



A Study on the Morphology and Molecular Biology of Tropical Strawberries

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The studies on the morpho-molecular characteristics of different strawberry genotypes were conducted at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. BARI Strawberry 1, BARI Strawberry 2, BARI Strawberry 3, FA 005, and Festival were used for this experiment. This study was undertaken to assess five tropical strawberry genotypes at morphological and molecular level. BARI Strawberry 2 was recognised as the preeminent genotype among five strawberry cultivars based on fruit production per plant (594.73 grammes), yield per

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hectare (19.39 tonnes), and fruit count per plant (32.42). The SSR technique was utilised to assess genetic variation and connections among five strawberry cultivars. Initially, ten primers were evaluated, and finally, three primers—EMFv104, ARSFL-10, and ARSFL-15—were selected for analysis. ARSFL-10 produced the fewest polymorphic alleles (3), while the markers EMFv104 and ARSFL-15 generated the most polymorphism alleles (4). The allele frequency for each location ranged from 0.4 (EMFv104) to 0.6 (ARSFL-10). The PIC values ranged from 0.672 on EMFv104 to 0.4992 on ARSFL-10. EMFv104 and ARSFL-15 demonstrated gene diversity scores of 0.72, whereas ARSFL-10 achieved a value of 0.56. The genotypes demonstrating the minimal genetic dissimilarity were BARI Strawberry 1 and Festival, with a value of 0.16667. Primer EMFv104 and ARSFL-15 are regarded as the most efficacious SSR markers for strawberry genotypes.

Keywords: Markers; SSR; genotyping; yield attributes.

1. INTRODUCTION

The strawberry (*Fragaria xananassa* Duch.), from the Rosaceae family, has traditionally been valued for its flavour and can be ingested fresh, frozen, or processed [1]. More than 20 species of *Fragaria* and countless cultivars are commercially produced in various nations [2]. The cultivars demonstrate considerable diversity in morphological traits [3]. One of the most widely grown berry species is the strawberry, which is grown in nearly every nation, including subtropical and temperate zones as well as high altitude tropical regions [4]. In temperate and subtropical climates, strawberries rank among the most significant fruit crops for fresh consumption and food processing. Varietal trials of strawberry have been conducted in Bangladesh [5], although there is a deficiency of sustainable strawberry cultivars appropriate for the country's climate. Strawberries are rich in essential chemicals, such as ellagic acid, folic acid, coumaric acid, janthomycin, phytosterols, and carotenoid precursors of vitamins A, C, and E [6]. Strawberry is a popular fruit, consumed either fresh or processed into juice, confections, jam, syrup, and wine. Strawberries, relative to other fruits, provide the distinct benefit of early and remarkably high yields per unit area, as the crop is ready for harvest six months after planting (Sharma et al., 2001). 286 tons of strawberries are produced in Bangladesh from 2019 to 2020 [7]. Comprehensive genetic studies on *Fragaria x ananassa* have resulted in the development of multiple molecular marker maps owing to its economic significance. The aim was to create improved cultivars demonstrating superior disease resistance, fruit quality, and other characteristics [8,9]. Strawberry has a base chromosome number of 7, and it naturally exhibits four ploidy levels: diploid, tetraploid, hexaploid, and octoploid [10]. The cultivated strawberry, *Fragaria xananassa*, is an octoploid

($2n = 8x = 56$) and has received considerable focus in molecular and genetic studies [11]. Molecular markers have emerged as essential instruments for assessing genetic diversity and interactions. Molecular markers in strawberries have been developed and employed to define germplasm collections [12] and for genetic mapping [13]. Simple sequence repeats (SSRs) have become the preferred markers for creating linkage maps in the genus *Fragaria* because to their considerable variability, codominance, and prevalence in the genome [8,9]. Bassil et al. [14] found that, Polymorphism was high and the number of presumptive alleles of 13 expressed sequence tag–simple sequence repeats (EST–SSRs) in 70 strawberry cultivars ranged from five to 32 per primer pairs, averaging 16.1.

This study was undertaken with the following objectives, considering the previously mentioned facts:

- To morphologically characterize several strawberry genotypes.
- To determine the genomic characteristics of different strawberry genotypes.

2. MATERIALS AND METHODS

The experiment was conducted at the research facility of the Department of Horticulture and the Department of Genetics at BSMRAU, Gazipur, from December 2017 to April 2018, to investigate the morphological characteristics of several strawberry genotypes, namely Festival (V1), BARI Strawberry 1 (V2), BARI Strawberry 2 (V3), BARI Strawberry 3 (V4), and FA 005 (V5). Of the five genotypes, Festival saplings were produced using tissue culture, whereas the other saplings originated from the mother plant. The experiment was executed via three replications and a

Randomised Complete Block Design (RCBD). Three blocks representing the three replications utilised to partition the entire experimental area. Five strawberry genotypes were randomly assigned in each repetition. The distance between plots was 0.5 meters, and the separation between replications was 0.6 meters. Each unit plot was 1.5 m by 1 m, yielding an area of 1.5 m². The total area of the experiment was 13 m multiplied by 6 m, resulting in 78 m². The aggregate number of plots was fifteen. Strawberry saplings were planted on December 7, 2017. All cow manure and phosphorus fertilisers were utilised in the preparation of the beds. Total nitrogen and potassium were administered in equal portions at 20-day intervals, starting 15 days after planting, during six applications [15]. The requisite intercultural operations were performed as mandated. Data were obtained from three randomly chosen plants in each plot. The STATISTIX 10 program was employed to generate and perform statistical analysis on the data. Following the execution of the Least Significant Difference (LSD) test, a comparison of means was conducted. The experiment was performed in the Advanced Plant Breeding Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur. The genetic investigation included five strawberry genotypes: V1-Festival, V2-BARI Strawberry 1, V3-BARI Strawberry 2, V4-BARI Strawberry 3, and V5-FA005. In the examination of genetic diversity among five genotypes, ten SSR markers (FxaHGA02P13, FxaAGA21F11, EMFv104, EFMvi136, ARSFL-10, ARSFL-11, ARSFL-15, ARSFL-16, EMFn125, EMFn134) demonstrating distinct amplifications were utilised. The altered Cetyl Trimethyl Ammonium Bromide (CTAB) technique was utilised for DNA extraction [16]. The CTAB method is a commonly employed protocol for DNA extraction. PCR was performed utilising 10µl reactions, consisting of 3µl DNA, 1µl of 10X reaction buffer, 2µl of 25 mM MgCl₂, 0.8µl of 25 mM dNTP, 0.5µl of each 10 µM forward and reverse primer, and 0.2µl of Taq DNA polymerase. DNA was transferred from the dilution plate to the PCR plate using a single-channel pipette. Each cycle consists of 45 seconds of initial denaturation at 95°C, 45 seconds of annealing at 55°C, and 1 minute of extension at 72°C, concluding with a final 5 minutes of extension at 72°C after 36 cycles. The PCR product was maintained in the thermal cycler at 15°C when required. The gel was synthesised by dissolving 1.2g of agarose

powder in 80ml of TAE buffer. Subsequently, 3 µl of Ethidium Bromide was added for visualisation purposes. Ethidium bromide was used for its high sensitivity, cost-effectiveness, and reliable DNA visualization, despite its toxicity. The agarose was dissolved in the buffer before being heated to a near-boiling temperature, while avoiding actual boiling. The agarose was allowed to cool sufficiently before pouring the solution into a mould, as high heat could lead to deformation or breakage of the mould. A comb was placed into the cast to create wells for sample loading, and the gel must be completely solidified before usage. The power supply unit was disabled, and the plates were removed from the tank. The agarose gel was carefully removed and positioned in the exposure cabinet of the gel documentation system. The gel was relocated within the exposure box to implement the necessary adjustments. A gel barrier was utilised to preserve the alignment of the gel. The gel image resolution can be adjusted via the camera settings. The gel was exposed to UV light, and the resulting image was saved as a JPEG file. The Power Marker software was employed to calculate the major allele frequency, gene diversity, and polymorphic information content (PIC) for each locus of SSR markers. The genetic distance of each variety was computed, and the neighbor-joining (NJ) tree was utilized to form clusters. The un weighted neighbor-joining tree was generated utilizing DARwin software version 5.0.158.

3. RESULTS AND DISCUSSION

3.1 Strawberry Morphological Analysis Using Many Genotypes

All genotypes exhibited a gradual increase in plant height during their life cycles. Marked variation among strawberry genotypes was observed eighty days after planting. At 80 days, genotype V1 (Festival) had the highest plant height of 23.17 cm, whereas genotype V3 (BARI Strawberry 2) displayed the lowest plant height of 17.67 cm (Fig. 1). The variation in plant height may be ascribed to genetic composition. The plant height recorded in this experiment was partially aligned with the findings of Rahman [17] and Asrey et al. [18], Riyaphan et al. [19] noted significant variability in the height of strawberry plants in Thailand, measuring between 10 and 20 cm during mid-harvest.

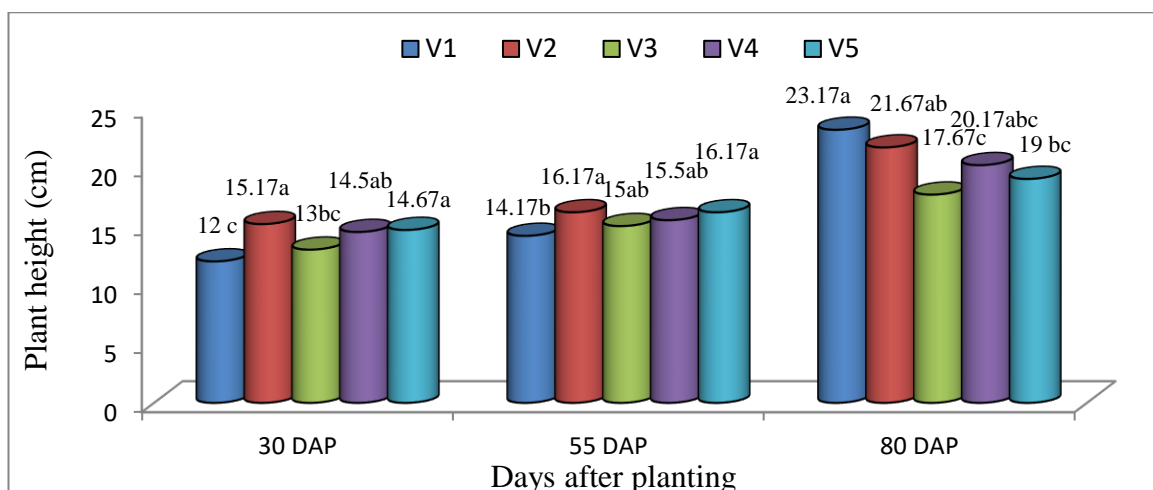


Fig. 1. Different kinds of strawberry plants have different effects on their height (cm) at different days after planting (DAP)

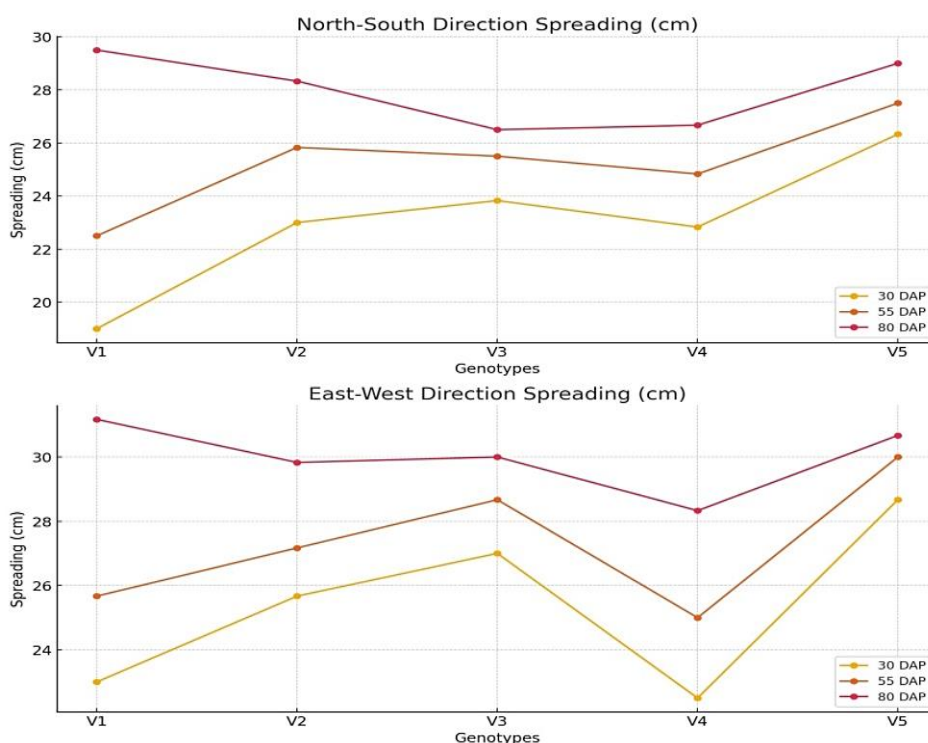


Fig. 2. Influence of strawberry genotypes on plant spread (NS) (cm) in various conditions

At 30 and 55 days after planting, significant genetic variation was seen in the plant canopy in both east-west and north-south orientations. Minimal variation was noted 80 days after planting; at this point, V1 (Festival) demonstrated the most plant canopy in the north-south direction (29.5 cm), while V3 (BARI Strawberry 2) exhibited the least (26.5 cm). In the east-west orientation, V1 (Festival) exhibited the maximum plant canopy at 31.17 cm, whilst V4 (BARI

Strawberry 3) displayed the lowest at 28.33 cm (Fig. 2). The disparity in plant size was ascribed to variations among genotypes. Rahman [17] noted a significant variation in the spread of strawberry plants, ranging from 27.00 to 30.11 cm. Asrey et al. [18] observed significant variability in plant dispersal, supporting the present findings. Thereafter, the plant canopy commenced a steady decline in April. This drop is attributable to a rise in air temperature.

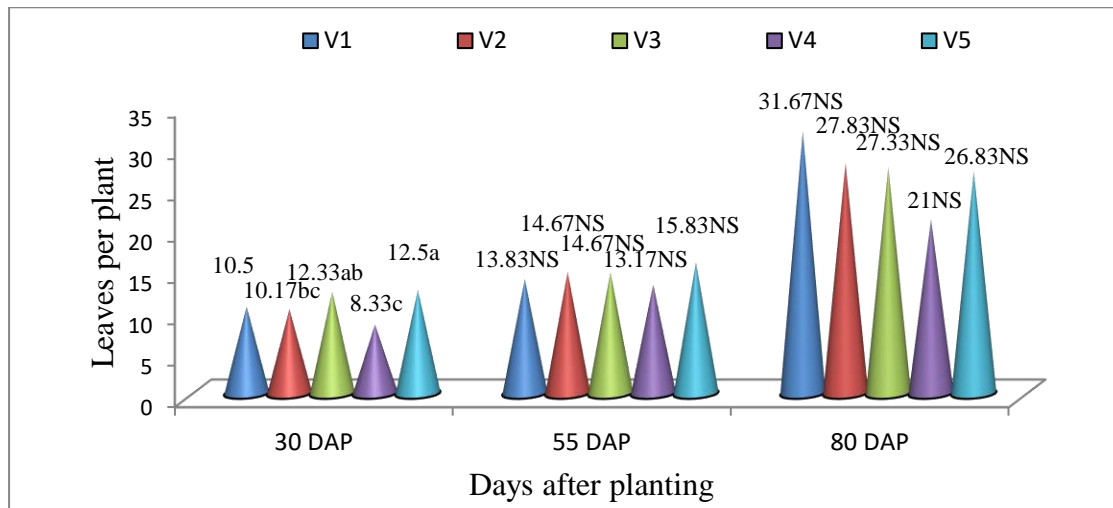


Fig. 3. Strawberry leaf quantity per plant influenced by genotypes at different DAP

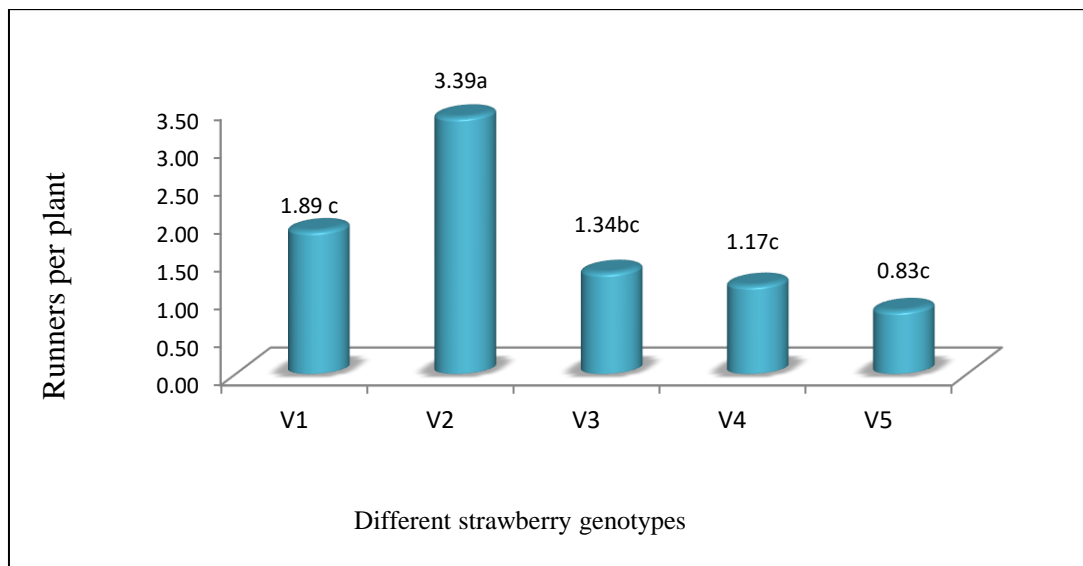


Fig. 4. The quantity of runners produced by each strawberry plant as influenced by genotypes

The quantity of leaves is essential for photosynthesis and exhibits significant diversity. The primary factor contributing to the differences in leaf number per plant among various genotypes is their inherent traits. Thirty days post-planting, genotype V4 (BARI Strawberry 3) exhibited the fewest leaves at 8.33, whereas genotype V5 (FA 005) displayed the most leaves at 12.5, with a statistically significant difference between them. However, after 80 days, genotype V1 (Festival) recorded the highest leaf count at 31.67, while genotype V4 (BARI Strawberry 3) had the lowest at 21 (Fig. 3), with a statistically negligible difference between the two. The findings of the current study were significantly lower than those published by Rahman [17], who observed a range of 60.52 to 49.00 leaves per

plant across different genotypes, as well as the data of leaves per plant documented by Asrey et al. [18]. This may result from the delayed planting of runner seedlings and several environmental factors.

Significant variation across the genotypes was observed in the number of runners produced per plant. Among the genotypes, V2 (BARI Strawberry 1) produced the highest number of runners (3.39), whilst V5 (FA 005) produced the lowest (0.83) (Fig. 4). Sweety [20] detected the greatest quantity of runners from BARI Strawberry 1 in her experiment. This result confirms the findings of Pérez-de-Camacaro et al. [21]. The number of crown plants and runners showed significant diversity across the strawberry cultivars in the United Kingdom.

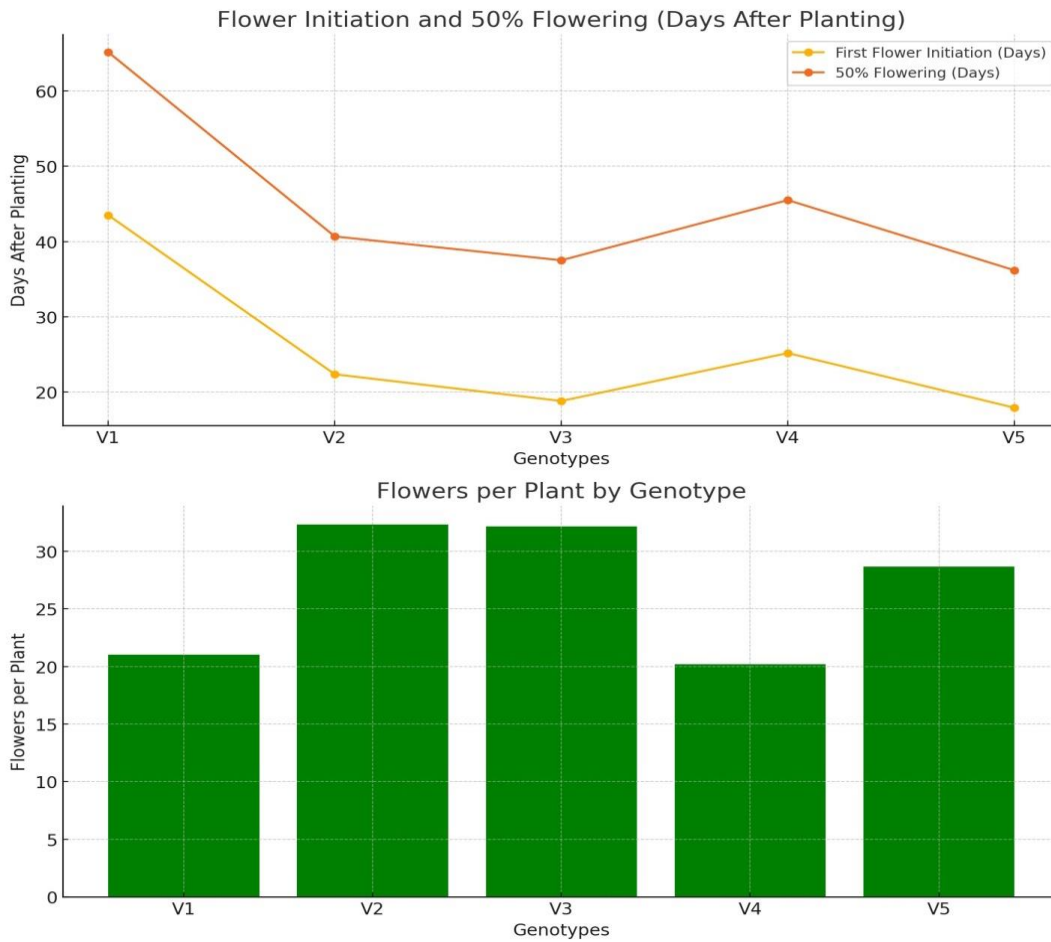


Fig. 5. Floral attributes of various strawberry genotypes

A notable variation was seen among the genotypes about the time to flowering. The genotype V5 (FA 005) displayed the earliest flowering onset at 17.90 days post-planting, whereas the genotype V1 (Festival) exhibited the later initiation at 43.50 days post-planting. The genotypes exhibited significant heterogeneity in the time to reach 50% bloom initiation. The genotype V1 (Festival) required a maximum of 65.17 days for 50% flower initiation, but genotype V5 (FA 005) needed a minimum of 36.17 days, which is statistically similar to genotype V3 (37.50 days). The quantity of flowers per plant exhibited significant variation across the various strawberry varieties. During the trial, V2 (BARI Strawberry 1) exhibited the highest bloom count at 32.33, whilst V4 (BARI Strawberry 3) displayed the lowest count of 20.17, which was statistically comparable to V1 (Festival) with a count of 21.03 (Fig. 5). Michel et al. [22] identified a linear association between the total flower count per plant and the number of inflorescences (trusses). They identified an

average of 25.90 flowers per inflorescence. Sønsteby and Heide [23] reported a diminished number of inflorescences with an augmented quantity of flowers per plant.

Significant variation across the genotypes in the time to initial fruit set was observed. The initial fruit set was recorded in genotype V5 (FA 005) at 41.42 days after planting, whereas the latest occurrence was noted in genotype V1 (Festival) at 68.00 days after planting (Fig. 6). Asrey et al. [18] noted a significant variation in the fruit count per plant among the cultivars, spanning from 25.33 to 40.66. The result corresponds with the present findings. The average yield of harvested fruits per plant exhibited considerable heterogeneity among the genotypes (Fig. 6). The highest yield of fruits (32.42) was recorded from V3 (BARI Strawberry 2), which was statistically similar to V2 (BARI Strawberry 1) (31.98). The genotype V4 (BARI Strawberry 3) produced the lowest fruit yield (19.29). Each genotype displayed notable variations in diameter and

length, presumably attributable to their intrinsic traits (Fig. 6). V5 (FA 005) produced the longest fruits at 5.13 cm, but V2 (BARI Strawberry 1) provided the shortest at 4.19 cm. The fruits from V5 (FA 005) exhibited the thickest portions measuring 3.99 cm and the thinnest at 3.28 cm (BARI Strawberry 1). This outcome is consistent with prior research by Rahman [17] and Asrey et al. [18]. Asrey et al. [18] observed considerable diversity in strawberry fruit size across genotypes, with fruit length spanning from 3.49 to 4.21 cm and fruit width from 2.91 to 3.40 cm among several cultivars. Rahman [17] indicated that the fruit length ranged from 2.17 to 4.26 cm, and the breadth varied from 1.85 to 4.04 cm. A crucial determinant of output in all fruit-bearing plants, including strawberries, is the weight of each individual fruit. The genotypes greatly influenced the fruit weight. V3 (BARI Strawberry 2) produced the heaviest fruits at 21.20 g, while V2 (BARI Strawberry 1) produced the lightest fruit at 14.88 g. Macit et al. [24] observed significant variation in the weight of strawberries in Turkey. The variance in fruit size may be ascribed to differences in cultivar and environmental circumstances. V3 (BARI Strawberry 2) had the maximum production per plant at 594.73g, whilst V4 (BARI Strawberry 3) displayed the lowest yield per plant at 309.62g. The intrinsic characteristics of genotypes may explain the differences in yield per plant. Rahman [17] found that the output per strawberry plant fluctuated significantly, between 442.50 and 129.85 grammes. Crespo [25] indicated that the fruit output per strawberry plant varied significantly across the examined varieties, with ranges of 179.00 to 312.40 g, 640.60 to 656.20 g, and 386.00 to 624.00 g per plant, respectively. The yield of strawberries per plot was significantly affected by genotype. V4 (BARI Strawberry 3) had the lowest fruit production per plot (1.55 kg), whereas V3 (BARI Strawberry 2) produced the most (2.91 kg). Biswas et al. [26] conducted an experiment demonstrating that the yield per plot of five strawberry genotypes varied from 2.25 kg to 9.04 kg, somewhat validating prior findings. Diverse strawberry genotypes markedly affect the yield of fruit per hectare. Genotype V3 (BARI Strawberry 2) attained the maximum yield of 19.39 tonnes per hectare, whereas genotype V4 (BARI Strawberry 3) yielded the minimum at 10.31 tonnes per hectare (Fig. 6). Chandler et al. [27] indicated that the output of three strawberry varieties per hectare ranged from 14.81 to 22.38 tonnes, providing

robust corroboration for the findings of the present study [28].

3.2 Molecular Characterization of Strawberries Utilizing SSR Markers

Across five strawberry genotypes, eleven alleles were found at the locations of three microsatellite markers. There was an average of 3.67 alleles per locus, with a range of 3–4. This information is presented in Fig. 7. In terms of polymorphism allele yield, markers EMFv104 and ARSFL-15 produced the highest number (4) and ARSFL-10 the lowest (3). At each location (ARSFL-10), the frequency of the main allele varied between 0.4 (EMFv104) and 0.6 (ARSFL-15). Calculating the PIC values for each SSR locus, using the alleles generated by each marker as the basis for this research, allowed us to examine polymorphism among the five strawberry genotypes. For ARSFL-10, the PIC value was 0.4992, while for ARSFL-15 and EMFv104, it was 0.672, as shown in Table 1. Anticipated heterozygosity can be measured via gene diversity. Among the five strawberry varieties, the average degree of genetic diversity was 0.56. Fig. 7 shows that the gene diversity values ranged from 0.56 for ARSFL-10 to 0.72 for EMFv104 and ARSFL-15.

The relatedness of strawberry genotypes was assessed using a shared SSR allele dissimilarity matrix. Table 1 displays pairwise genetic dissimilarity estimates varying from 0.16667 to 1. The nearest genotypes detected were BARI Strawberry 1 and Festival, demonstrating a genetic dissimilarity score of 0.16667. The greatest dissimilarity was noted between BARI Strawberry 3 and Festival, BARI Strawberry 1 and BARI Strawberry 3, BARI Strawberry 3 and BARI Strawberry 2, FA005 and Festival, FA005 and BARI Strawberry 1, and FA005 and BARI Strawberry 3, each exhibiting a dissimilarity value of 1. Biswas et al. [26] genotyped thirty-three strawberry accessions utilizing thirty-five SSR markers to observe genetic diversity or relatedness among the accessions studied and phenotyped based on leaf total antioxidant (TA) content and trichome density and they identified 120 alleles with an average of 3.43 alleles per locus. Shannon indices, which are indicative of measure of diversity ranged from 0.1461 for the marker ARSFL_9 to 1.6635 for the marker FG1a/b.

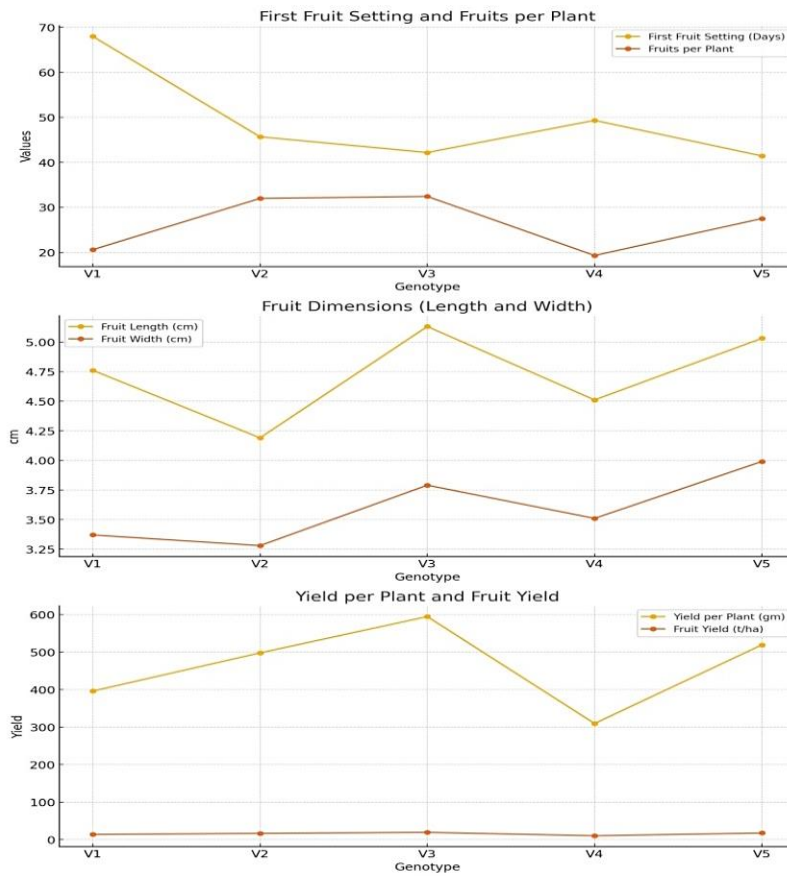


Fig. 6. Yield and yield-related characteristics of diverse strawberry genotypes

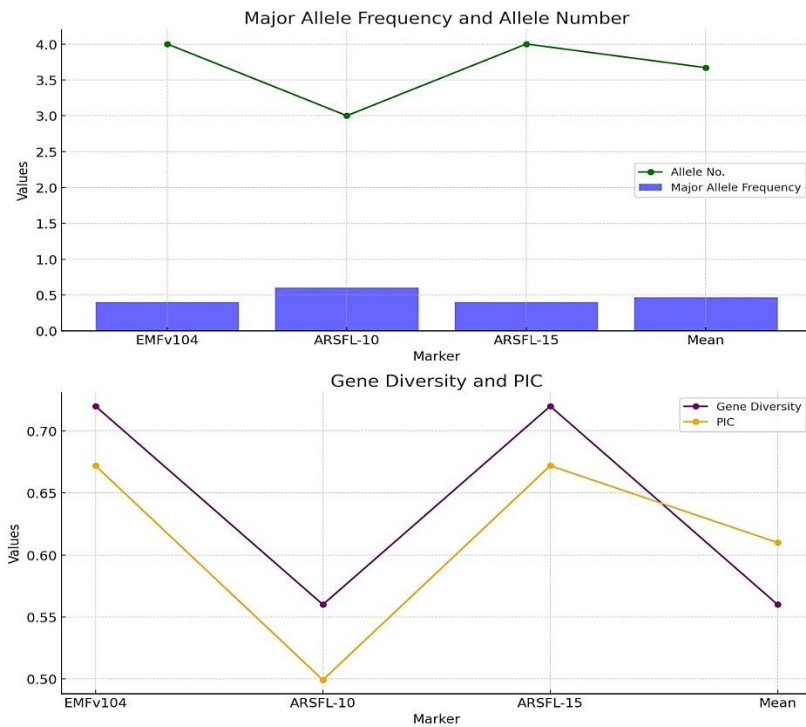


Fig. 7. Evaluation of Polymorphism from SSR Profiles

Table 1. Discordance between five strawberry genotype pairs

Genotypes	V1	V2	V3	V4	V5
V1	0				
V2	0.16667	0			
V3	0.4545	0.4545	0		
V4	1	1	1	0	
V5	1	1	1	0.5	0

Note: Festival (V1), BARI Strawberry 1 (V2), BARI Strawberry 2 (V3), BARI Strawberry 3 (V4), and FA 005 (V5)

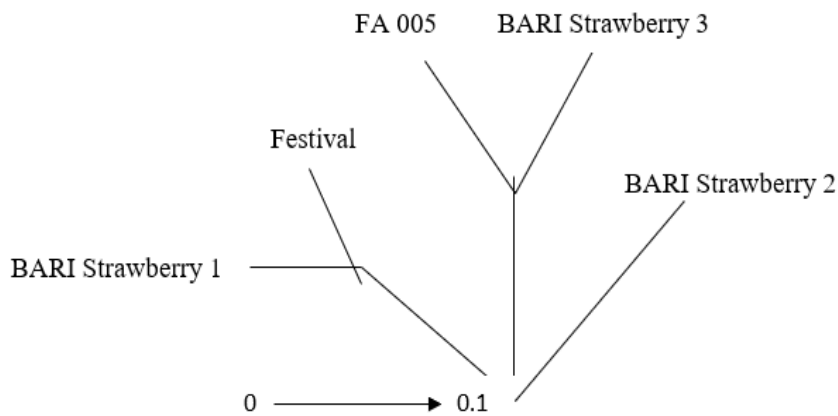


Fig. 8. An un weighted neighbor-joining tree that shows the genetic relationships between five genotypes of strawberries based on alleles found using three SSR markers

The un weighted Neighbor-Joining tree seen in Fig. 8 was produced using DARwin software version 5.0.158, demonstrating the genetic links among the five strawberry genotypes based on 10 SSR primers. Genotypes derived from genetically similar types are classified together. The strawberry genotypes were classified into three main categories: group I, group II, and group III, as seen in Fig. 5. Group I consisted of two genotypes: Festival and BARI Strawberry 1. Group II consisted of two genotypes: BARI Strawberry 3 and FA 005. Group III comprised exclusively BARI Strawberry 2.

The results demonstrate that strawberry genotypes display variation at both morphological and molecular levels. BARI Strawberry 2 and FA 005 were recognised as promising cultivars because to their yield performance in Bangladeshi circumstances. The V3 genotype (BARI Strawberry 2) demonstrated the highest yield according to field performance. This genotype can be cultivated abundantly in Bangladesh. Primer EMFv104 and ARSFL-15 are the most effective SSR markers for strawberry genotypes.

4. CONCLUSION

The current study made an in-depth research on the morphology and molecular features of five genotypes of tropical strawberry cultivated under agro-climatic conditions of Bangladesh. However, in our study, we found that the genotype BARI Strawberry 2 is overall a promising genotypic category (plant and hectare) with high yield performance. This improved performance is regarded as due to the particular morphological characters of this genotype such as stem height, foliage, and fruit size. Similarly, EMFv104 and ARSFL-15 were also found to be useful as genetic markers due to the ability of SSR markers in estimating genetic differences among strawberry genotypes used for the study. Genetic dissimilarity analysis revealed that BARI Strawberry 1 is relatively less distant from Festival, which can serve as potential crossing material in future strawberry breeding programs. The study shows that genotypes such as BARI Strawberry 2 have the potential to adapt well locally and at the same time meet high productivity, which could be helpful in expanding the cultivation of strawberry even in tropical

climates. The identified SSR markers can be used for debugging, diversity analysis, and further MAS to develop new cultivars of the strawberry with desirable traits. The results of this study could be used as a basis for giving scientific recommendations on the production of strawberries in regions like Bangladesh.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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