
Assessment of Field Performance and Genetic Diversity Analysis of Tissue Culture Variants of Strawberry

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Abstract The present experiment was conducted to select suitable high yielded stable strawberry variants through micro propagation. Reproducible protocols for *in vitro* proliferation of plantlets through shoot tip and nodal segment explants had shown significant variation in callus induction as influenced by BAP 1.0 mg L⁻¹. The concentration of BAP exhibited significant influence on the percentage of callus induction. The regenerated shoots were cultured on shoot induction media containing different combined concentrations of BAP and NAA 1.0 BAP and 0.5 mg L⁻¹ and those were sub-cultured on MS medium supplemented with combination of BAP 1.5 mg L⁻¹ and IBA 1.0 mg L⁻¹ in order to allow root. Among the tissue culture variants of strawberry giving emphasis on key yield parameter e.g. petiole length, days to flowering, number of fruits plant⁻¹, % brix and weight of individual fruit tissue culture variants G-9, G-10 and G-11 were selected as superior variants for cultivation across all the environments. Significant variation among the variants was found for all the characters. It was found that yield plant⁻¹ was positively correlated with petiole length, days to opening of first flower, number of fruits plant⁻¹, % brix and weight of individual fruit. Path analysis revealed that petiole length, crown spread plant⁻¹, pollen sterility, no. of fruits plant⁻¹, ascorbic acid content of individual fruit and weight of individual fruit had positive direct effects on fruit yield plant⁻¹. The genotypes were grouped into three clusters based on Euclidean distance following Ward's method and highest intra cluster distance was found in cluster III and inter cluster distance was observed between genotypes of cluster I and III.

Keywords: Strawberry, micro propagation, tissue culture variants, co-relation of coefficient, path analysis, diversity analysis

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Introduction

Strawberry (*Fragaria x ananassa* Duch.) is one of the most important fruit plants for both fresh consumption and food processing in the temperate and subtropical areas, with a global production of over 4.1 million tons and a production area of about 255000 ha (FAO, 2008). The United States is the world's largest producer of strawberries, accounting for 28% of world supply. At a distant second, Spain accounted for 8%, followed by Russia, Korea, Japan and Poland accounting for 5 to 6% each (FAOSTAT, 2007). In Bangladesh there is no statistics about the area and production of this crop, since it has recently been introduced into the country. But there has been a bright prospect of farming strawberry, a high-value crop, everywhere in the country except the coastal districts because it gives early and very high returns per unit area compared to other fruits because the crop is ready to harvest within six months after planting.

Regeneration protocols of strawberry are species specific to their regeneration capacity (Passey *et al.*, 2003). Selection of the proper hormone combination, explants, and cultivar are the keys of successful regeneration of strawberry (Barcelo, 1998; Jimenez-Bermudez, 2002). Several reports indicated the possibility of in vitro regeneration of strawberry microplants via callus or cell suspension culture or another culture (Stvensson and Johansson, 1994). Different hormonal combinations and shoot tips explant sources influence the number of regenerated plants (Adak *et al.*, 2001). A pretreatment in darkness is vital for callus induction and plantlet regeneration (Popescu *et al.*, 1997). Therefore, regeneration of strawberry is influenced by explants, hormonal combinations, light and season of the crop growth. The effectiveness of increasing yield depends on the extent of variability in yield that controlled by genetic factor. Information on correlation coefficient between yield and its contributing characters has always been helpful as a basis for selection for yield in a breeding program. Thus, determination of correlation between the characters are a matter of considerable importance in selection practices, since it helps in construction of selection indices and also permit the prediction of correlated response.

The present study was planned to assess their field performance for commercial cultivation and to analyze diversity for further breeding programs. The objectives of study were to assess the field performance of strawberry somaclones and to analyze the genetic variation, relationship and stability of strawberry somaclones.

Material and methods

The planting material (shoot tip and nodal segment) of *Fragaria x ananassa* Duch. were collected from previous cultured superior eighteen plant materials, from Department of Genetics and Plant Breeding, Bangladesh Agricultural University, for the establishment of culture.

In vitro propagation of strawberry: Shoot tip and nodal segments were collected from 2 weeks old runners from eighteen superior variants, then sterilized and cultured onto the medium recommended by Boxus, 1999 supplemented Indolebutyric acid (IBA 1.0 mg L⁻¹), α -naphthaleneacetic acid (NAA 0.5 mg L⁻¹), 6- Benzylamino purine (BAP 1.0 and 1.5 mg L⁻¹), glucose (40.0 g dm⁻³) and Bacto-Difco agar (6.4 g dm⁻³), Afterwards, formation and performed better variants. Culture medium attached to the roots was gently washed out with running tap water. The plantlets were then transplanted into polybags contains potting mixture with garden soil, sand and cow dung in the ratio of 1:2:1. Immediately after transplantation, the plants along with the poly bags covered with moist polythene bag to prevent desiccation. After two to three days, the polythene bags were perforated to expose the plants to natural environment. After proper hardening of the variants that were used for field assessment.

Then the fifteen to twenty days seedlings of the eighteen variants were sown on 10 November 2012 at the experimental Farm, Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Mymensingh. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The plot size was 4m x 2.7m with 8 rows. The distance regarding block to block was 1m, plot to plot was 75cm, line to line was 30cm and plant to plant within rows was 5-7cm. Inter cultural operations and other agronomic practices were done at proper time. Harvesting was begun on last of February 2013 completed on 26 April 2013. Five plants were selected randomly from each unit plot for data collection in such a way that the border effect could be avoided for the highest precision. Data on the following parameters were recorded from the sample plants during the course of experiment: plant height, number of compound leaves plant⁻¹, petiole length, leaf area, crown spread of plant, days to first flowering, number of flowers plant⁻¹, number of pollen sterility plant⁻¹, number of fruits plant⁻¹, weight of individual fruit and weight of fruits plant⁻¹. The biochemical analysis of qualitative characters of strawberry was performed in the laboratory of the Department of Molecular Biology and Biochemistry, Bangladesh Agricultural University, Mymensingh. The biochemical parameters which were taking into consideration for determination are pH, Total soluble solid (TSS), and ascorbic acid content.

Statistical Analysis

Analysis of variance was performed using the plant breeding statistical program (PLBSTAT, Version 2N, Utz 2007) with the following model:

$$Y_{ij} = \mu + r_j + \epsilon_{ij}$$

Estimation of correlation coefficient

The genotypic and phenotypic correlations were estimated by the formula suggested by Miller *et al.* (1958).

$$\text{Genotypic correlation, } r_{g1.2} = \frac{\text{CoV} \cdot g_{1.2}}{\sqrt{\delta^2 g_1 \times \delta^2 g_2}}$$

Similarly, phenotypic correlation,

$$r_{p1.2} = \frac{\text{CoV} \cdot p_{1.2}}{\sqrt{\delta^2 p_1 \times \delta^2 p_2}}$$

Estimation of path co-efficient

Direct and indirect path coefficients were calculated as described by Lynch & Walsh (1998) as

$$r_{yi} = P_{yi} + \sum_{\substack{i'=1 \\ i' \neq i}}^k r_{ii'} P_{yi'} \quad \text{for } i \neq 1$$

Analysis of Genetic divergence

Genetic divergence plays a vital role in existing germplasm in mode and source of origin. Mahalanobis' D^2 -statistics may be applied for such study. It also measures the distance for a number of traits between two populations. First the difference between the means in respect of the pooled effect of all characters between different populations was tested.

i. Calculation of D^2 values

The Mahalanobis' distance (D^2) values were calculated from transformed uncorrelated means of characters according to Rao (1952) and Singh and Chaudhury (1985). For each combination the mean deviation, i.e. $Y^1_i - Y^2_i$ with $i = 1, 2 \dots p$ was estimated and the D^2 was calculated as sum of the squares of these deviations, i.e. $\sum (Y^1_i - Y^2_i)^2$. The D^2 values were estimated for all possible pairs of combinations between genotypes.

ii. Clustering

The D^2 values of genotypes were arranged in order of relative distances from each other by the method suggested by Tocher (Rao 1952) and Singh and Chaudhary (1985) was used for cluster formation.

iii. Calculation of average intra-cluster distances

Average intra-cluster distances were calculated by the following formula as suggested by Singh and Chaudhury (1985).

$$\text{Average intra-cluster } D^2 = \frac{\sum D^2}{n}$$

iv. Calculation of average inter-cluster distances

Average inter-cluster distances were calculated by the following formula as suggested by Singh and Chaudhury (1985).

$$\text{Average intra-cluster } D^2 = \frac{\sum D^2_{ij}}{n_i \times n_j}$$

v. Estimation of contribution of individual characters towards divergence

In all the combinations each character was ranked on the basis $d_i = y^j_i - Y^k_i$ values. Rank 1 was given to the highest mean difference and rank p to the lowest mean difference, where p is the total number of characters. Thus, the number of times appearing first in ranking was calculated for each character and finally a table was prepared and the percent contribution was calculated.

Results and Discussion

The analyses of variance of 18 tissue culture variants of strawberry for all characters under study are shown in Table 1. Analysis of variance for the characters revealed that there were significant variations among the tissue culture variants for all the characters. This indicates that there was a genotypic variation among the tissue culture variants for the characters. The mean performances of the 18 tissue culture variants were evaluated for all characters are presented in Table 2. Among the 18 tissue culture variants, G-14 had shown highest plant height (21.00 cm) and G-9 and G-13 shown shortest (16.00 cm) plant type. G-12 had shown highest (29.00) followed by G-1 and G-7 where as G-13 and G-16 had shown lowest (21.00) followed by G-14, G-15 and G-18 number of compound leaves plant⁻¹. Higher petiole length (19.33 cm) had exhibited in G-9 and G-3 had the shortest (14.00) petiole length. This difference may be due to the difference in physiological conditions. G-4 had shown the highest (71.30 cm) leaf area and G-1 had shown the lowest (67.33 cm) leaf area. The difference in leaf area caused by treatment of tissue culture derived plants by different hormone in various developmental stages. G-12 had shown the highest (21.17 cm) crown spread of plant and the tissue culture variant G-16 had showed the lowest (24.10 cm) crown spread of plant. Among the tissue culture variants, G-1, G-7, G-8 had showed the early flowering (38 days). In contrast, the maximum days to flowering (42 days) required for the tissue culture variant G-11. The higher number of flowers (45.00) was exhibited in the tissue culture variant G-9 and the tissue culture variants G-15 had the lowest (39.67) number of flowers. The tissue culture variants G-2, G-5, G-18 had ranging from 12 to 13.5% pollen sterility. In contrast, the maximum pollen sterility (21.63%) had showed for the tissue culture variant G-1. The higher number of fruits plant⁻¹ (24.67) was obtained from the tissue culture variant G-18. On the other hand, the lower number of fruits plant⁻¹ (18.00) was recorded in the tissue culture variant G-1 planting. The weight of individual fruit ranged from 14g to 23g. The maximum weight of individual fruit (22.84 g) was found in G-10 and minimum weight of individual fruit (14.52 g) was observed from in the tissue culture variant G-3. Among the tissue culture variant, G-9 had showed the highest (3.51) pH and the tissue culture variant G-18 had shown the lowest (3.34) p^H. The maximum ascorbic acid content(32.35 mg/100g) was found in the tissue culture variant G-6 and minimum ascorbic acid content (25.77 mg/100g) was found in the tissue culture variant G-2. % brix of fruits strawberry varied from 16% to 18 %. The maximum % brix (17.77) in G-16 and G-17 was recorded from tissue culture variants and the minimum (16.61) was obtained in tissue culture variants G-2. Thus among the tissue culture variants giving emphasis on yield parameter e.g. petiole length, days to

flowering, number of fruits plant⁻¹, % brix and weight of individual fruit tissue culture variants G-9, G-10 and G-11 were selected as superior variants for cultivation across all the environments.

Relationship between physiological and yield contributing characters was studied through analysis of correlation between them. The correlation coefficients between all the fourteen characters were presented in Table 3. It appears from table 10 that yield plant⁻¹ was positively significantly correlated with weight of individual fruit ($r=0.49^*$), petiole length ($r=0.50^*$), Days to opening of first flower ($r=0.53^*$) and number of fruits plant⁻¹ ($r=0.53^*$). Among them, petiole length, Days to opening of first flower, number of fruits plant⁻¹ and weight of individual fruit suggesting that genotypes with high partitioning efficiency gave increase in yield plant⁻¹ and those characters were positively and significantly correlated with yield plant⁻¹. Similar results were obtained by Ara *et al.* (2009); Sakila *et al.* (2007). Among the yield contributing character number of compound leaves plant⁻¹, leaf area and crown spread of plant plant⁻¹ were negatively and non-significantly correlated with yield plant⁻¹. Among the yield contributing character plant height, number of flowers plant⁻¹, pollen sterility, pH, ascorbic acid content of individual fruit, and % brix were positively and non-significantly correlated with yield plant⁻¹.

Study of correlation at yield components levels exhibited that plant height showed positive and insignificant correlation with character number of compound leaves plant⁻¹, petiole length, leaf area, pollen sterility, ascorbic acid content and % brix whereas no. of fruits plant⁻¹ was significantly positive correlated with plant height. Plant height had also showed negative and insignificant correlation with crown spread of plant plant⁻¹, days to opening of first flower, number of flowers plant⁻¹, pH and weight of individual fruit. Similar results were obtained by Singh *et al.* (2000). Number of compound leaves plant⁻¹ showed positive and insignificant correlation with number of flowers plant⁻¹, pollen sterility and pH whereas crown spread plant⁻¹ was significantly positive correlated. It also showed negative and insignificant correlation with petiole length, leaf area, days to opening of first flower, no. of fruits plant⁻¹, ascorbic acid content and weight of individual fruit and % brix was significantly negative correlated. Similar results were obtained by Ara *et al.* (2009). Petiole length showed negatively and insignificant correlation with crown spread of plant plant⁻¹ and days to opening of first flower. Again petiole length also showed positive and insignificant correlation with leaf area, number of flowers plant⁻¹, pollen sterility, pH, ascorbic acid content of individual fruit, % brix and weight of individual fruit whereas weight of individual fruit had positive and significant correlation with petiole length. Similar results were obtained by Ara *et al.* (2009). Leaf area showed positive and insignificant

correlation with crown spread of plant plant⁻¹, pH, ascorbic acid content of individual fruit and weight of individual fruit. Leaf area also showed negatively and insignificant correlation with rest characters. Similar results were obtained by Kumar *et al.* (2013). Crown spread of plant plant⁻¹ had positive and insignificant correlation with days to opening of first flower and pH. It also showed negatively significant correlation with number of fruits plant⁻¹ and showed positive and insignificant correlation with number of flowers plant⁻¹, pollen sterility, ascorbic acid content of individual fruit, % brix and weight of individual fruit. Similar results were obtained by Sean and Douglas (2000). Days to opening of first flower showed negatively insignificant with number of flowers plant⁻¹ and positive and significant correlation with weight of individual fruit. Similar results were obtained by Kumar *et al.* (2013). Number of flowers plant⁻¹ showed negatively insignificant correlation with weight of individual fruit and showed positive and insignificant correlation with rest characters.

Similar results were obtained by Ara *et al.* (2009); Kumar *et al.* (2013). Pollen sterility showed negatively insignificant correlation with character number of fruits plant⁻¹. Number of fruits plant⁻¹ showed negatively insignificant correlation with weight of individual fruit. P^H showed positive and insignificant correlation with character ascorbic acid content of individual fruit and showed negatively insignificant correlation with character % brix and weight of individual fruit. Similar results were obtained by Ara *et al.* (2009).

Ascorbic acid content of individual fruit showed highly positive and significant correlation with character % brix. Similar results were obtained by Ara *et al.* (2009); Kumar *et al.* (2013). % brix showed positive and insignificant correlation with character weight of individual fruit. Similar results were obtained by Ara *et al.* (2009); Kumar *et al.* (2013).

The path coefficient analysis (Table 4) was performed using correlation coefficient to determine direct and indirect influence considering thirteen characters. Among them petiole length and ascorbic acid content of individual fruit had high positive direct effects on weight of fruits yield plant⁻¹. Among the characters, ascorbic acid content of individual fruit and weight of individual fruit had high positive direct effects on fruit yield plant⁻¹. Plant height, number of compound leaves plant⁻¹, leaf area, days to opening of first flower, number of flowers plant⁻¹, pH, % brix showed negative direct effect on weight of fruits yield plant⁻¹. The highest direct effect of petiole length and ascorbic acid content of individual fruit had high positive direct effects on weight of fruits gave a significant positive correlation inducing that among all the traits under study these trait contributed maximum for fruit yield. The residual effect was 0.027 indicating that the thirteen characters contributed 99.97 percent of variability in grain yield plant⁻¹ studied in path analysis. Similar results were in

accordance with studies of Singh *et al.* (2000); Sean and Douglas (2000).

Both correlation and path co-efficient studies revealed that number of fruits plant⁻¹, ascorbic acid content of individual fruit and weight of individual fruit were the most important components for getting higher yield. Recent breeding research also emphasized giving importance of these characters.

Using Euclidean distance following Ward's method, the tissue culture variants were grouped into distinct clusters. Based on D^2 -value, the genotypes were grouped into three clusters (Table 5). Cluster I, II and III had different no. of genotypes. The cluster II contained eight genotypes which is the largest one and the cluster III contained only four genotypes which is the smallest one. Also the cluster I contained six genotypes. The average intra and inter cluster distances are presented in Table 6. It was observed that inter cluster distance were always higher than those of intra cluster distance. The intra cluster distance of cluster III had (2.353608583) which was the highest value. However, cluster III contained only four genotypes. The second highest (1.328135553) intra cluster distance of the cluster II it contained eight no. of genotypes and lowest intra cluster distance of (1.0753505358) the cluster I it contained six genotypes.

The mutual relationships among the three clusters are presented in the diagram (Fig. 1). The average inters and intra cluster distance (Table 6) have been used to denote cluster distance. The maximum inter cluster distance was observed between genotypes of cluster I and III (0.78) followed by clusters II and III (0.65). Thus, somaclonal variation among genotypes drawn from these widely divergent clusters with high yield potential would likely to produce heterotic combinations and wide variability in segregating generations.

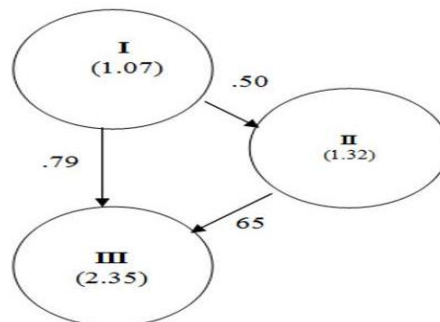


Figure 1. Cluster diagram showing the average intra and inter cluster distances ($D = \sqrt{D^2}$ values) of 18 tissue culture variants of strawberry. The values along the lines inter cluster distances and the values within the circle indicate intra cluster distances

Dendrogram based on Ward's method indicated grouping of 18 tissue culture variants of strawberry into three clusters (Fig. 2). G-1, G-2, G-3, G-4, G-5 and G-8 were grouped in cluster I with high genetic (1.07) distance, while G-6, G-7, G-9, G-12, G-13, G-14, G-17 and G-18 in cluster II with genetic distance (1.32) and G-10, G-11, G-15 and G-16 in cluster III with highest genetic distance (2.35).

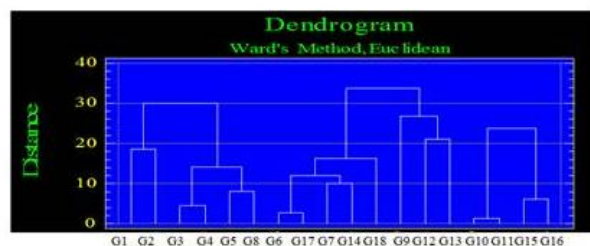


Figure 2. Dendrogram based on Ward's method indicated grouping of 18 tissue culture variants of strawberry

The mean values of each cluster for eleven characters are presented in Table 7. There was wide range of variation in the cluster mean values for all the characters. The mean values of all characters for the respective character were categorized into low (L), intermediate (I) and high (H) classes.

Conclusion and Recommendation

Among the tissue culture variants giving emphasis on key yield parameter e.g. petiole length, days to flowering, number of fruits plant⁻¹, % brix and weight of individual fruit tissue culture variants G-9, G-10 and G-11 were selected as superior variants for cultivation across all the environments. Both correlation and path co-efficient studies revealed that number of fruits plant⁻¹, ascorbic acid content of individual fruit and weight of individual fruit were the most important components for getting higher yield. Recent breeding research also emphasized giving importance of these characters. Therefore, it could be concluded that for further research program, especially for hybridization, genotype could be selected from different clusters that might provide maximum heterosis regarding yield. In conclusion, the result of the present experiment revealed that the variability existed among the selected tissue culture variants of strawberry were present. Among these variants the superior genotypes may be used in future breeding program. This variability may be used for the selection of superior genotypes for commercial cultivation

at farmer's level as well as for breeding new genotypes of strawberry in our country.

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Table 1. Analysis of variance for plant characters of 18 the tissue culture variants of strawberry

Items	df	Plant height (cm)	No. of compound leaves	Petiole length (cm)	Leaf Area (cm)	Crown spread / plant (cm)	Days to flowering	No. of flowers /plant	Pollen sterility (%)	No. of fruits/ plant	pH content
Tissue culture variants	17	5.85**	19.91* *	8.96**	2.80*	2.32**	3.78**	7.69**	26.41*	10.73* *	0.004* *
Replication	2	0.39	19.57	1.23	5.44*	1.83	1.40	1.40	33.46	0.13	0.0007
Error	34	1.19	7.75	2.02	1.39	0.62	1.27	1.25	10.60	1.80	0.0013

Table 2. Analysis of variance for plant characters of 18 the tissue culture variants of strawberry

Tissue culture variants	Plant height (cm)	No. of compound leaves	Petiole length (cm)	Leaf Area (cm)	Crown spread / plant (cm)	Days to flowering	No. of flowers /plant	Pollen sterility (%)	No. of fruits/ plant	pH content
G-1	16.33 de	28.33 a	14.63 d	67.33d	26.67 a-c	38.67 d	43.00 a-d	21.67 a	18.00 e	3.397 b-e
G-2	18.00 b-e	27.33ab	15.53 cd	68.50b-d	25.83 a-d	39.00 cd	40.00 ef	12.67 d	21.33 cd	3.430 bc
G-3	17.00 c-e	25.33a-c	14.00 d	70.27ab	27.07 ab	39.33 b-d	40.00 ef	14.33 cd	20.67 cd	3.417 b-d
G-4	16.3 de	24.67a-c	16.23 b-d	71.30 a	26.67 a-c	40.00 a-d	42.00 b-e	19.00 a-d	20.67 bc	3.407 b-d
G-5	18.00 b-e	24.67a-c	14.40 d	69.58 a-d	27.07 ab	40.00 a-d	42.00 b-e	13.00 d	21.67 bc	3.400 b-e
G-6	19.00 bc	24.33a-c	16.37 b-d	70.17 a-c	26.07 a-d	39.00 cd	41.00 d-f	18.67a-d	22.00 bc	3.443 bc
G-7	20.00 ab	28.00 a	18.00 a-c	69.33 a-d	25.47 c-f	38.00 d	44.00 ab	20.00 a-c	24.00 ab	3.383 c-e
G-8	17.00 c-e	25.33a-c	16.08 b-d	69.37 a-d	26.50 a-d	38.00 d	43.33 a-c	16.33 a-d	19.00 de	3.383 c-e
G-9	16.00 e	24.00a-c	19.33 a	68.90 b-d	25.47 c-f	40.00 a-d	45.00 a	20.00 a-c	24.00 ab	3.513 a
G-10	17.00 c-e	22.33bc	15.57 cd	69.47a-d	26.00 a-d	41.33 ab	41.33 c-f	18.00 a-d	20.67 cd	3.400 b-e
G-11	19.00 bc	23.00cd	17.70 a-c	68.47 b-d	25.70 a-e	42.00 a	41.33 c-f	18.00 a-d	21.67 bc	3.367 de
G-12	18.33 b-d	29.00 a	14.53 d	67.87 cd	27.17 a	41.33 ab	44.00 ab	20.00 a-c	24.00 ab	3.407 b-d
G-13	16.00 e	21.00 c	14.13 d	67.90 cd	25.57 b-f	41.00 a-c	44.00 ab	15.00 b-d	22.00 bc	3.447 b
G-14	21.00 a	21.67c	18.10 a-c	69.10 a-d	24.30 ef	40.00 a-d	41.67 c-f	20.33 a-c	24.00 ab	3.383 c-e
G-15	17.00 c-e	21.33 c	14.57 d	68.90 b-d	25.90a-d	40.00 a-d	39.67 f	21.33 ab	20.33 c-e	3.437 bc
G-16	16.67 de	21.00 c	14.17 d	68.40 b-d	24.10 f	39.33 b-d	41.00 d-f	20.00 a-c	22.00 bc	3.357 de
G-17	18.00 b-e	22.67a-c	18.43 ab	69.07 a-d	25.40 c-f	39.33 b-d	42.00 b-e	16.00 a-d	24.00 ab	3.433 bc
G-18	18.33 b-d	21.67c	17.57 a-c	68.23 b-d	25.07d-f	39.00 cd	44.00 ab	13.33 d	24.67 a	3.343 e
CV (%)	6.16	11.46	8.85	1.71	3.05	2.84	2.65	18.45	6.11	1.06
Max	21.00	29.00	19.33	71.30	27.17	42.00	45.00	21.67	24.67	3.513
Min	16.00	21.00	14.00	67.33	24.10	38.00	39.67	12.67	18.00	3.343
LSD (0.05)	1.81	4.62	2.36	1.96	1.31	1.87	1.86	5.40	2.22	0.06

Table 3. Coefficients of Correlation among different yield components of 18 tissue culture variants of strawberry

Traits	Plant height(cm)	No. of compound leaves plant ⁻¹	Petiole length(cm)	Leaf area (cm)	Crown spread of plant plant ⁻¹ (cm)	Days to opening of first flower	No. of flowers plant ⁻¹	Pollen sterility (%)	No. of fruits plant ⁻¹	pH	Ascorbic acid content of individual fruit	% brix	Weight of individual fruit(gm)
No. of compound leaves plant ⁻¹	0.11												
Petiole length(cm)	0.43	-0.06											
Leaf area(cm)	0.05	-0.05	0.11										
Crown spread of plant plant ⁻¹ (cm)	-0.29	0.61**	-0.44	0.25									
Days to opening of first flower	-0.06	-0.29	-0.10	-0.14	0.07								
No. of flowers plant ⁻¹	-0.08	0.20	0.36	-0.33	-0.01	-0.05							
Pollen sterility (%)	0.03	0.06	0.12	-0.09	-0.17	0.07	0.1						
No. of fruits plant ⁻¹	0.51*	-0.15	0.57*	-0.09	-0.47*	0.13	0.38	-0.07					
pH	-0.37	0.05	0.11	0.12	0.19	0.10	0.09	0.07	0.04				
Ascorbic acid content	0.46	-0.27	0.16	0.31	-0.03	0.44	0.04	0.15	0.45	0.02			
% brix	0.21	-0.53*	0.11	-0.12	-0.38	0.38	0.11	0.15	0.45	-0.15	0.67**		
Weight of individual fruit(gm)	-0.01	-0.31	0.17	0.01	-0.03	0.52*	-0.13	0.20	-0.09	-0.03	0.21	0.27	
Weight of fruits /plant(kg)	0.28	-0.35	0.50*	-0.04	-0.31	0.53*	0.12	0.13	0.53*	0.04	0.45	0.49*	0.79**

* and ** indicate significant at 0.05 and 0.01 probability, respectively

Table 4. Partitioning of phenotypic correlations into direct and indirect effects of thirteen important characters by path analysis

Items	Plant height (cm)	No. of compound leaves / plant	Petiole length (cm)	Leaf area (cm ²)	Crown spread of plant / plant (cm)	Days to opening of first flower	No. of flowers / plant	Pollen sterility (%)	No. of fruits / plant	pH	Ascorbic acid content of individual fruit	% brix	Weight of individual fruit (gm)	Correlation to yield / plant (kg)
Plant height (cm)	-0.07106	-0.0012	0.02288 ₈	-0.00222	-0.00871	0.00049 ₆	0.00370 ₅	0.00040 ₄	0.33117	0.00781	0.0283	-0.01125	-0.01334	0.287
No. of compound leaves / plant	-0.00782	-0.0109	-0.00334	0.00261 ₇	0.01825 ₈	0.00221 ₅	-0.00919	0.00073 ₁	-0.09987	-0.0011	-0.01667	0.02734 ₈	-0.25929	-0.357
Petiole length (cm)	-0.03112	0.00069 ₈	0.05225₅	-0.00528	-0.01319	0.00081 ₇	-0.01669	0.00140 ₇	0.36982 ₈	-0.00247	0.01012	-0.00578	0.14339 ₉	0.504*
Leaf area (cm ²)	-0.00355	0.00064 ₃	0.00621 ₈	-0.04436	0.00742 ₉	0.00106 ₉	0.01513 ₈	-0.00108	-0.05863	-0.00251	0.01915	0.00614 ₁	0.01333 ₉	-0.041
Crown spread of plant / plant (cm)	0.02074 ₈	-0.00667	-0.0231	-0.01104	0.02983₄	-0.00053	0.00064	-0.00191	-0.30733	-0.00392	-0.0023	0.01976 ₃	-0.02918	-0.315
Days to opening of first flower	0.00461 ₉	0.00316 ₁	-0.00559	0.00621	0.00208 ₈	-0.00764	0.00251 ₅	0.00081 ₈	0.08504 ₈	-0.00211	0.02672 ₅	-0.01971	0.43686 ₈	0.533*
No. of flowers / plant	0.00575 ₆	-0.00219	0.01907 ₃	0.01468 ₂	-0.00042	0.00042	-0.00046	0.00109 ₁	0.24934 ₄	-0.00186	0.00272 ₇	-0.00583	-0.11505	0.122
Pollen sterility (%)	-0.00263	-0.00073	0.00674 ₁	0.00439 ₁	-0.00522	-0.00057	-0.00457	0.01091₁	-0.0451	-0.00149	0.00902 ₉	-0.008	0.16924 ₄	0.132
No. of fruits / plant	-0.03652	0.00169	0.02999 ₅	0.00403 ₆	-0.01423	-0.00101	-0.0177	-0.00076	0.00644₃	-0.00102	0.02745 ₂	-0.02353	-0.0767	0.536*
pH	0.02678 ₈	-0.00058	0.00621 ₈	-0.00537	0.00563 ₉	-0.00078	-0.00412	0.00078 ₆	0.03157 ₁	-0.02072	0.00121 ₂	0.00784 ₃	-0.02501	0.046
Ascorbic acid content of individual fruit	-0.03318	0.00299 ₈	0.00872 ₇	-0.01402	-0.00113	-0.00337	-0.00206	0.00162 ₆	0.29186 ₈	0.00029 ₂	0.0606	-0.34727	0.18008	0.457

% brix	-0.01549	0.00577 7	0.00585 3	0.00527 8	-0.01143	-0.00292	-0.00517	0.00169 1	0.2938	0.03148 7	0.40783	-0.0516	0.22427	0.494*
Weight of individual fruit(gm)	0.00113 7	0.00339	0.00898 8	-0.00071	-0.00104	-0.004	0.00631 2	0.00221 5	-0.05928	0.00062 1	0.13089	-0.1388	0.00833 7	0.790**

Residual effect = 0.027

* and ** indicate significant at 0.05 and 0.01 level of probability, respectively.

Bold figures indicate the direct effect

Table 5. Clustering pattern of 18 tissue culture variants of based on Euclidean distance following Ward's method and the member present in each respective cluster

Cluster number	Number of genotypes	Percent	Name of genotypes
I	6	33.33	G-1, G-2, G-3, G-4, G-5 and G-8
II	8	44.44	G-6, G-7, G-9, G-12, G-13, G-14, G-17 and G-18
III	4	22.22	G-10, G-11, G-15 and G-16

Source: Primary data collected through questionnaire

Table 6. Average intra and inter cluster D^2 and D values of three clusters by Euclidean method

Cluster number	I	II	III
I	17.34 (1.07)	107.60 (.50)	56.06 (.79)
II		49.39 (1.32)	47.33 (.65)
III			14.12 (2.35)

Source: Primary data collected through questionnaire

Table 7. Cluster mean for 14 yield and yield related characters in 18 tissue culture variants of strawberry

Cluster	I	II	III
Plant height (cm)	17.11(L)	18.3325(H)	17.4175(I)
No.of compound leaves plant-1	25.9433(H)	24.1675(I)	22.0825(L)
Petiole length(cm)	15.145(L)	17.0575(H)	15.5025(I)
Leaf area(cm)	69.3917(H)	68.8212(I)	68.81(L)
Crown spread of plant plant-1(cm)	26.635(H)	25.565(I)	25.425(L)
Days to opening of first flower	39.1667(L)	39.7075(I)	40.665(H)
No. of flowers plant-1	41.7217(I)	43.2088(H)	40.8325(L)
Pollen sterility (%)	16.1667(L)	17.9163(I)	19.3325(H)
No. of fruits plant-1	20.2233(L)	23.5838(H)	21.1675(I)
pH	3.40667(I)	3.4175(H)	3.3925(L)
Ascorbic acid content of individual fruit (mg/100g)	28.5833(L)	30.93(H)	30.2825(I)
% brix	16.9917(L)	17.53(I)	17.6225(H)
Weight of individual fruit(gm)	16.9533(L)	17.1737(I)	20.4825(H)
weight of fruit plant-1 (kg)	0.343333(L)	0.4075(I)	0.4325(H)

H= High value; I= Intermediate value; L= Low value