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

Haque, S.*, Iqbal, S., Jahan, G. S., Islam, N. and Nath, U. K.

Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh.

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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

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.

* **Coresponding author**: Haque, S.; **Email:** shahinsarvi@gmail.com

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 has been planned to assess their field performance for commercial cultivation and to analyze diversity for further breeding programs. The objectives of present study are as follows:

- To assess the field performance of strawberry somaclones.
- To analyze the genetic variation, relationship and stability of strawberry somaclones.

Materials 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 Gnetics 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 Indolebutaric acid (IBA 1.0 mg L^{-1}), α-naphthaleneaceticacid (NAA 0.5 mg L^{-1}), 6- Benzylamino purine (BAP 1.0 and 1.5 mg L^{-1}), glucose (40.0 g dm) and Bacto-Difco agar (6.4 g dm), 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:

$$Y_{ij} = g_i + r_j + \varepsilon_{ij}$$

Estimation of correlation coefficient

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

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

Similarly, phenotypic correlation,

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

Estimation of path co-efficient

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

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

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 …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).

$$\text{Average intra-cluster } D^2 = \frac{\sum D^2 \text{ ij}}{ni \times nj}$$

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 actual moisture contents of the samples were 4.2, 6.5 and 8.3% (wet basis) but for easy reference in this report, the levels are referred to as 4, 6, and 8%. The oil point pressure of fine and coarse dika kernel particles as influenced by the pre-treatments are presented in Tables 1 and 2 respectively. In Table 1, the lowest pressure at which oil flow was observed was 0.41 MPa (at 50 ^oC, 8% MC and 45 min of heating) which differs significantly ($p<0.05$) from mean pressure at all other combination of pre-treatment. The highest pressure was 0.65 MPa (at 30 ^oC, 4% MC and 15 min of heating), which was not significantly different from values obtained at a heating temperature of 30 and 40 ^oC. It may therefore be inferred that in the range of experimental values, for fine particles, oil point pressure ranged between 0.65 and 0.41 MPa.

Considering Table 2, for coarse particles, the lowest pressure at which oil flow was observed was 1.51 MPa, (at 50 ^oC, 8% MC and 45 min of heating). This value is significantly different ($p<0.05$).from values of mean pressure at other combinations of pre-treatment. However, the highest was 2.11 MPa (at 30 ^oC, 4% MC and 15 min) which stands out above all other pre-treatment. It may be inferred summarily that for coarse particles, oil point pressure ranged

between 1.51 and 2.11 MPa. These results show that for both particle sizes of dika kernel, oil point pressure reduced as heating temperature, heating time and moisture content increased. In the presence of excess moisture the liquid phase bears the entire load itself being incompressible and does not exert any pressure on the oil bearing particles; the oil bearing cells get swollen. This increases the pressure on the cell wall, further application of pressure ruptures and forces them to discharge their contents and adversely affects oil expression (Bamgboye and Adejumo, 2011).

In previous studies, Ajibola *et al.* (2000) and Akintunde *et al.* (2001) stated that when an oilseed is subjected to heat treatment, moisture loss creates a void which serves as migratory space for the contents of the oil bearing cells thereby facilitating the rupture of oil bearing cells as heating progresses. In addition, this lowers viscosity of oil and coagulates the protein, thus enabling oil to emerge from the oil-bearing cells into the inter-kernel void (Adeeko and Ajibola, 1990). Moreover, dika seed oil normally solidifies at room temperature, above which it melts due to its high myristic acid content (Ogunsina *et al.* 2012). When temperature increases or heating is prolonged, the tendency is for oil to flow more readily from the oil bearing cells. Similar results had been reported for cashew kernel, locust bean, soybean and groundnut (Ogunsina *et al.* 2008; Owolarafe *et al.*, 2003; Ajibola *et al.*, 2000; Adeeko and Ajibola, 1990).

Figure 3 further established that the average oil point pressure for fine particles was far less than that of coarse particles. This conforms with expectation and previous reports that mechanical oil expression is achievable at relatively lower pressure with fine particles than with coarse particles (Akintunde *et al.*, 2001; Fasina and Ajibola, 1990). Figure 4 shows the relationship between dika kernel particle sizes and oil yield under varying pressures. It was observed that oil yield of fine particles was more than that of coarse particles as applied pressure varied from 5 MPa to 15 MPa. The highest oil yield (36.6%) was obtained with fine particles at 15 MPa while the lowest oil yield (17.5%) was obtained at 5 MPa for coarse particles. This may be due to the fact that a larger surface area of fine particles was exposed to moisture, heat and pressure. The oil cells of fine particles ruptured and collapsed more quickly under the applied pressure since larger surface area were exposed to the applied pressure. This result corroborates previous findings for soybean, groundnuts, and conophor nut (Akintunde *et al.*, 2001; Adeeko and Ajibola, 1990; Fasina and Ajibola, 1989).

The relationship between moisture content, applied pressure and oil yield of dika kernel is shown in Fig. 5. The most common trend was that oil yield increased as moisture content and pressure increased. For fine particles,at 10

and 15 Mpa of applied pressure, oil yield decreased as the MC varied between 6 and 8%. Generally for fine particles, the mucilage that dika kernel contains contains will normally get swollen in the presence of moisture; and this blocks oil passage which consequently hinder oil flow thereby reducing oil yield. Similar behavior may be found with oil seeds with high mucilage content such as flax and okra seeds. The least oil yield recorded for coarse particles was at 4% MC and 5 Mpa whereas the highest was obtained for fine particles at 4% at 15 MPa. 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. PH 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 D2-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 (1.07535050358) the cluster I it contained six genotypes.

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	Ascorbic acid content mg/100g	% brix	weight of individual fruit (gm)	weight of fruits /plant (kg)
Tissue culture variants	17	5.85**	19.91**	8.96**	2.80*	2.32**	3.78**	7.69**	26.41*	10.73**	0.004**	9.93**	0.35**	13.6**	8518.24**
Replication	2	0.39	19.57	1.23	5.44*	1.83	1.40	1.40	33.46	0.13	0.0007	2.27	0.13	0.36	231.75
Error	34	1.19	7.75	2.02	1.39	0.62	1.27	1.25	10.60	1.80	0.0013	2.03	0.10	2.14	1284.3

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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	Ascorbic acid content mg/100g	% brix	weight of individual fruit (gm)	weight of fruits /plant (kg)
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	26.56 ef	16.72 cd	16.47 fg	296.7 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	25.77 f	16.61 d	16.16 fg	343.2 c-e
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	30.87 a-d	17.13 a-d	14.52 g	301.3 e
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	29.34 b-d	17.01 b-d	16.74 e-g	346.8 c-e
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	30.62 a-d	17.18 a-d	19.63 b-d	425.0 ab
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	32.35 a	17.50 ab	17.44 c-f	383.9 b-d
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	31.23 a-c	17.50 ab	15.88 fg	380.3 b-d
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	28.34 de	17.30 a-c	18.20 c-f	343.9 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	29.40 b-d	17.14 a-d	19.43 b-e	465.3 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	31.67ab	17.53 ab	22.84 a	473.3 a
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	30.94 a-d	17.66 ab	21.40 ab	462.5 a
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	31.59 ab	17.64 ab	17.41 c-f	417.0 ab
G-13	16.00 e	21.00 c	14.13 d	67.90	25.57 b-f	41.00 a-c	44.00	15.00	22.00	3.447 b	30.94 a-d	17.77 a	15.37 fg	338.0 de

				cd			ab	b-d	bc					
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	32.29 a	17.42 ab	17.36 c-f	415.7 ab
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	29.94 a-d	17.63 ab	20.06 bc	408.1 a-c
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	28.58 c-e	17.67 a	17.63 c-f	387.2 b-d
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	30.27 a-d	17.77 a	17.67 c-f	424.9 ab
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	29.37 b-d	17.50 ab	16.83 d-g	415.9 ab
CV (%)	**6.16**	**11.46**	**8.85**	**1.71**	**3.05**	**2.84**	**2.65**	**18.45**	**6.11**	**1.06**	**4.76**	**1.89**	**8.21**	**9.18**
Maximum	**21.00**	**29.00**	**19.33**	**71.30**	**27.17**	**42.00**	**45.00**	**21.67**	**24.67**	**3.513**	**32.35**	**17.77**	**22.84**	**473.3**
Minimum	**16.00**	**21.00**	**14.00**	**67.33**	**24.10**	**38.00**	**39.67**	**12.67**	**18.00**	**3.343**	**25.77**	**16.61**	**14.52**	**296.7**
LSD (0.05)	**1.81**	**4.62**	**2.36**	**1.96**	**1.31**	**1.87**	**1.86**	**5.40**	**2.22**	**0.06**	**2.37**	**0.54**	**2.43**	**59.46**

Table 3. Coefficients of Correlation among different yield components of 18tissue 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)	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	0.022888	-0.00222	-0.00871	0.000496	0.003705	0.000404	0.33117	0.00781	0.0283	-0.01125	-0.01334	0.287
No.of compound leaves /plant	-0.00782	**-0.0109**	-0.00334	0.002617	0.018258	0.002215	-0.00919	0.000731	-0.09987	-0.0011	-0.01667	0.027348	-0.25929	-0.357
Petiole length(cm)	-0.03112	0.000698	**0.052255**	-0.00528	-0.01319	0.000817	-0.01669	0.001407	0.369828	-0.00247	0.01012	-0.00578	0.143399	0.504*
Leaf area(cm)	-0.00355	0.000643	0.006218	**-0.04436**	0.007429	0.001069	0.015138	-0.00108	-0.05863	-0.00251	0.01915	0.006141	0.013339	-0.041
Crown spread of plant/plant(cm)	0.020748	-0.00667	-0.0231	-0.01104	**0.029834**	-0.00053	0.00064	-0.00191	-0.30733	-0.00392	-0.0023	0.019763	-0.02918	-0.315
Days to opening of first flower	0.004619	0.003161	-0.00559	0.00621	0.002088	**-0.00764**	0.002515	0.000818	0.085048	-0.00211	0.026725	-0.01971	0.436868	0.533*
No. of flowers/plant	0.005756	-0.00219	0.019073	0.014682	-0.00042	0.00042	**-0.00046**	0.001091	0.249344	-0.00186	0.002727	-0.00583	-0.11505	0.122
Pollen sterility(%)	-0.00263	-0.00073	0.006741	0.004391	-0.00522	-0.00057	-0.00457	**0.010911**	-0.0451	-0.00149	0.009029	-0.008	0.169244	0.132
No. of fruits/plant	-0.03652	0.00169	0.029995	0.004036	-0.01423	-0.00101	-0.0177	-0.00076	**0.006443**	-0.00102	0.027452	-0.02353	-0.0767	0.536*
pH	0.026788	-0.00058	0.006218	-0.00537	0.005639	-0.00078	-0.00412	0.000786	0.031571	**-0.02072**	0.001212	0.007843	-0.02501	0.046
Ascorbic acid content of individual fruit	-0.03318	0.002998	0.008727	-0.01402	-0.00113	-0.00337	-0.00206	0.001626	0.291868	0.000292	**0.0606**	-0.34727	0.18008	0.457
% brix	-0.01549	0.005777	0.005853	0.005278	-0.01143	-0.00292	-0.00517	0.001691	0.2938	0.031487	0.40783	**-0.0516**	0.22427	0.494*
Weight of individual fruit(gm)	0.001137	0.00339	0.008988	-0.00071	-0.00104	-0.004	0.006312	0.002215	-0.05928	0.000621	0.13089	-0.1388	**0.008337**	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

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**	107.60	56.06
	(1.07)	(.50)	(.79)
II		**49.39**	47.33
		(1.32)	(.65)
III			**14.12**
			(2.35)

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.

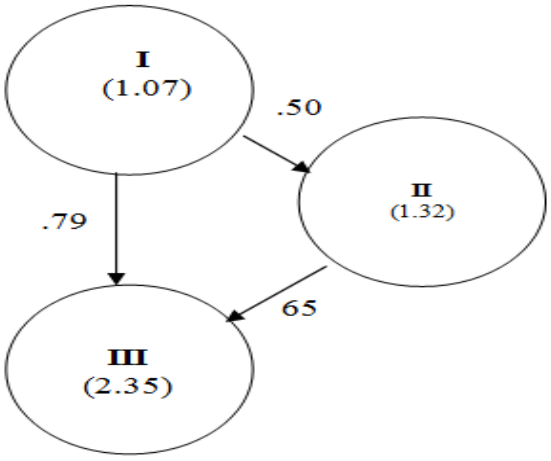

Figure 1. Cluster diagram showing the average intra and inter cluster distances (D = $\sqrt{D^2}$ values) of 18 tissue culture variants of strawberry. The values along the lines inter cluster distances and the values within the circle indicate intra cluster distances

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).

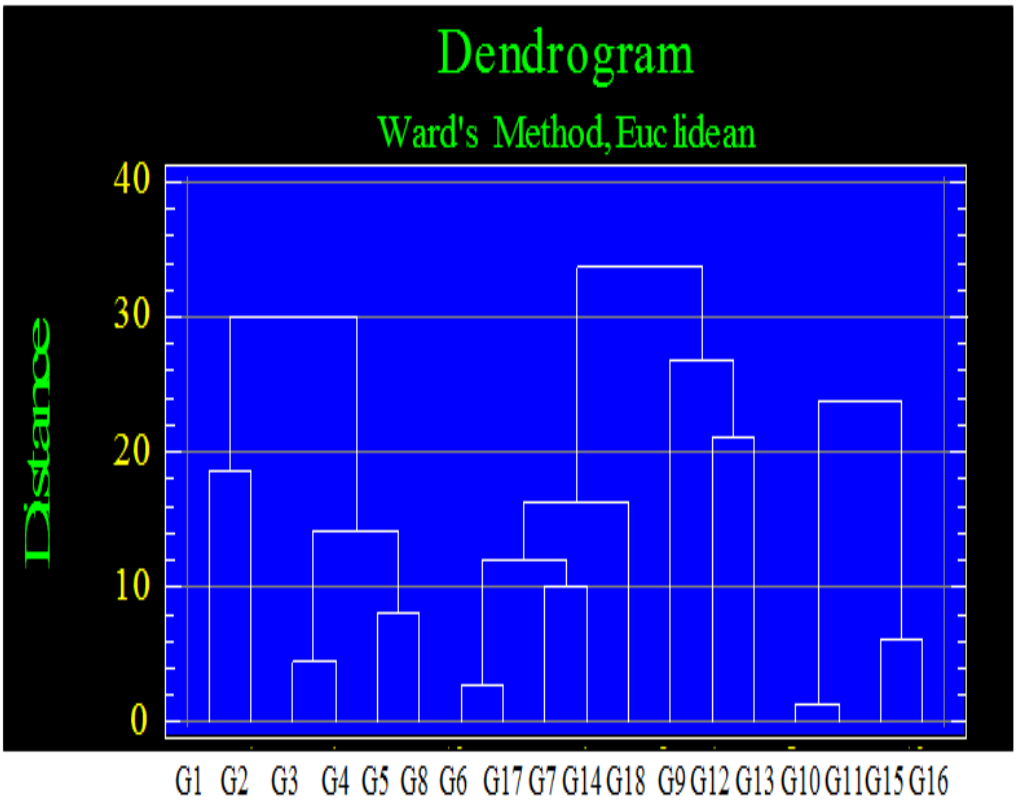

Figure 2. Dendrogram based on Ward's method indicated grouping of 18 tissue culture variants of strawberry

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.

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

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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