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Morphological and Molecular Characterization of Tropical Strawberry

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Key words: Yield, Gene diversity, Simple sequence repeat marker, Major allele frequency

Abstract

The experiment was conducted to assess five tropical strawberry genotypes at phenotypic and molecular level. Among the five strawberry genotypes (BARI Strawberry 1, BARI Strawberry 2, BARI Strawberry 3, FA 005 and Festival), BARI Strawberry 2 was found to be the best in respect of fruit per plant (32.42), fruit yield per plant (594.73 gm) and yield per hectare (19.39 ton). Ten SSR primers were initially screened for molecular characterization and finally MFv104, ARSFL-10 and ARSFL-15 markers were selected for the analysis. EMFv104 and ARSFL-15 produced the maximum number of polymorphic alleles (4) while ARSFL-10 produced three polymorphic alleles. The major allele frequency at each locus ranged from 0.4 (EMFv104) to 0.6 (ARSFL-10). The PIC values varied from 0.4992 on ARSFL-10 to 0.672 on EMFv104. The gene diversity ranged from 0.56 (ARSFL-10) to 0.72 (EMFv104 and ARSFL-15). BARI Strawberry 1 and Festival were the closest genotypes with the lowest genetic dissimilarity value of 0.16667. EMFv104 and ARSFL-15 can be used as polymorphic markers for assessing genetic diversity of different strawberry genotypes.

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a member of the Rosaceae family and it has traditionally been a popular delicious fruit for its flavor, taste, fresh use, freezing and processing (Sharma 2002). Strawberry is one of the most important fruit plants for both fresh consumption and food processing in the temperate and subtropical areas. It is rich in various life saving proteins as well as carotene precursor of vitamin A, vitamin C, vitamin E, alagic acid, folic acid, kumaric acid, janthomycin and phytosterol (Anon. 2008).

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The fruit can be eaten raw or used in making juice, desserts, jam, syrup and wine. Due to its economic importance, *Fragaria* × *ananassa* has been the subject of much genetic research aimed at developing superior cultivars with enhanced disease resistance, fruit quality, and other characters, prompting the development of a number of molecular marker maps for this species (Rousseau-Gueutin et al. 2008, Sargent et al. 2009). The cultivated strawberry, *Fragaria* × *ananassa*, is an octoploid (2n = 8x = 56) and has been the focus of an increasing number of molecular and genetic studies (Folta and Devis 2006). Molecular markers have been developed into powerful tools to analyze genetic relationships and genetic diversity. Molecular markers in strawberry have been developed and used to characterize germplasm collections (Gil-Ariza et al. 2009) and for genetic mapping (Spigler et al. 2008). Simple sequence repeats (SSRs) have been the marker of choice for different crops (Huda et al. 2019) including the genus *Fragaria* for diversity analysis, and linkage map development (Rousseau-Gueutin et al. 2008, Sargent et al. 2009) due to their abundance in the genome, their co-dominant and highly polymorphic nature.

Several breeding efforts have been taken to develop new varieties with enhanced taste and flavor or extended harvest period and shelf life improvement (Capocasa et al. 2008, Faedi et al. 2000). The prerequisite to the success of these breeding program is to have the accessibility to variable genetic resources. Accordingly, it is imperative to assess the available plant genetic resources for effective development and management of new cultivars. Generally the assessment and evaluation is carried out based on the morphological traits such as the structure of leaves and flowers, fruit characteristics, and flowering habit (Nielsen and Lovell 2000). Yet, the morphological methods are dependent on the environmental conditions. Hence, molecular markers were developed with a view to assessing the genetic variability, linkage mapping, and identification of different crop genotypes (Chambers et al. 2013, Govan et al. 2008, Isobe et al. 2013).

SSR markers for strawberry were first developed using primer pairs that amplified characterized regions such as expressed sequence tags (ESTs) or genomic libraries, in contrast to other primer pairs that amplified anonymous DNA fragments (Monfort et al. 2006, Sargent et al. 2009). The completion of the genome sequence of diploid *F. vesca* (Shulaev et al. 2011) allowed robust SSRs to be developed and mapped on the Fragaria reference map (Rousseau-Gueutin et al. 2011, Sargent et al. 2011, Zorrilla-Fontanesi et al. 2011). In present study, authors characterized five tropical strawberry genotypes at the phenotypic and molecular level. The genotyping results will provide a molecular basis for future breeding programs and will facilitate the development of novel strawberry cultivars with increased genetic diversity.

Materials and Methods

The experiment was laid out in a randomized complete block design (RCBD) with three replications. Five strawberry genotypes, namely Festival (V_1), BARI Strawberry 1 (V_2), BARI Strawberry 2 (V_3), BARI Strawberry 3 (V_4) and FA 005 (V_5) were included in the

experiment. Among the five genotypes, Festival saplings were grown by tissue culture while the rest of the saplings were grown from the mother plant. Necessary intercultural operations were done as and when required. The data were recorded from randomly selected three plants from each plot. Fifteen morphological traits, i.e. plant height, plant spread, number of leaves per plant, runner number per plant, first flower initiation, days to 50% flowering, flowers per plant, days to first fruit setting, fruits per plant, fruit length, fruit width, fruit weight, yield per plant, fruit yield per plot, fruit yield, were recorded. The recorded data were compiled and analyzed statistically by using STATISTIX 10 program. The mean comparison was done following the least significant difference test (LSD).

Molecular characterization

Five strawberry genotypes, V_1 - Festival, V_2 - BARI Strawberry 1, V_3 - BARI Strawberry 2, V_4 - BARI Strawberry 3 and V_5 - FA 005, were characterized at the molecular level. Ten SSR markers (FxaHGA02P13, FxaAGA21F11, EMFv104, EFMvi136, ARSFL-10, ARSFL-11, ARSFL-15, ARSFL-16, EMFn125, EMFn134) were initially selected for the study. Three primers (EMFv104, ARSFL-10 and ARSFL-15) with clear polymorphic amplifications were finally included in the analysis.

DNA was extracted following modified cetyl trimethyl ammonium bromide (CTAB) method (Ferrari et al. 2007). CTAB is a standard method for DNA extraction which is followed in most cases. PCR was performed in 10 μl reactions containing 3 μl DNA, 1 μl 10X reaction buffer, 2 μl 25 mM $MgCl^2$, 0.8μl of 25 mM dNTP, 0.5 μl each of 10 μM forward and reverse primers and 0.2 μl of Taq DNA polymerase.

After initial denaturation for 5 min at 95°C, each cycle comprises 45 sec denaturation at 95°C, 45 sec annealing at 55°C, and 1 min extension at 72°C with a final extension for 5 min at 72°C at the end of 36 cycles. The PCR product was preserved at 15°C in the thermal cycler in case of necessity. The gel was prepared by dissolving the 1.2 g agarose powder in 80 ml TAE buffer. The gel was exposed to UV light and the gel image was saved as a jpeg file.

Major allele frequency, gene diversity and polymorphic information content (PIC) for each locus of SSR markers were calculated using Power Marker software. Genetic distance was calculated for each variety, which was used for cluster development using neighbor-joining (NJ) tree. The un-weighted neighbor joining tree was constructed using DARwin software 5.0.158.

Results and Discussion

Morphological characterization of strawberry influenced by different genotypes

Regarding the plant height, all the genotypes showed increasing trend over all throughout their life cycle. Significant variation was observed among different strawberry genotypes at 80 days after planting. At 80 days, the highest plant height

showed by the genotype V_1 (Festival) was 23.17 cm and the lowest plant height of 17.67 cm was recorded from V_3 (BARI Strawberry 2)

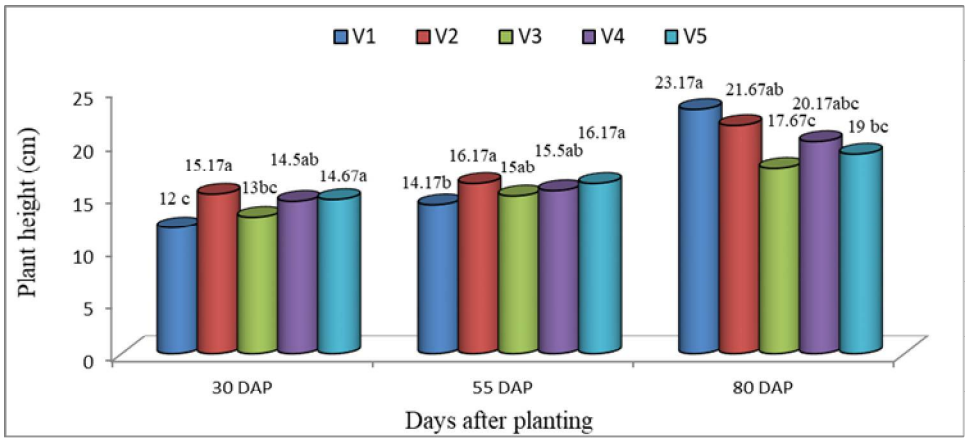

Fig. 1. Plant height (cm) of strawberry as influenced by different genotypes at different days after planting. V_1 = Festival, V_2 = BARI Strawberry 1, V_3 = BARI Strawberry 2, V_4 = BARI Strawberry 3, V_5 = FA 005.

Significant variation among the genotypes was observed at 30 and 55 days after planting for plant canopy both in north-south and east-west direction. But insignificant variation was observed at 80 days after planting and that time, the highest plant canopy in north-south direction (29.5 cm) was recorded in V_1 (Festival) and the lowest (26.5 cm) in V_3 (BARI Strawberry 2) and the highest plant canopy in east-west direction (31.17 cm) was recorded by V_1 (Festival) and the lowest (28.33 cm) was found from V_4 (BARI Strawberry 3) (Table 1). The variation in plant size was due to the variation in genotypes.

Table 1. Plant spread (cm) as influenced by the genotypes of strawberry at different days after planting.

Genotypes	Spreading (cm)					
	North-South direction			East-West direction		
	30 DAP	55 DAP	80 DAP	30 DAP	55 DAP	80 DAP
V_1	19 c	22.50 c	29.50 a	23 b	25.67 bc	31.17 a
V_2	23 b	25.83 ab	28.33 a	25.67 ab	27.17 abc	29.83 ab
V_3	23.83 ab	25.5 ab	26.50 a	27 a	28.67 ab	30 ab
V_4	22.83 b	24.83 bc	26.67 a	22.5 b	25 c	28.33 b
V_5	26.33 a	27.50 ab	29 a	28.67 a	30 a	30.67 ab
Level of significance	**	**	NS	**	**	NS
CV (%)	7.47	5.01	6.57	6.94	5.91	4.24

Means bearing same letter(s) in a column do not differ significantly at 1% level of probability. NS - Non significant.

Leaf number is important for photosynthesis and it differed significantly. The leaf number per plant in different genotypes varied mainly due to inherent characters of the genotypes. On 30 days after planting the minimum no. of leaves were found 8.33 by the genotype V_4 (BARI Strawberry 3) and the maximum was observed 12.5 by the genotype V_5 (FA 005) where the difference was statistically significant. But, on 80 days of planting, the maximum number of leaves were counted 31.67 by the genotype V_1 (Festival) and the lowest number was counted 21 by the genotype V_4 (BARI Strawberry 3) (Fig. 2) and this variation was statistically insignificant.

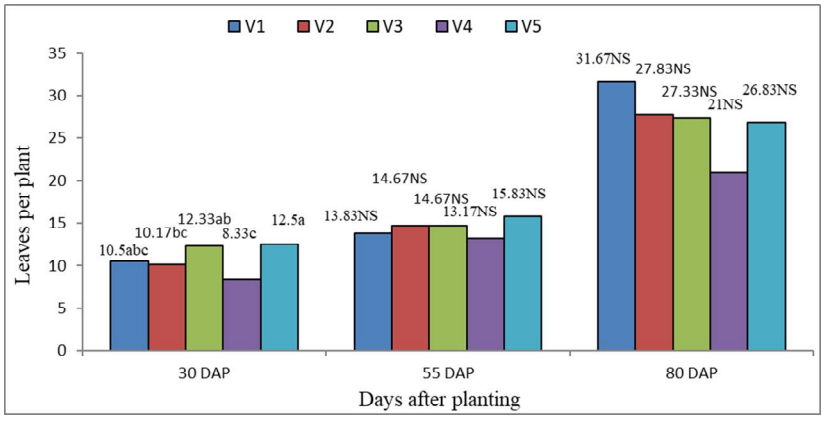

Fig. 2. Leaves per plant of strawberry as influenced by genotypes at different days after planting. V_1 = Festival, V_2 = BARI Strawberry 1, V_3 = BARI Strawberry 2, V_4 = BARI Strawberry 3, V_5 = FA 005.

Significant variation at the 1% level of significance among the genotypes was observed in respect of runner number per plant. Among the genotypes V_2 (BARI Strawberry 1) produced the highest number of runner (3.39) while V_5 (FA 005) produced the lowest runner (0.83) (Fig. 3).

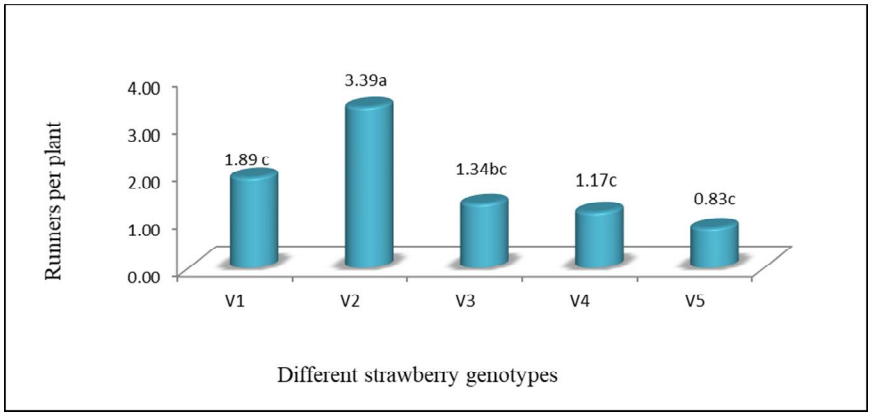

Fig. 3. Runners per plant of strawberry as influenced by genotypes. V_1 = Festival, V_2 = BARI Strawberry 1, V_3 = BARI Strawberry 2, V_4 = BARI Strawberry 3, V_5 = FA 005.

Significant variation at the 1% level of significance among the genotypes in relation to days to flowering was observed. The first flowering initiation was recorded earliest in the genotype V_5 (FA 005) which was 17.90 days of planting while the most lately was found 43.50 days of planting by the genotype V_1 (Festival). Days to 50% flower initiation differed significantly among the genotypes. The maximum days required for 50% flower initiation by the genotype V_1 (Festival) was 65.17 and the minimum days required for 50% flower initiation by the genotype V_5 (FA 005) was 36.17 which was statistically similar with the genotype V_3 (37.50). Significant variation was also observed in number of flowers per plant among different strawberry genotypes. During the study period, the highest number of flowers per plant (32.33) was observed in V_2 (BARI Strawberry 1) while the lowest were 20.17 in V_4 (BARI Strawberry 3), which was statistically similar with 21.03 in V_1 (Festival) (Table 2).

Table 2. Table of floral characteristics of different strawberry genotypes.

Genotypes	First flower initiation (DAP)	50% flowering (DAP)	Flowers per plant
V_1	43.50 a	65.17 a	21.03 c
V_2	22.35 c	40.67 c	32.33 a
V_3	18.80 d	37.50 d	32.13 a
V_4	25.17 b	45.50 b	20.17 c
V_5	17.90 d	36.17 d	28.67 b
Level of significance	**	**	**
CV (%)	3.67	3.27	6.46

Means bearing same letter(s) in a column do not differ significantly at 1% level of probability.

Significant variation at the 1% level of significance among the genotypes in relation to days to first fruit setting was observed. The first fruit setting was recorded earliest in the genotype V_5 (FA 005) which was 41.42 days of planting while the most lately was found 68.00 days of planting by the genotype V_1 (Festival) (Table 3).

Average number of harvested fruits per plant exhibited wide range of variation among the genotypes (Table 3). The highest number of fruits (32.42) was harvested from V_3 (BARI Strawberry 2), which was statistically similar with V_2 (BARI Strawberry 1) (31.98). The lowest number of fruits (19.29) was produced by the genotype V_4 (BARI Strawberry 3). In respect of length and diameter, all the genotypes varied significantly probably due to the inherent characters of genotype (Table 3). The longest fruits (5.13 cm) were produced by V_5 (FA 005) and the shortest fruits (4.19 cm) were produced by V_2 (BARI Strawberry 1). The thickest (3.99 cm) fruit was found in V_5 (FA

005) and the thinnest (3.28 cm) in V_2 (BARI Strawberry 1). Individual fruit weight is one of the most important yield contributing characters in all fruits plants including strawberry. The genotypes significantly influenced the fruit weight. The heaviest fruits (21.20 g) were produced by V_3 (BARI Strawberry 2) while the lightest fruit was found 14.88 g in V_2 (BARI Strawberry 1). The highest yield/plant (594.73g) was recorded from V_3 (BARI Strawberry 2) and the lowest yield/plant was 309.62g fruits/plant recorded by the V_4 (BARI Strawberry 3). The variation in yield/plant was might be due to the inherent character of the genotypes. The genotype exerted significant influence on the strawberry yield per plot. The V_3 (BARI Strawberry 2) produced the highest fruit per plot which was 2.91 kg and the lowest (1.55 kg) was produced by the V_4 (BARI Strawberry 3). Fruit yield per hectare was significantly influenced at the 1% level of significance by different strawberry genotypes. The genotype V_3 (BARI Strawberry 2) produced the highest strawberry yield of 19.39 ton per hectare and the lowest was harvested by the genotype V_4 (BARI Strawberry 3) which was 10.31 ton per hectare (Table 3).

Table 3. Yield and yield contributing characteristics of different strawberry genotypes.

Genotype	First fruit setting (DAP)	Fruits/ plant	Fruit length (cm)	Fruit width (cm)	Fruit weight (gm)	Yield/ plant (gm)	Fruit yield/ plot (kg)	Fruit yield (t/ha)
V_1	68.00 a	20.58 b	4.76 ab	3.37 c	18.62 ab	396.18 c	2.06 c	13.73 c
V_2	45.67 c	31.98 a	4.19 c	3.28 c	14.88 c	498.00 b	2.50 b	16.69 b
V_3	42.17 d	32.42 a	5.13 a	3.79 ab	21.20 a	594.73 a	2.91 a	19.39 a
V_4	49.33 d	19.29 b	4.51 bc	3.51 bc	16.04 bc	309.62 d	1.55 d	10.31d
V_5	41.42 d	27.50 a	5.03 ab	3.99 a	19.71 a	519.34 b	2.64 ab	17.60 ab
Level of significance	**	**	**	**	**	**	**	**
CV (%)	3.06	10.19	5.98	5.50	9.79	5.91	7.69	7.69

Means bearing same letter (s) in a column do not differ significantly at 1% level of probability. NS- Non Significant.

Considering all the above mentioned morphological attributes, BARI Strawberry 2 performed the best among the studied genotypes. It showed the best performance in all the yield and yield-related characters except the first fruit setting and the fruit width. It had the highest number of fruits, individual fruit weight, which is one of the most important yield contributing characters, yield per plant (gm), fruit yield per plot (Kg), and also fruit yield per hectare.

Molecular characterization of strawberry using SSR markers

A total of 11 alleles were detected at the loci of 3 microsatellite markers across 5 strawberry genotypes. The allele number per locus varied from 3 to 4, with an average of 3.67 alleles (Table 4). The marker EMFv104 and ARSFL-15 produced the highest number of polymorphic alleles (4) while ARSFL-10 produced the least number of polymorphic alleles (3). The frequency of the major allele at each locus ranged from 0.4 (EMFv104) and

ARSFL-15 to 0.6 (ARSFL-10). The level of polymorphism among the 5 strawberry genotypes was evaluated by calculating PIC values for each SSR locus. This calculation was based on the alleles produced by each marker. The PIC values varied from 0.4992 on ARSFL-10 to 0.672 on ARSFL-15 and EMFv104 (Table 4).

Gene diversity is the measure of expected heterozygosity. The average gene diversity among the 5 strawberry genotypes was 0.56. The gene diversity values ranged from 0.56 (ARSFL-10) to 0.72 (EMFv104 and ARSFL-15) as shown in Table 4.

Table 4. Assessment of polymorphism from SSR profiles.

Marker	Major allele frequency	Allele No.	Gene diversity	PIC
EMFv104	0.4	4	0.72	0.672
ARSFL-10	0.6	3	0.56	0.4992
ARSFL-15	0.4	4	0.72	0.672
Mean	0.47	3.67	0.56	0.61

A dissimilarity matrix shared SSR alleles were used to determine the level of relatedness among the strawberry genotypes. Pair-wise genetic dissimilarity estimates ranged from 0.16667 to 1 is shown in Table 5. It was found that BARI Strawberry 1 and Festival was the closest genotypes with the lowest genetic dissimilarity value of 0.16667.

On the other hand, the highest level of dissimilarity was observed between BARI Strawberry 3 and Festival, BARI Strawberry 1 and BARI Strawberry 3, BARI strawberry 3 and BARI Strawberry 2, FA005 and Festival, FA 005 and BARI Strawberry 1, FA 005 and BARI Strawberry 3 genotypes with a dissimilarity index of 1.

Table 5. Dissimilarity matrix among pairs of five strawberry genotypes.

Genotypes	Festival	BARI Strawberry 1	BARI Strawberry 2	BARI Strawberry 3	FA 005
Festival	0				
BARI Strawberry 1	0.16667	0			
BARI Strawberry 2	0.4545	0.4545	0		
BARI Strawberry 3	1	1	1	0	
FA 005	1	1	1	0.5	0

Dendrogram presented in Fig. 4 was constructed using DARwin software 5.0.158 which revealed genetic relatedness among the 5 strawberry genotypes using three SSR primers. Genotypes that are derivatives of genetically similar types clustered together. The strawberry genotypes were clustered into three major groups, i.e., group I, group II and group III as shown in Fig. 4. Group I consisted of 2 genotypes: Festival and BARI Strawberry 1. Group II consisted of 2 genotypes: BARI Strawberry 3 and FA 005. Group III comprised of only one genotype, BARI Strawberry 2.

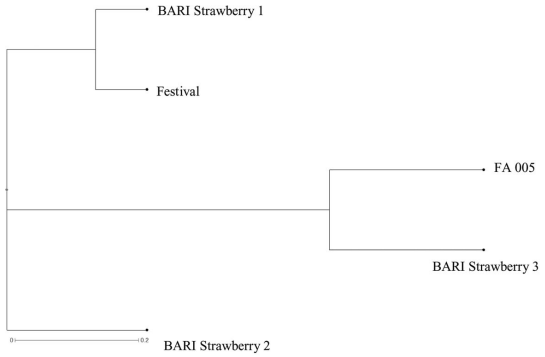

Fig. 4. Dendrogram showing the genetic relationships among 5 strawberry genotypes based on the alleles detected by three SSR markers.

Based on the findings, it may be concluded that the genotypes of strawberry showed variation in morphological as well as molecular level. On the basis of yield BARI Strawberry 2 and FA005 were found to be promising under Bangladesh condition. The genotype, BARI Strawberry 2 was the highest yielder on the basis of the field performance. So, this genotype can be cultivated widely in Bangladesh or new variety can be developed using this genotype as parent for better agronomic characteristics. Primer EMFv104 and ARSFL-15 can be used as polymorphic markers for assessing genetic diversity of different strawberry genotypes.

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