Annals of Bangladesh Agriculture
Journal homepage: bsmrau.edu.bd/aba

ORIGINAL ARTICLES

Morpho-biochemical traits improvements in cherry tomato using EMS mutagen

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ARTICLE INFO.

Keywords:
antioxidant, mutagen, trait, treatment, correlation.

Received : 21 April 2024
Revised : 11 June 2024
Accepted : 30 June 2024
Published : 15 July 2024

Citation:
Hasan, M., R. Parvin, A. S. M. Hasibuzzaman and M. A. Haque. 2024. Morpho-biochemical traits improvements in cherry tomato using EMS mutagen. *Ann. Bangladesh Agric.* 28(1): 145-156

ABSTRACT

The approach of chemical mutagenesis has proven effective in enhancing crop genetic variation. Out of the many different chemical mutagens available, ethyl methanesulfonate (EMS) is commonly used in crops because of its high frequency of causing DNA changes. Various concentrations, ranging from 1.2% to 2% of EMS were administered to cherry tomatoes to compare their effects with a control group. The objective was to determine these concentrations' effectiveness in improving the cherry tomatoes' morphological and biochemical characteristics. Significant variations were noticed in the morphological and biochemical characteristics compared to the control. A concentration of 1.4% EMS was linked to characteristics associated with earliness, but 1.2% EMS positively impacted the quantity of fruits produced per plant. The use of 2% EMS treatment resulted in a higher number of branches per plant, but it did not have any discernible positive effect on yield. The correlation studies indicated a moderate positive link between the height of plants and the quantity of fruits produced per plant. A concentration of 1.2% EMS showed positive effects on lycopene and ascorbic acid, but a concentration of 1.4% EMS resulted in improved levels of chlorophyll a, chlorophyll b, and carotenoids. Chlorophyll b showed good performance with a concentration of 1.6% EMS; however, a concentration of 2% EMS was more effective in increasing phenolic and antioxidant content. The experiment's results revealed that EMS-induced mutagenesis is a valuable tool for enhancing agronomic and yield-related traits and biochemical features. In addition, mutants possessing yield-related beneficial characteristics will be accessible to researchers for high-level investigation. They will also function as genetic resources for advancing and improving breeding strategies.

Introduction

Cherry tomatoes are a great choice for cultivation because they can withstand both hot and cold weather and are resistant to diseases. Due to their many health benefits, including a high concentration of antioxidant and phytochemical compounds like lycopene, ß carotene, flavonoids, vitamin C, and many other vitamins and minerals, as well as their delicious

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https://doi.org/10.3329/aba.v28i1.73498
ISSN 1025-482X (Print)/2521-5477 (Online)

flavor, low-calorie count, and ability to keep fruit fresh even when heated, they have gained popularity around the world (Campestrini *et al.*, 2019; Yang *et al.*, 2023). Therefore, cherry tomato breeding efforts should focus on improving nutritional quality and health benefits alongside yield improvement. Because of their nutritional worth, versatility in cooking, and use in both homemade and packaged foods, cherry tomato consumption has increased substantially in recent decades (Paulsen *et al.*, 2019; Richardson and Arlotta, 2022). Furthermore, the greatest obstacles to maintaining the global supply are the changing climate and the increase in the global population size, among other things. Given the speed with which the climate is altering, it is crucial to develop cherry tomato cultivars that are both nutritional and produce abundant harvests. Established cherry tomatoes have very little variation in their genes, making it difficult to generate high-yielding cultivars with better flavor and resilience to biotic and abiotic challenges. One possible solution to the constraint is to use introgression breeding. However, the high-yielding genetic base is occasionally severely compromised by the introgression of the genome. Furthermore, the process of introgression breeding takes a lot of time. A further restriction is the instability of cultivated cultivars when it comes to crossing. One of the most encouraging ways to increase genetic diversity and speed up the development of crops is through mutation breeding. Researchers have used induced mutagenesis in several different crop species (Bado *et al.*, 2015; Yali and Mitiku, 2022).

As climate change is unpredictable, novel varieties must be created on an ongoing schedule to ensure environmentally friendly farming. Breeders can't take advantage of numerous things due to the low rate of genetic diversity caused by spontaneous mutations; instead, they must resort to artificial mutations. Furthermore, mutation breeding can be used to isolate mutants with numerous traits, and mutant varieties have a far better chance of survival in environments with rapidly changing climates (Raina *et al.*, 2018; Zakir, 2018).

Mutagenesis, the efficient generation of mutations, can be triggered by a mutagen or happen spontaneously. Physical mutagenesis and chemical mutagenesis are two main categories that describe the various effective approaches that have been established for inducing genetic changes. In addition to improving the odds of obtaining acceptable phenotypic variation, mutagens are useful for studying genotypic changes linked to phenotypes and annotating gene function. Ethyl methane sulfonate (EMS) and gamma rays are two examples of the chemical and physical mutagenic agents that have been utilized in agricultural research to produce mutations. Because it is so good at causing deletions and point mutations in chromosomal segments, EMS is the chemical mutagen that breeders use the most. Evidence suggests that EMS can increase traits such as disease resistance, fruit quality, and male sterility in addition to morphological changes. When more traditional methods of breeding proved ineffective, EMS mutagenesis proved to be an effective alternative in multiple instances (Riaz and Gul, 2015; Chaudhary *et al.*, 2019).

When utilizing chemical mutagens, it is essential to optimize doses. Unfortunately, research on the most effective dose of EMS mutagen for causing crop mutations is lacking. The frequency of beneficial mutants detected in the advanced generations is heavily influenced by the optimal dose of seed treatment used to generate the mutant population. The efficacy and efficiency of different mutagen concentrations can be observed well by looking at the mutation frequency represented as a function of the dose and biological damage. Therefore, finding the optimal EMS dose for its efficient use in cherry tomato breeding was the primary objective of the study.

Materials and Methods

Plant material and EMS treatment

The study utilized healthy, disease-free seeds of an exotic cherry tomato collected from the Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh.

First, distilled water was used to pre-soak the seeds beforehand. On blotting paper, the seeds were subsequently dried. The seeds were submerged in 0 (control), 1.2%, 1.4%, 1.6%, and 2% EMS solution for two hours to allow to set the treatment doses (Chen *et al.*, 2023; Ye *et al.*, 2020). To make certain that the mutagen was exposed uniformly, the liquid containing the seeds was shaken periodically. The treated seeds were properly cleaned four times with water from the faucet to get rid of any remaining chemicals. After that, the seeds were placed in a tray with soil and dried with blotting paper. The plants were later moved to the field. The following morphological parameters were measured:

Days to first flowering, days to first fruit set, days to first harvest, branch per plant, plant height, fruits per plant, fruit diameter, fruit length, individual fruit weight. Every sample was unique because of mutations; so, the morphological and biochemical traits were measured per plant. An average of 13 plants were used per treatment to assess the efficacy of the EMS treatment.

Aside from the morphological assessment, the following biochemical traits were measured, as described by Islam *et al.* (2022): total phenol, antioxidant, fruit chlorophyll a and chlorophyll b, lycopene, carotenoid, ascorbic acid, Na, and K content of the fruits.

Biochemical procedures

Total phenol determination

The following amounts of gallic acid were used to make standard solutions: 0 mg/mL, 0.0125 mg/mL, 0.025 mg/mL, 0.050 mg/mL, 0.100 mg/mL, and 0.150 mg/mL. Following the proper dilution process, either the test extracts (0.1 mL) or the gallic acid standards (0.1 mL) were transferred into test vials that had a total volume of 15 mL. After the Folin-Ciocalteu reagent was added to each test tube, the contents of each tube were shaken with a mixing vortex (0.2 N, 3.0 mL) to ensure that the material were evenly distributed. After waiting for one minute, 2.0 milliliters of Na_2CO_3 in water with a concentration of nine percent by weight (w/v) was introduced to the solution, and then it was permitted to cool down to the ambient temperature. After being present for a period of two hours, the absorbance was measured at a wavelength of 765 nm. Analyzing the absorbance of the extract samples and comparing it to the absorbance of the gallic acid standard solutions allowed for the determination of the total phenolic compounds that were present in the sample. Three observations were made on each sample.

Antioxidant determination

The final volume of 4.1 ml was accomplished by mixing 0.1 ml of the sample extraction with 1 ml of $CuC1_2$ solution, 1 ml of neocuproine alcoholic solution, 1 ml of NH4Ac buffer solution, and 1 ml of water. The mixture was then stirred until it reached the desired concentration. After waiting for thirty minutes, the absorbance at 450 nm was measured, and the results were contrasted to the blank assay (Islam *et al.*, 2024).

Chlorophyll determination

A fresh sample weighing 0.2 grams was placed in a test tube, and then a mortar and pestle were used to combine the sample with 10 milliliters of acetone containing 80%. To obtain the chlorophyll that had been removed from the acetone solution, the homogenate was filtered through the Whatman filter paper of the first grade. The homogenate was washed off with 5 milliliters of 80% acetone multiple times. As a result of adding 80% acetone, the total volume increased up to 25 milliliters. The amount of chlorophylls a and b, as well as the overall amount of chlorophyll that was present in the samples, were determined by measuring the optical density at 663 and 645 nm.

Quantification of lycopene

The desiccated sample, weighing between 0.3-0.6g in powder form, was measured in a container. Subsequently, 5 ml of a BHT-acetone mixture with a concentration of 0.05% w/v, 5 ml of ethanol, and 10 ml of hexane were introduced. The beaker was positioned within a receptacle filled with ice on a magnetic

stirring plate, agitated for a duration of 15 minutes, and supplemented with 3 milliliters of distilled water. The mixture was agitated for 5 minutes on ice and then left at ambient temperature for 5 minutes to facilitate the distinction between the two sections. The lycopene-containing top layer was extracted using a pipette and transferred to a test tube. Aluminum foil was used to wrap the tubes having lycopene isolates. The reading was made at 445,471, and 502 nm against blank. Hexane served as the blank solution.

Carotenoid determination

A quantity of two grams of fruit pulp was placed into an empty beaker. A volume of 5 milliliters of acetone was introduced. The container was sealed tightly and stored in a dark environment at a temperature of 40ºC for a duration of 24 hours. A volume of 3 milliliters of the liquid remaining after centrifugation was transferred into a cuvette. The measurement of absorbance was taken at a wavelength of 444 nm, with acetone serving as the reference solution.

Ascorbic acid determination

One gram of the material was used to derive ascorbic acid applying a 4% solution of trichloroacetic acid. The volume was then adjusted to 10 milliliters utilizing the same solution. The liquid portion acquired after spinning at 2000 revolutions per minute for 10 minutes was mixed with a small amount of activated charcoal, forcefully agitated, and left undisturbed for 5 minutes. The charcoal bits were separated using spinning, and portions were utilized for an estimate.

Mineral estimation

The presence of sodium, magnesium, calcium, manganese, iron, copper, and potassium, along with other minerals, was also detected in fruits. For the cherry tomato samples, 0.5 grams of dried extracts were sifted into a 250-millilitre processing tube. A volume of 5 milliliters of strong nitric acid (65%) was added and left undisturbed overnight. Subsequently, the substance was subjected to a temperature of 60 degrees celsius until the emission of brown smoke ceased. Subsequently, a 2 ml aliquot of $HClO_4$ acid was introduced to the samples at a temperature of 150^0C to reduce their size. The samples underwent filtration using a Whatman No. 42 paper filter and were subsequently diluted in a 50 ml volumetric flask to achieve the desired volume. The mineral's amounts were determined using an Atomic Absorption Spectrophotometer.

The data analysis was conducted using the R program.

Results

Morphological changes due to EMS treatment

Nine morphological traits namely days to first flowering, days to first fruit set, days to first harvest, branch per plant, plant height, fruits per plant, fruit diameter, fruit length, and individual fruit weight were measured to discriminate the performance of different doses of EMS-treatment. The GT biplot analysis was used for the visualization of the association between the morphological traits found among the EMS treatments (Fig. 1). An acute angle between two qualities indicates a positive association, whereas an obtuse angle indicates an opposite relationship (Yan and Reid, 2018). By considering the association between the plant traits, acute angles were observed between control and fruit length and individual fruit weight; 1.2% EMS treatment and fruits/plant; 1.4% EMS treatment and days to first flowering and fruit set and fruit diameter; 1.6% EMS treatment and plant height; 2% EMS treatment and branch/plant.

Based on the nine assessed morphological traits, a correlation matrix was developed (Fig. 2). A strong positive correlation was observed between days to first flowering and individual fruit weight (0.85, r^2 = 0.64) and days to first fruit set (0.79, r^2 =0.51); between individual fruit weight and fruit diameter (0.81, r^2 = 0.55. A moderate correlation was observed between days to first flowering and fruit diameter (0.73, r^2 = 0.38), between plant height and fruits per plant (0.66, r^2 = 0.26), between days to first harvest and individual fruit weight (0.60, r^2 = 0.15), between days to first

flowering and fruit diameter (0.73, $r^2 = 0.38$). On the contrary, a strong negative correlation was identified between first flowering and branch/plant (-0.82, $r^2 = 0.58$); days to first fruit set and fruits/plant (-0.95, $r^2 = 0.87$); days to first harvest and branch/plant (-0.81, $r^2 = 0.55$); individual fruit weight (-0.941, $r^2 = 0.84$) and branch/plant.

Figure 3 illustrates the alterations in morphological characteristics resulting from EMS treatment. The 1.4% EMS treatment showed a detrimental effect on the days to first flowering compared to the control. The other treatments did not show any notable differences. EMS treatment also had a negative impact on days to first fruit set. The mutagenic treatments did not influence the days to first harvest. A notable difference was observed in fruits per plant. Compared to the control, 1.4% and 2% EMS treatments had a negative impact, while 1.6% of EMS treatment performed the best. Individual fruit weight did not change after the EMS treatment application. Not much difference was observed in the branch per plant. A slightly declining trend was observed in 1.4% EMS treatment, while the 2% EMS was better compared to the control. 1.6% EMS was responsible for higher plant height. Fruit diameter did not have any changes, while fruit length was the highest in control.

Biochemical changes due to EMS-treatment

Nine biochemical traits namely total phenol, antioxidant, fruit chlorophyll a and chlorophyll b, lycopene, carotenoid, ascorbic acid, Na, and K content of the fruits were measured to discriminate the performance of different doses of EMS treatment. The GT biplot analysis was used for the visualization of the association between the biochemical traits found among the EMS treatments (Fig. 4). According to Yan and Reid (2018), an acute angle between two qualities indicates a positive association, whereas an obtuse angle indicates an opposite association. By considering the association between the plant biochemical traits, acute angles were observed between control and K content; 1.2% EMS treatment and lycopene and ascorbic acid; 1.4% EMS treatment and chlorophyll a, chlorophyll b, and carotenoid; 1.6% EMS treatment and chlorophyll b; 2% EMS treatment and phenolic content and antioxidant.

A correlation matrix was developed based on the nine assessed biochemical traits (Fig. 5). A strong

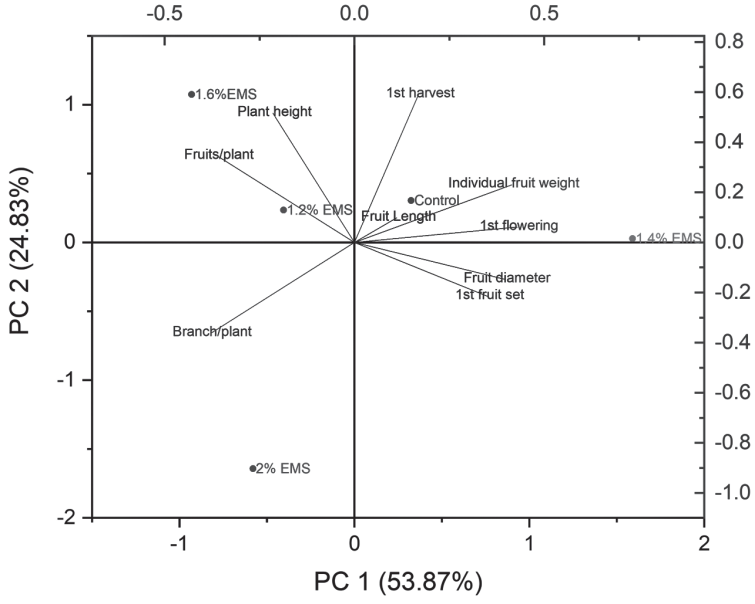

Fig. 1. Biplot of morphological traits and mutagenic treatments. The morphological traits are written in blue, while the EMS treatments are shown in red color.

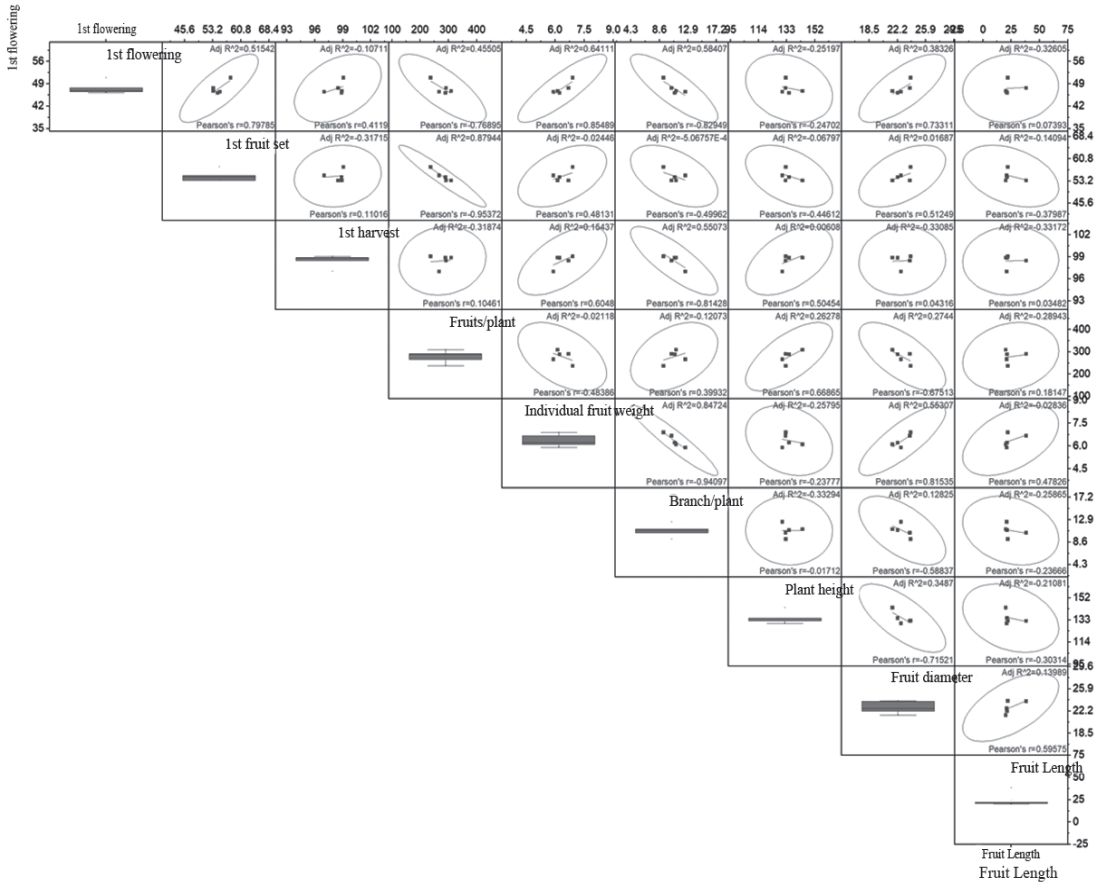

Fig. 2. A graphical representation of the correlation of the studied nine morphological traits.

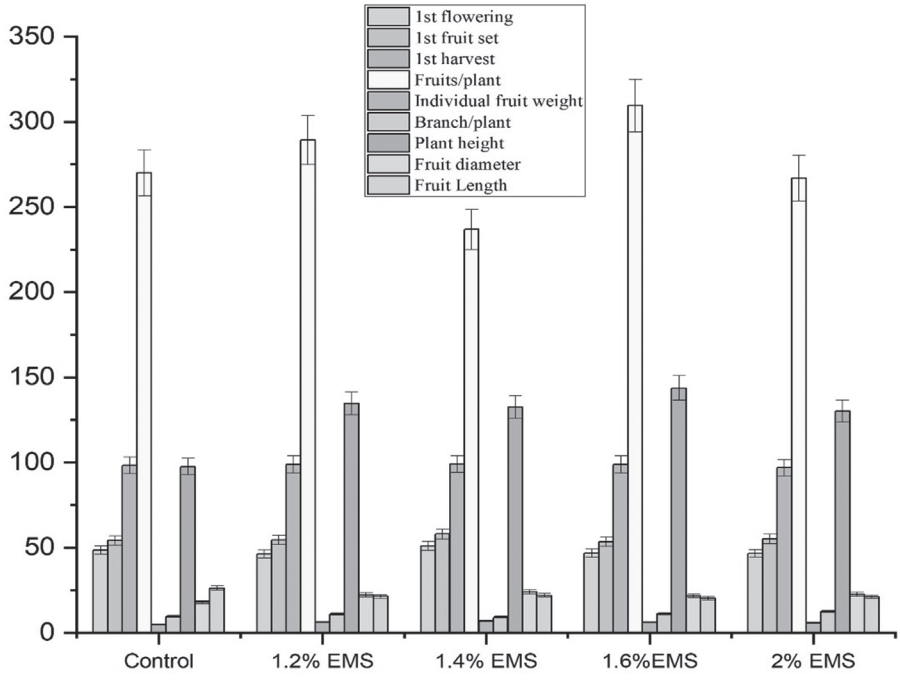

Fig. 3. EMS-treatment induced changes in morphological performances.

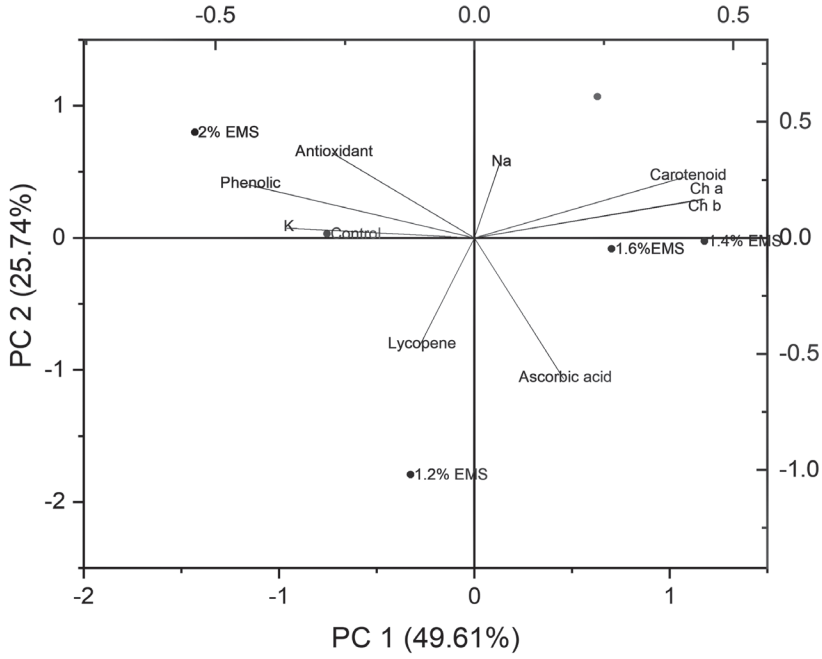

Fig. 4. Biplot of biochemical traits and mutagenic treatments. The biochemical traits are written in blue, while the EMS treatments are shown in red color.

positive correlation was observed between phenolics and antioxidant (0.82, r^2 = 0.57); chlorophyll a and chlorophyll b (0.99, r^2 = 0.99) and carotenoid (0.99, r^2 = 0.98). On the contrary, a strong negative correlation was identified between phenolics and ascorbic acid (-0.81, r^2 = 0.54), chlorophyll b (-0.77, r^2 = 0.47), and carotenoid (-0.73, r^2 = 0.38).

Fig. 6. represents the changes in biochemical traits due to EMS treatment. Compared to the control, Na content did not show any notable changes after the EMS treatment. K content was decreased upto 1.6% EMS treatment, but in 1.6% it was at par with the control. Phenolics did not show much difference in EMS treatment. Antioxidants had a significant change in EMS treatment. Compared to the control, 1.6% EMS treatment had a negative impact of EMS treatment, while 2% EMS treatment performed the best. Ascorbic acid also showed a tremendous change after the EMS treatment. Compared to the control, only 2% of EMS was inferior. The other three treatments performed better than the control. 1.2% EMS performed the best. Lycopene, chlorophyll a, and chlorophyll b did not have notable changes. 1.2% and 1,4% EMS treatment showed better performance in carotenoid when compared with the control.

Discussion

Mutagenesis is a potent and efficient technique for generating genetic diversity, extensively employed for enhancing the traits of plants. Mutation breeding offers the primary benefit of enhancing specific traits in a variety without altering the underlying genetic makeup. To obtain optimal outcomes in mutagenesis, it is necessary to use appropriate concentrations of mutagens. In the present study, the data recorded on the induced mutagenesis by chemical mutagen EMS in cherry tomatoes showed a wide range of variability at the morphological and biochemical levels.

This paper first analyzed the association of morphological traits and EMS doses. Distinct variations were observed. The highest dose, 2% EMS, had a single trait association: branch per plant. The number of branches per plant was greater when the dose was the maximum. However, it did not influence the number of fruits per plant or fruit length

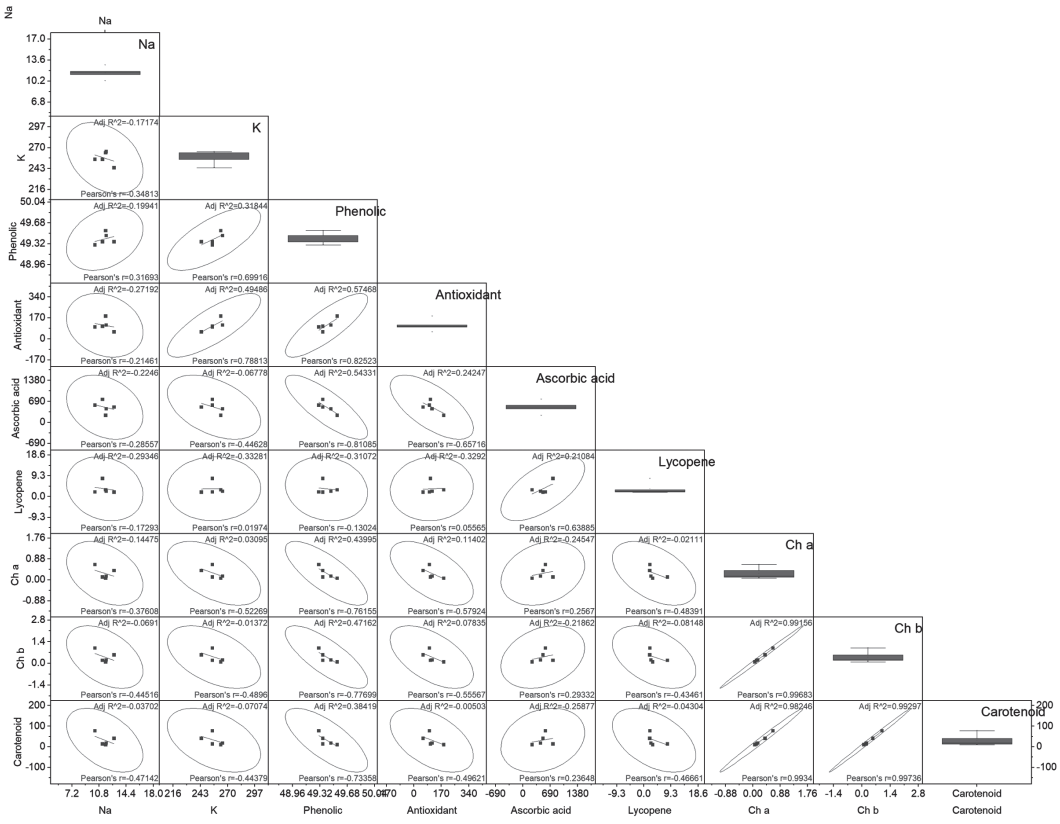

Fig. 5. A graphical representation of the correlation of the studied nine biochemical traits.

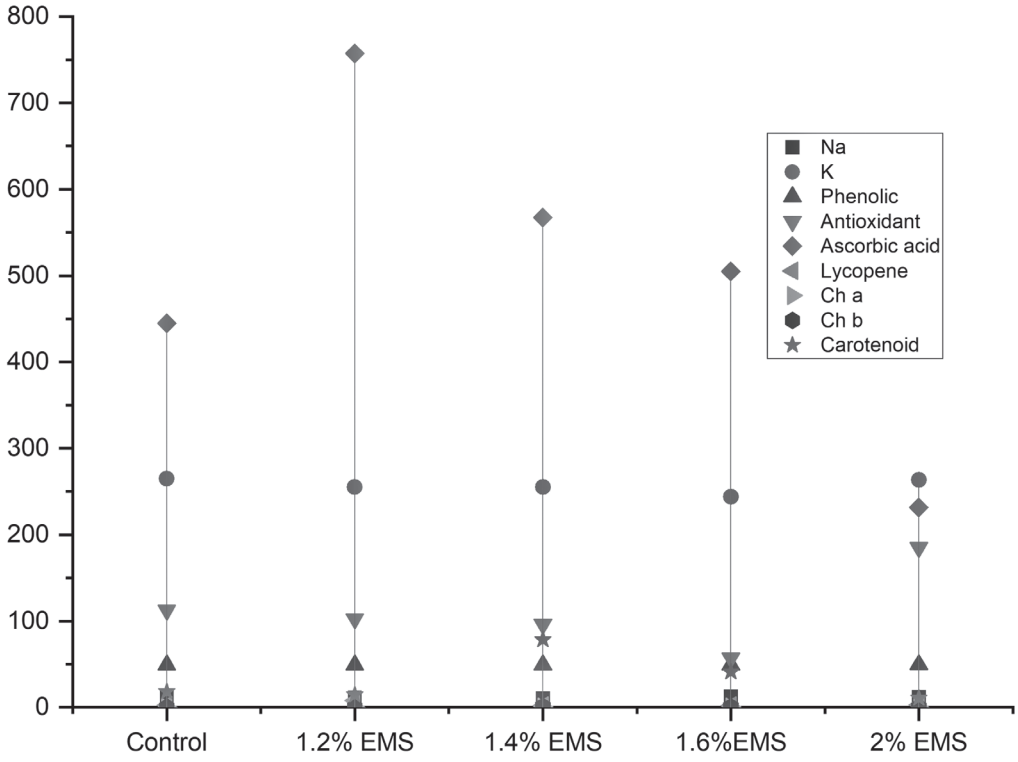

Fig. 6. EMS-treatment induced changes in biochemical performances.

or diameter. It is necessary to optimize the number of branches because an increase in branch numbers might lead to a decrease in the quality of goods owing to competition among branches (Mäkinen *et al.*, 2001; Bak and Karadeniz, 2021). Distinct variations in the morphological characteristics were noted when various doses of EMS were administered. None of the studied morphological traits were associated with more than one EMS dose. 1.4% EMS was associated with traits related to earliness such as days to first flowering and days to first fruit set. Earliness does not correlate with higher fruit yield, nor does it affect the number of fruits per plant or the weight of individual fruits (Gelmesa *et al.*, 2012; Shokat *et al.*, 2015; Hernández-Bautista *et al.*, 2020). 1.2% EMS had a stimulatory effect on the number of fruits per plant.

The association of the traits with the doses can be effectively utilized if the trait correlation studies are analyzed (Amin *et al.*, 2015; Ahmed *et al.*, 2020; Kalpande *et al.*, 2022). The correlation studies in this paper suggested a moderate positive correlation between plant height and the number of fruits per plant. As 1.6% EMS was related to plant height, with the proper selection pressure, the number of fruits per plant can be increased.

The application of EMS treatment might occasionally result in adverse consequences on the morphological characteristics (Dhakshanamoorthy *et al.*, 2010; Kumari *et al.*, 2016; Altindal and Altindal, 2018). When compared to the control, which did not receive EMS treatment, a 1.4% EMS treatment had a detrimental impact on the days to first flowering. Comparable results were noted for the number of fruits produced per plant. Nevertheless, the EMS treatment exhibited a contrasting trend of 1.6% for the number of fruits per plant, so demonstrating the effectiveness of optimizing dosage for different traits. No significant alterations were observed in certain traits when comparing the EMS treatments with the control (i.e. days to first harvest). This is also obvious, as the heritable changes in traits completely depend on the changes in alleles. If the mutation did not change any allelic combination that is responsible for a specific trait, the trait will not show any heritable changes during field trials. On the other hand, the beneficial changes are very important. Regarding the number of branches per plant, the 2% EMS treatment outperformed the control. However, it did not have any beneficial effect on the yield.

Additionally, biochemical analysis was conducted to examine the alterations in traits resulting from EMS treatments. Furthermore, noticeable variations were also detected. The experiment demonstrated a strong correlation between control and the K, whereas the EMS treatments exhibited associations with many other biochemical traits. The changes were prominent. The lycopene and ascorbic acid positively responded to the 1.2% EMS treatment, however, the chlorophyll a, chlorophyll b, and carotenoid concentrations performed better with the 1.4% EMS treatment. Carotenoid is a very important antioxidant in cherry tomatoes. It has been demonstrated that consuming tomatoes and tomato-containing foods can lower the chance of developing chronic illnesses including malignancy and heart disease (Agarwal and Rao, 2000). For chlorophyll b, 1.6% EMS performed well, whereas 2% EMS performed better regarding phenolic and antioxidant content. It was clear that plant genotypes with EMS treatment could have more antioxidant content. Increased antioxidant synthesis aids in the plant's ability to withstand environmental stress. Stress is a common occurrence for plants since they are highly vulnerable to environmental challenges. This results in an oxidative stress that produces more reactive oxygen species (ROS). These reactive oxygen species are essential to oxidative signaling because they trigger a fast and potent reaction that leads to plant sensitization and ultimately, tolerance to a challenging atmosphere. However, reactive oxygen species affects the major cellular components. According to several

studies (Llauradó Maury *et al.*, 2020; Méndez *et al.*, 2021; Fuentes-Cardenas *et al.*, 2022; Islam *et al.*, 2022), crops can control the amount of ROS which helps them avoid oxidative stress nonetheless enables oxidative signaling. Consequently, administering EMS treatment to cherry tomatoes can greatly enhance their defense mechanisms.

The results of the experiment validated the effectiveness of EMS induced mutagenesis as a viable technique for exploring new factors that can enhance yield and yield-related traits, as well as biochemical features. Furthermore, researchers will have access to mutants that contain beneficial characteristics related to crop productivity, allowing for in-depth analysis. Additionally, they will serve as a genetic reservoir for the advancement and enhancement of breeding strategies.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, M.H. and M.A.H.S.; methodology, M.H.; validation, M.H, resources, M.H.; data curation, R.P. and ASMH; writing—preparation of the initial draft, M.H; writing, review and editing, M.H., M.A.H.S, R.P. and A.S.M.H. All authors have reviewed the manuscript in its current form and given their approval.

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