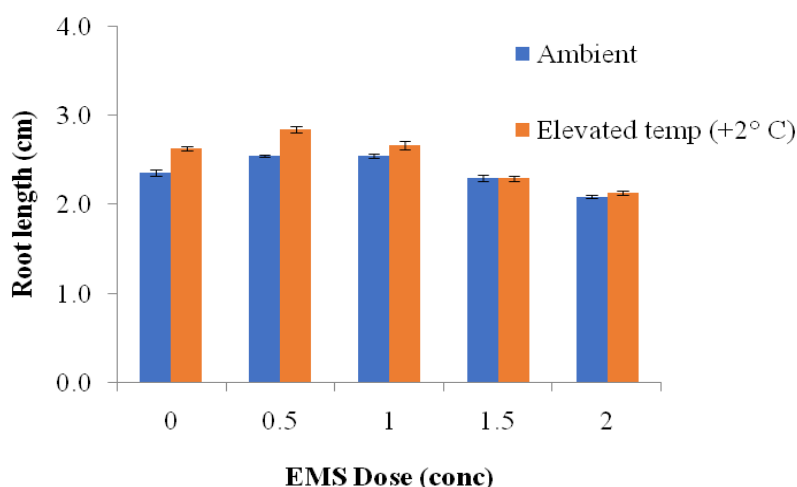


Fig 2: Effect of EMS dose (%) and temperature on root length of summer onion

Root length of onion increased after the application of 0.5% dose of EMS but with increment of the EMS concentration, root length of onion gradually decreased (ca. 3.20 cm, 3.58 cm, 3.22 cm, 3.04 cm and 2.94 cm in control, 0.5%, 1.0%, 1.5% and 2.0% concentration of EMS respectively). The interaction effects of different growing conditions and EMS doses on root length of onion was non-significant.

Shoot Length (cm)

Shoot length of onion was significantly greater ($p < 0.01$, Fig 3) in elevated temperature condition than ambient temperature showing the mean value of 3.45 cm in elevated condition and 3.22 cm in ambient condition respectively. Furthermore, the shoot length was significantly influenced by the concentrations of EMS at ($p < 0.05$, Fig 3).

Shoot length increased after the application of 0.5% dose of EMS. Conversely, with increment of the EMS concentration shoot length gradually decreased. The interaction effects of growing temperature conditions and EMS concentration on shoot length of onion was found non-significant.

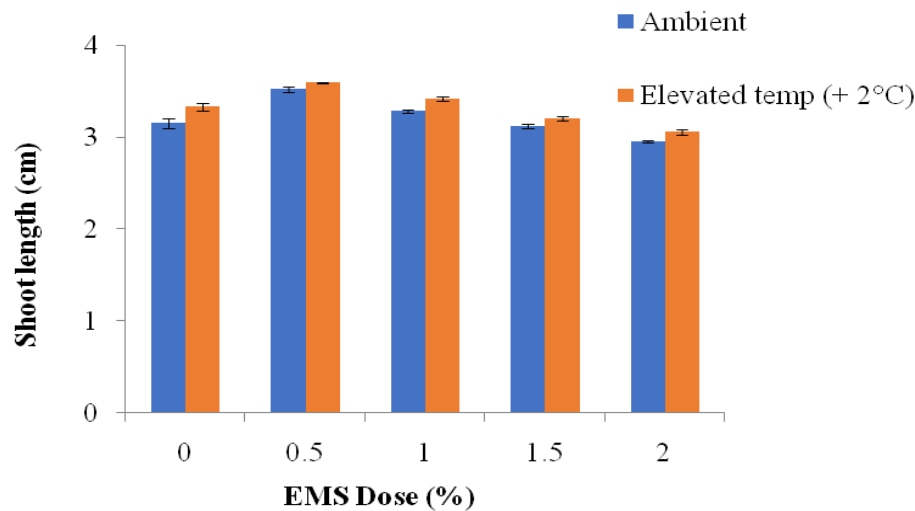


Fig 3: Effect of EMS dose and temperature on shoot length of summer onion

Plant Height (cm)

Under ambient temperature condition, the highest plant height 71.3 cm was recorded with 0.5% concentration of EMS at 45 DAT, which decreased with increasing concentrations of EMS (Table1). Similarly, the highest plant height 68.7 cm was recorded with 0.5% concentration of EMS at elevated temperature, which decreased with increasing concentrations of EMS. The interactive effect of temperature and EMS concentrations on plant height was found non-significant.

Table 1: Interactive effect of temperature and EMS concentrations on plant height

Condition	EMS (%)	Plant height (cm)			
		15 DAT	30 DAT	45 DAT	60 DAT
Ambient temperature	0.0	34.33	43.33	57.33	54.67
	0.5	41.33	48.33	71.33	64.67
	1.0	36.33	44.67	66.67	59.67
	1.5	34.00	43.33	61.33	57.67
	2.0	31.33	41.33	58.33	53.33
Elevated temperature	0.0	32.33	42.33	56.33	52.33
	0.5	38.67	49.33	68.67	66.33
	1.0	35.67	47.33	65.67	63.33
	1.5	33.33	45.33	63.67	60.67
	2.0	31.00	40.33	55.67	54.67
Level of significance		NS	NS	NS	NS
CV (%)		6.0	5.0	5.90	6.60

Effect of EMS Concentrations on Leaf Characteristics and Chlorophyll Contents of Summer Onion Under Ambient and Elevated Temperature

At ambient temperature, the highest number of leaves/plants, leaf length, leaf diameter, leaf area, leaf area index and chlorophyll contents were recorded with 0.5% EMS, which decreased with increasing concentrations of EMS (Table 2).

On the contrary, elevated temperature caused lower values of the above-mentioned leaf parameters compared to ambient temperature condition, although no significant interactions were found among the EMS concentrations and temperature levels.

Table 2: Interactive effects of growing temperature and EMS concentrations on leaf characters and Chlorophyll contents

Condition	EMS (%)	Leaves plant ⁻¹ (no)	Leaf length (cm)	Leaf diameter (cm)	Leaf area (cm ²)	Leaf area index (LAI)	Chlorophyll content (μmolm ⁻²)
Ambient temperature	0.0	5.08	52.36	1.14	81.10	3.32	7.40
	0.5	6.51	58.83	1.38	85.06	3.38	7.96
	1.0	5.32	53.50	1.22	82.66	3.15	7.47
	1.5	5.05	51.02	1.12	81.05	2.98	7.26
	2.0	4.77	49.78	0.98	78.66	2.87	6.90
Elevated temperature	0	5.10	50.94	1.08	78.62	2.75	7.25
	0.5	5.89	56.44	1.35	83.54	2.91	7.68
	1.0	5.26	51.06	1.12	80.37	2.82	7.40
	1.5	5.13	48.72	1.05	78.27	2.71	7.14
	2.0	4.71	46.08	0.98	76.39	2.68	6.82
Level of significance		NS	NS	NS	NS	NS	NS
CV (%)		3.50	6.05	3.22	4.25	3.75	5.21

At ambient temperature, the highest bulb length (6.52 cm) and diameter (5.04 cm), single bulb weight (38.3 g) and total bulb yield (14.95 t/ha) of onion were recorded with 0.5% EMS (Table 3), thereafter decreased with increasing concentrations of EMS.

However, the mentioned yield parameters and total bulb yield were decreased under elevated temperature condition at all levels of EMS compared to ambient temperature. Above all, the interaction effect of temperatures and EMS concentrations on split bulb percentage, bulb length and diameter, single bulb weight and total bulb yield of onion was found non-significant.

Table 3: Interactive effects of temperatures and EMS concentrations on split bulb, bulb length and diameter, single bulb weight and total bulb yield of onion

Temperature	EMS (%)	Neck thickness (cm)	Split bulb (%)	Bulb length (cm)	Bulb diameter (cm)	Single bulb weight (g)	Total bulb yield (t h ⁻¹)
Ambient	0.0	1.12	2.00	6.16	4.30	32.20	12.60
	0.5	1.33	1.92	6.52	5.04	38.30	14.95
	1.0	1.16	2.03	6.33	4.90	34.30	13.10
	1.5	1.08	2.12	6.20	4.65	33.55	12.60
	2.0	0.96	2.16	6.12	4.25	30.80	12.00
Elevated	0.0	1.02	1.97	5.10	3.30	32.00	12.30
	0.5	1.26	1.80	5.44	3.88	37.90	14.40
	1.0	1.10	1.94	5.12	3.60	33.50	12.90

	1.5	0.98	2.04	5.08	3.45	32.30	12.10
	2.0	0.87	2.10	4.94	3.20	28.20	11.90
Level of significance		NS	NS	NS	NS	NS	NS
CV (%)		3.35	4.98	6.42	5.00	3.83	6.78

- Maximum single bulb weight and total bulb yield were recorded with 0.5% EMS application
- bulb weight and total bulb yield decreased with increasing concentrations of EMS (1.0-2.0%)
- Total bulb yield decreased under elevated temperature condition irrespective of EMS concentrations
- Interaction of temperature and EMS concentrations on onion seeds germination, seedling growth and yield components were found non-significant

DISCUSSION

The present study showed that the germination of onion seeds, plant height, root and shoot length significantly increased at 0.5% EMS, however gradually decreased with the increment of EMS concentrations. These findings were supported by Singh *et al.*, (2021) [6]. The highest seed germination observed at 0.5% EMS may be due to stimulating effect of lower dose of chemical mutagen. The decrease in onion seeds germination above the 0.5% EMS application may be due to mutagenic effects which delayed the physiological and biological processes necessary for seed germination (Khan *et al.*, 2012) [7]. Under greenhouse condition, we recorded the highest germination of onion seeds at 29°C, which was supported by Rayyan *et al.* (2012) [8], while Ansari (2009) reported onion seeds germinate within 13-28°C [9]. In this study, a reduction in shoot length of onion was found with increasing concentration of EMS above 0.5% level, which may be due to uneven damage or injury to the meristematic cells as a result of genetic injury. Joshi *et al.* (2011) also observed stimulatory effect on seedling shoot length at low dose of EMS and inhibitory effect on it with increasing dose of EMS in onion [10]. The stimulation of seedling root length at 0.5% mutagen has also been noticed in onion, however, the inhibition of root growth was also recorded with the increase of EMS dose in our study, which coincides with other researchers' findings [10]. It was reported that prolong use of EMS may suppress mitotic division and affect nuclear condensation thereby irregular distribution of chromosomes, bridges and fragmentation which hinder the growth [11]. In this study, the highest plant height (70.24 cm) was recorded with 0.5% concentration of EMS at 45 days, which decreased with the increasing concentration of EMS. Similar results were obtained in regards to plant height [12, 13]. At 0.5% concentration of EMS, the maximum number of leaves (5.46) was recorded under ambient condition compared to the number of leaves (4.40) obtained under elevated temperature, thereafter decreased with the increasing concentrations of EMS, being supported by other research findings [14, 15]. Leaf length and diameter were recorded highest at 0.5% concentration of EMS, which decreased gradually with increasing concentrations of EMS. Significant differences were also observed in seedling height, fresh true leaf weight, seedling weight, and plant survival with increasing EMS concentration and time [16]. Higher variations in the form of dwarf seedlings were recorded after treatment with 1.5% EMS for 24 h. LAI was significantly influenced by different concentration of EMS. LAI was recorded highest under the treatment of 0.5% concentration of EMS. Thereafter, with the increasing concentration of EMS, LAI of onion decreased and the combined effect of different growing conditions and EMS doses on LAI of onion was found non-significant [17]. Chlorophyll content in the leaves of onion was recorded significantly higher with 0.5% concentration of EMS at ambient condition than that of elevated temperature condition. Further with the

increment of EMS concentration, chlorophyll content of onion leaf decreased. Neck diameter of the bulb of onion was significantly greater in ambient condition than that of elevated condition and maximum neck diameter of bulb was recorded at 0.5% concentration of EMS. Bulb length and bulb diameter of onion were significantly higher in ambient condition than that of elevated condition at 0.5% concentration of EMS. The cumulative effect of temperature and EMS was found non-significant on bulb length and bulb diameter, being supported by Munoz *et al.* (1995) [18]. In this study, single bulb weight and total bulb yield of onion were found comparatively greater under ambient field condition than that of greenhouse grown elevated temperature condition. The main effect of growth environment was significant ($P < 0.05$) for fresh weight of bulb. In general, higher temperatures accelerate, and lower temperatures may delay bulb development. which could be accompanied by changes in physiological activity of onion plants. Steer [19] observed a significant interaction of cultivar, daylength and temperature on onion bulbing, in which case bulbing was most rapid at the highest temperature and longest day length.

Maximum single bulb weight was recorded with 0.5% concentration of EMS, which was decreased with the increasing concentration of EMS. The interaction effect of growing conditions and EMS concentrations on single bulb weight of onion was found non-significant, which was supported by Hwang [20]. It was also reported that average yields of BARI piav-2, BARI piav-3 and BARI piav-5 summer onion cultivars were 12.72, 12.33 and 12.18 t/ha respectively, about two times higher than the present average yield of 8 t/ha in Bangladesh [21]. The application of 120 kg K ha⁻¹ from Potassium Sulphate along with a blanket dose of N100 P50 S20 B1.5 Zn 4.0 kg ha⁻¹ and 5.0-ton cow dung ha⁻¹ was found suitable for maximizing the bulb yield of summer onion var. BARI piav-3 [22]. Total bulb yield of onion significantly varied under different growing conditions. Total bulb yield was greater in ambient condition than that of elevated condition. The maximum total bulb yield was recorded at 0.5% concentration of EMS, whereas decreased with increasing concentration of EMS. The interaction effect of growing temperature conditions and EMS concentrations on total bulb yield of onion was non-significant. Effect of EMS on bulb yield of onion was partially supported by the findings of Mahajan V. [23]. It was also reported that 0.6% EMS was found optimum for onion cultivar Bhima Dark Red [6], which may be used by onion breeders for development of onion mutants to create new breeding material for screening against various biotic and abiotic stresses in the changing climatic conditions. Considering the effects of environmental factors on the growth and development of onion, Budathoki (2012) reported that high mean temperature (>25 °C) suppressed leaf emergence whereas temperature between 17.7 °C to 25 °C favoured leaf emergence and their subsequent growth prior to bulbing [24].

CONCLUSION

It was found that germination percentage of onion seed significantly increased under elevated temperature at 29°C (+2°C higher than ambient) at 0.5% EMS, however, decreased with increasing EMS concentrations. The maximum plant height (70.24 cm) and leaves number/plant were recorded at 0.5% concentration of EMS, which decreased with increasing concentration of EMS. Leaf diameter, leaf area, LAI and chlorophyll content of the leaves and neck diameter of bulb onion were found significantly higher in ambient condition than that of elevated condition. With the increasing concentration of EMS chlorophyll content of onion leaf

decreased. Both root and shoot length increased with 0.5% concentration of EMS, however, decreased with higher concentrations of EMS (1.0-5.0%). Maximum total bulb yield was recorded with 0.5% concentration of EMS at ambient condition, which decreased at elevated temperature. From the above findings, it may be concluded that EMS at 0.5% concentration was found suitable for the improvement of onion growth parameters and total bulb yield under ambient temperature, although total bulb yield decreased at elevated temperature.

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