Scientia Horticulturae 122 (2009) 409–416

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Development and evaluation of *in vitro* somaclonal variation in strawberry for improved horticultural traits

M.K. Biswas [a,b,*], M. Dutt [c], U.K. Roy [b], R. Islam [b], M. Hossain [b,*]

[a] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, 430070, Wuhan, PR China
[b] Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh
[c] Citrus Research and Education Center, UF/IFAS,700 Experiment Station Road, Lake Alfred, FL 33850, United States

ARTICLE INFO

Article history:
Received 8 January 2009
Received in revised form 20 May 2009
Accepted 2 June 2009

Keywords:
In vitro culture
Micropropagation
Organogenesis
Somaclonal variation
Fragaria sp.

ABSTRACT

Strawberry cultivation is not popular in Bangladesh due to the unpredictable climatic conditions and lack of proper cultivars. Using somaclonal variation, several new promising selections were generated and evaluated for their flowering and fruiting ability, adaptability and sustainability. To induce variation, plants were regenerated using various tissue culture techniques. Our results suggested that a high concentration of BAP in culture medium successfully resulted in the induction of somaclonal variation. Among the tissue culture techniques adopted in this study, meristem culture was most effective for induction of somaclonal variation. Twenty five putative somaclones with better horticultural features were subsequently selected and field evaluated for three clonal generations. Several of the selections reverted back to their original phenotype within 2–3 vegetative propagations. Three of the stable selections were distinct from each other in terms of fruit and other horticultural characters, and have potential for commercial cultivation in Bangladesh.

1. Introduction

The strawberry (*Fragaria* sp. family Rosaceae) is an important and popular fruit produced in temperate and sub-tropical climates and is well liked due to its fragrance, taste and nutritional properties. There are more than twenty *Fragaria* species and numerous cultivars commercially cultivated in almost thirty seven countries (Gaafar and Saker, 2006). The cultivars vary remarkably in size, color, flavor, shape, degree of fertility, season of ripening, liability to disease and constitution of plant.

In vitro micropropagation is an important tool for crop improvement in plant breeding and is a common application for induction of somaclonal variation. Somaclonal variation can be defined as variation among plants regenerated from *in vitro* culture (Larkin and Scowcroft, 1988) and is a common phenomenon in plant tissue culture (Skirvin et al., 1993). Somaclonal variation is caused by changes in chromosome number (polyploidy or aneuploidy), damage to chromosomes (insertions, deletions, translocations, mutations, etc.), or changes in methylation of chromatin (Evans et al., 1984; Kaeppler and Phillips, 1993; Peschke and Phillips, 1992; Phillips et al., 1994). Chromosome damage can occur through crossing over, late replication in heterochromatic regions, transposable elements, point mutations, or chromosome rearrangements (Evans et al., 1984; Peschke and Phillips, 1992). Tissue culture activation of retrotransposons has also been demonstrated in *Oryza sativa* to induce somaclonal variation (Hirochika et al., 1996). The variation observed in tissue cultured clones are of two types, epigenetic and genetic. Changes in DNA methylation often give rise to epigenetic effects, which can cause expression of genes normally suppressed (Kaeppler et al., 2000). Epigenetic variation is often unstable and can disappear either after plants are removed from culture or within a few clonal or sexual generations (Kaeppler et al., 2000), while genetic variation is heritable (Skirvin et al., 1994). The success in applying somaclonal variation in plant breeding is therefore dependent on the genetic stability of the selected somaclones.

Somaclonal variation has been used as a useful tool in micropropagated bananas (Hwang and Ko, 1987; Sahijram et al., 2003). It was also observed in apple (Chevreau et al., 1998), blackberries (McPheeters and Skirvin, 1989) and peach (Hammerschlag and Ognianov, 1990) amongst other fruit crops. Strawberries too are amenable to *in vitro* somaclonal variation (Battistini and Rosati, 1991; Kaushal et al., 2004). Previous studies suggested that the concentrations of auxin in culture media and also number of subcultures are important factors for induction of somaclonal variation in an *in vitro* system (Gaafar and Saker, 2006). Somaclonal variation had both positive and negative impact in plant breeding. This resulted in the production of genetic variability which leads to

* Corresponding authors. Present address: National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, 430070, Wuhan, PR China. Tel.: +86 27 15926343329.
E-mail address: mkbcit@ymail.com (M.K. Biswas).

0304-4238/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2009.06.002

the expression of traits such as resistant to disease, herbicides and tolerance to environmental or chemical stress etc.

A majority of the strawberry cultivars are grown in the temperate parts of the world, although a few day-neutral cultivars can be grown in the sub-tropical regions. However, commercial cultivation is not popular in Bangladesh due to lack of proper cultivars. The objective of this study was to develop a new strawberry cultivar from *Fragaria* × *ananassa* Duch. through the induction of somaclonal variation and selection of stable variants which could be suitable for commercial cultivation. In this study different tissue culture techniques were applied to induce somaclonal variation. RAPD was carried out to investigate genetic stability of somaclones and field evaluation was performed to characterize the somaclonal variants up to three subsequent clonal generations.

2. Materials and methods

2.1. Plant material preparation

Fragaria × *ananassa* Duch. was selected for the establishment of *in vitro* cultures. Runner tips were collected from field grown stock plants and soaked in water containing a drop of Tween 80. Tips were subsequently rinsed under running tap water for 20 min to remove the surface contaminants followed by several rinses with sterile distilled water. The tips were then surface sterilized by dipping in 0.1% $HgCl_2$ solution (w/v) for 5 min. Terminal buds (3–4 mm), were dissected and cultured on semisolid MS (Murashige and Skoog, 1962) basal medium supplemented with 3% sucrose and 0.8% agar. The pH was adjusted to 5.8. This basal medium was used in subsequent experiments with addition of appropriate plant growth regulators. The explants were incubated in a growth chamber under 16/8 h light/dark cycle at 25 ± 2 °C. The regenerating shoots, leaves and nodal segments obtained from *in vitro* culture were used for subsequent experiments. A detailed experimental work flow is illustrated in Fig. 1.

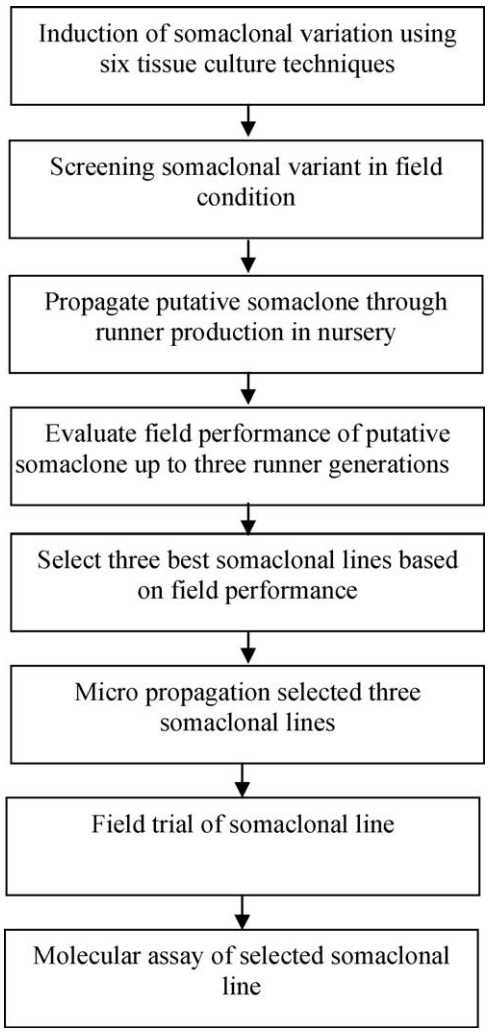

Fig. 1. Work flow of the experiment.

2.2. Treatments for induction of somaclones

2.2.1. Meristem culture

Meristem was isolated from 2 weeks old *in vitro* grown plants. Isolated meristems (0.3–0.5 mm) devoid of immature leaves and leaf primordia were excised using a binocular stereoscopic microscope. The meristems were subsequently transferred onto a filter paper bridge in test tubes containing liquid MS medium supplemented with 0.5 mg l^{-1} GA_3 for primary establishment. After 3–4 weeks of culture, shoots were subcultured onto semisolid MS medium supplemented with 0.5 mg l^{-1} GA_3 and 0.1 mg l^{-1} IBA, which were added for shoot and root induction.

2.2.2. Micropropagation

For micropropagation 2 weeks old *in vitro* grown plants were used. Shoots were cultured on MS medium supplemented with 2.0 mg l^{-1} BAP and 0.5 mg l^{-1} kinetin with a 4 weeks subculture interval. After 2nd and 12th subcultures, microshoots were excised and cultured on half strength MS medium for root induction and generation of microplantlets.

2.2.3. Direct shoot organogenesis from leaf

Young leaves from 3 weeks old *in vitro* plants were cultured on MS basal medium supplemented with 6 mg l^{-1} BAP. After 3 months regenerated plantlets were transferred onto root induction medium for generation of microplantlets.

2.2.4. Callus culture and subsequent plant regeneration

Young, fully expanded leaves and nodal segments were collected from 4-weeks old *in vitro* plants. The leaves were sliced into 1.0 cm^2 square pieces. Cut leaves as well as longitudinally sectioned nodes were placed on MS basal medium supplemented with 3 mg l^{-1} 2,4-D for callus induction. After 2 weeks, induced calli were subcultured onto shoot regeneration medium (MS media supplemented with 0.5 mg l^{-1} BAP). After another 12 weeks of culture, regenerated shoots were transferred on to root induction medium (half strength of MS media) and kept in growth chamber for microplantlet development.

2.2.5. Somatic embryogenesis

Somatic embryos were induced from leaves derived callus. Three weeks old leaves derived calli (as explain earlier) were transferred onto somatic embryogenesis medium and placed in dark. This medium consisted of 1.0 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BAP, supplemented with 25% proline. Somatic embryos were isolated and cultured onto shoot and root development medium, as explained earlier, and kept under 16/8 h light/dark cycle at 25 ± 2 °C.

2.3. Screening for somaclones from regenerated plant population

Well rooted *in vitro* plants were removed from culture vessel and washed to remove all traces of agar. Plants were subsequently transferred into pots containing sterilized sand and garden soil (1:1 ratio) and kept in growth chamber for 2 weeks. Hardened plantlets were transferred to nursery and maintained. Vigorous

plants were subsequently transplanted to the experimental field. In this study, plants regenerated from a particular tissue culture technique considered as a population. As a result we induced six populations those were arising from six tissue culture techniques as explained earlier.

Runner derived explants of *Fragaria* × *ananassa* Duch. was used as control plants in the field experiments. The experimental field design was a completely randomized block with three replications. Each replication consist one population arising from a particular tissue culture technique as explained earlier. In order to screen somaclonal variants from the six regenerated population, quantitative and qualitative phenotypic changes were considered. Phenotypic changes were observed among population up to fruiting and data were collected for canopy size, number of runners, days to flowering, number of flower per plant, number of fruit per plant, average fruit weight, and percent of plants surviving the summer.

2.4. Propagation of putative somaclones and selection of desirable somaclone

Twenty five somaclonal variant were identified from the six population based on the improved traits over the control. For the selection of those twenty five putative somaclone were considered the six important agronomic traits such as, number of flower cluster per plant, number of flower per cluster, number of fruit per cluster, average fruit weight, number of runner production, fruit shape. Putative somaclones were propagated by runner and maintained in nursery up to next cropping season. Runner derived plantlets were sown in field. The experiment was laid out in the randomized complete block design with three replications. Plantlets were planted at 35 cm (plant to plant distance) distance on 50 cm wide and 350 cm long raised bed. The soil of the experimental plots was specially amended with cowdung and coarse sand (1:1 v/v). Urea–TSP–MP was applied at the rate 75–60–75 kg/ha. The entire dose of TSP, MP and 50% urea was applied as top dressing into two equal installments at 30 and 60 days after planting. Intercultural operations such as earthing up, weeding and mulching were done as required. Field performance was evaluated up to three clonal generations. Based on the field performances of three clonal generations we identified three potential somaclones for commercial cultivation with improved agronomic traits.

2.5. Micropropagation of selected somaclone

Runner tips collected from selected somaclones were micropropagated as previously described. These plants were again hardened and re-evaluated under field condition to evaluated stability and performance.

2.6. Molecular assay for selected somaclonal variant

Leaves were collected from selected three somaclonal variants and control. Total genomic DNA was extracted from fresh leaves, using CTAB method as previously described by Sue et al. (1997).

Fifteen random primers were evaluated using polymerase chain reaction (PCR) as detailed in Table 1. The PCR reaction mix contained 25 ng genomic DNA as template. PCR cycle conditions were: 1 cycle at 92 °C for 3 min, 45 cycles of 92 °C for 30 s, 35 °C for 1 min, 72 °C for 2 min with a final cycle of 10 min at 72 °C. Amplified DNA products were separated by electrophoresis in a 1.5% (w/v) agarose gels in 0.5× TBE buffer, stained with 0.5 μg ml^{-1} of ethidium bromide (EtBr) and photographed under exposure to UV light.

Table 1
List of primer used in this study.

SL	Primer ID	Nucleotide sequence (5′–3′)
1	A-03	AGTCAGCCAC
2	A-15	TTCCGAACCC
3	AA-01	AGACGGCTCC
4	AA-19	TGAGGCGTGT
5	AF-06	CCGCAGTCTG
6	AL-04	ACAACGGTCC
7	D-16	AGGGCGTAAG
8	E-12	TTATCGCCCC
9	G-01	GGGAATTCGG
10	G-10	CCGATATCCC
11	H-14	ACCAGGTTGG
12	I-04	CCGCCTAGTC
13	O-05	CCCAGTCACT
14	T-04	GTCCTCAACG
15	J-19	GGACACCACT

2.7. Data collection and statistical analysis

In order to evaluate the field performance of somaclonal variant we measured both quantitative and qualitative traits. Quantitative traits were measured from five randomly selected plants from each replication. Qualitative traits such as fruit shape, fruit texture, leaves color, leaf shape were measured by visual observation. Quantitative data are subjected to the statistical analysis. Means were separated with Duncan's multiple range test ($P < 0.05$) were appropriate. Statistical parameters like percentage, mean, standard error were estimated by using the Microsoft Office Excel program and Duncan's multiple range test were estimated using SAS version 6.11 (SAS Institute, Cary, U.S.A.).

3. Results

Somaclonal variation was induced using *in vitro* micropropagated plants. Microplantlets were regenerated through different tissue culture techniques including micropropagation, direct shoot organogenesis from leaf, indirect organogenesis via callus culture and somatic embryogenesis (Fig. 2). Seventy five explants were used of each regeneration treatments. Our results showed that maximum number of plants regenerated from meristem culture. Direct organogenesis using cut leaf segments produced the lowest number of regenerants (Fig. 3). However, there was no statistical difference in regeneration of plants between the meristem culture and micropropagation. These treatments were however significantly different from others (leaf organogenesis, callus culture and somatic embryogenesis). There was also no difference in regeneration from callus culture or via somatic embryogenesis. The regenerated plantlets were subsequently evaluated for somaclonal variation under field conditions. Qualitative and quantitative traits observed in the putative somaclones and quantitative data are summarized in Table 2. In most cases somaclones were more vigorous than control. However, different morphological characters differed in the different populations and there was no set trend. We observed several phenotypic differences in the *in vitro* derived plants such as leaf shape in most plants regenerated from the twelfth subculture, direct regeneration from leaves, callus culture or somatic embryogenesis (Fig. 4H–N). Leaf petiole was shorter and thicker and lamina was comparatively bigger than control. Most of the leaves were lighter green and number of leaves was less than control. Plants regenerated via somatic embryogenesis had a significantly bigger canopy size while numbers of runners were more in plants derived from meristem culture (Fig. 4A–G). All the *in vitro* derived plants were late to flower when compared to control. However, most of the *in vitro* plants had significantly more flowers per plant and number of fruits. In

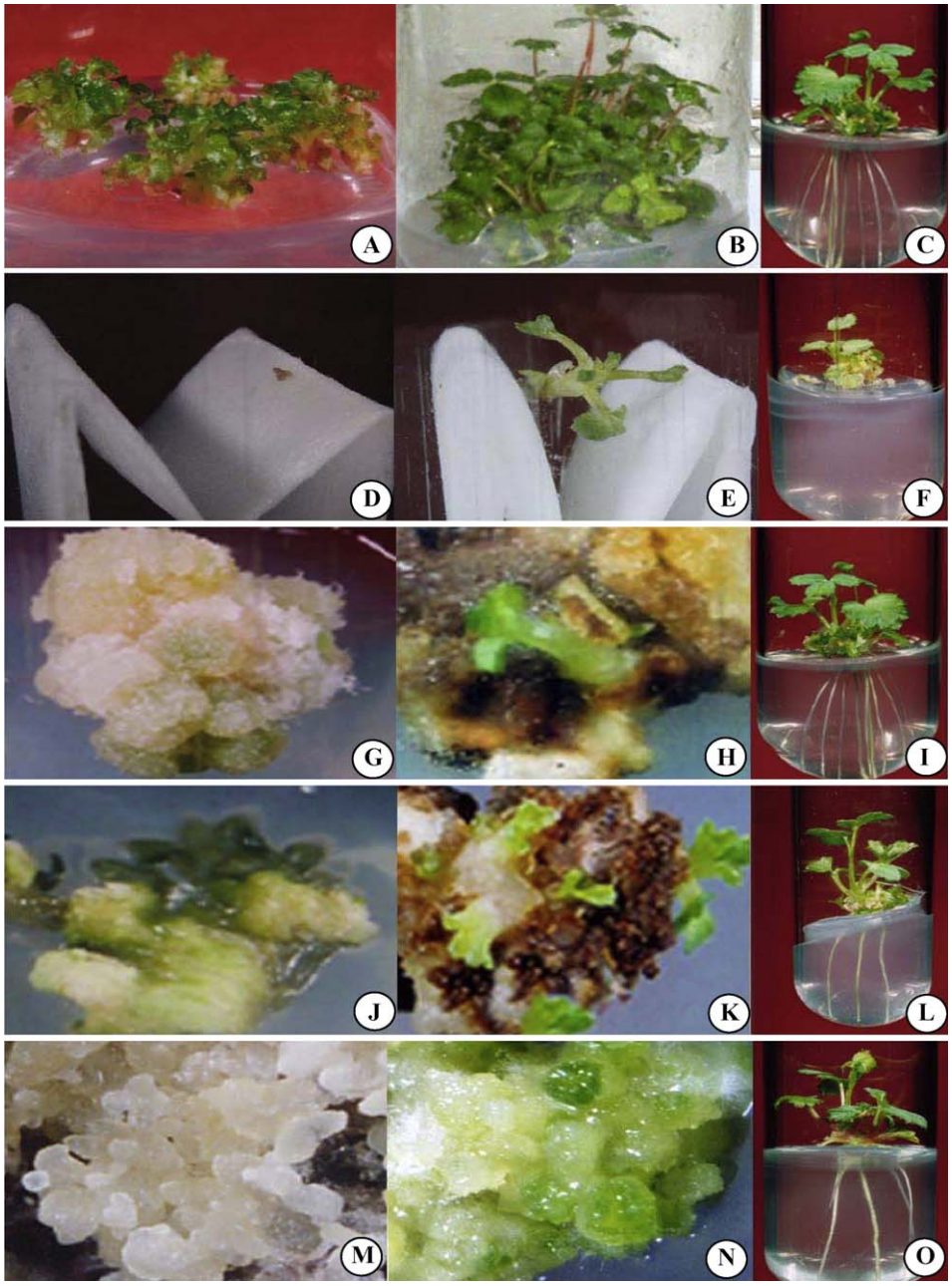

Fig. 2. Induction of somaclone using different tissue culture methods. A–C: micropropagation; D–F: meristem culture; G–I: plant regeneration via callus culture; J–L: direct plant regeneration from leaf; M–O: plant regeneration via somatic embryogenesis.

somaclones, flower cluster was more branched than control but number of cluster per plant was less than control. Stalk of flower cluster was comparatively thin in control and sepal was shorter than somaclones (Fig. 4V and W). A clear variation was observed for fruit shape among the putative somaclones. More distinguishable variation was found in somatic embryogenesis derived somaclones, which produced bigger fruit than other somaclones and control. Also, most of the somaclones produced fruits with a

Table 2
Field performance of tissue culture derived clones.

Methods to obtain somaclone	Canopy size (cm)	Number of runner	Days to flowering	Number of flower per plant	Number of fruit per plant	Average single fruit weight (g)	Percent of summer survival
Meristem culture	24.83 ± 0.87^{bc}	7.50 ± 0.76^{a}	79.50 ± 3.41^{b}	29.67 ± 1.20^{a}	9.33 ± 0.56^{a}	19.39 ± 1.67^{a}	51.67 ± 2.03^{a}
Second subculture	24.33 ± 0.80^{bc}	7.67 ± 0.76^{a}	78.83 ± 2.48^{b}	28.50 ± 2.64^{a}	9.17 ± 0.70^{a}	19.74 ± 2.43^{a}	53.67 ± 1.45^{a}
Twelfth subculture	25.33 ± 0.84^{b}	7.83 ± 0.70^{a}	78.33 ± 1.91^{b}	27.17 ± 1.30^{a}	9.17 ± 0.48^{a}	19.82 ± 1.82^{a}	54.50 ± 2.35^{a}
Direct organogenesis from leaf	22.17 ± 0.95^{c}	3.83 ± 0.91^{b}	88.17 ± 3.20^{a}	29.33 ± 2.40^{a}	8.83 ± 0.95^{a}	18.45 ± 2.11^{a}	33.67 ± 3.44^{b}
Regeneration from callus culture	22.33 ± 0.67^{c}	4.83 ± 0.83^{b}	89.67 ± 1.91^{a}	28.17 ± 2.39^{a}	8.50 ± 0.85^{a}	19.39 ± 1.94^{a}	36.00 ± 2.31^{b}
Somatic embryogenesis	30.83 ± 0.98^{a}	5.67 ± 0.71^{ab}	84.00 ± 2.99^{ab}	21.50 ± 0.76^{b}	5.50 ± 0.76^{b}	18.70 ± 1.82^{a}	35.83 ± 1.40^{b}
Control	$24.50 \pm 1.18b^{c}$	3.67 ± 0.88^{b}	65.17 ± 2.36^{c}	22.00 ± 0.82^{b}	9.50 ± 0.67^{a}	12.20 ± 1.64^{b}	38.33 ± 2.12^{b}

Means with same letter in a column are not significantly different at $P \geq 0.05$ based on DMRT test.

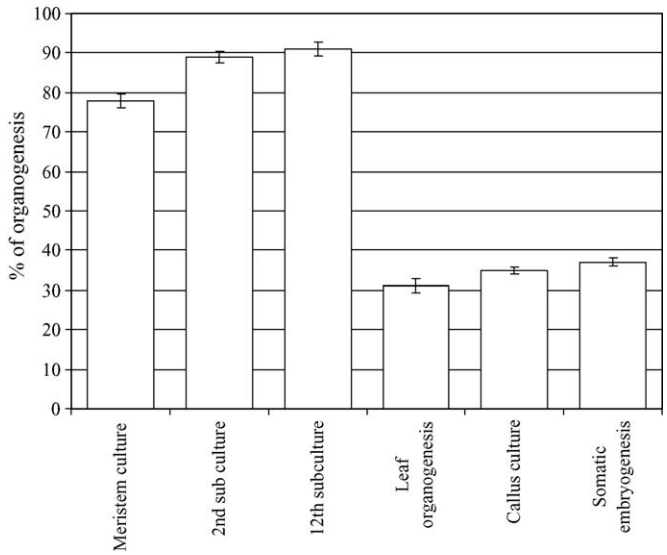

Fig. 3. Percentage of plant regeneration from different tissue culture methods.

different texture when compared to control fruits (Fig. 4O–U). Numbers of fruit from *in vitro* meristem derived plants were statistically similar to control. The average single fruit weight was also higher in these plants when compared to control. Over 50% of the plants derived from meristem tip culture also survived the summer compared to 38% for control.

Frequency of variant phenotypes in regenerated plant population is presented in Table 3. Most of the plants were similar to control. However, we did observe variation in the different populations. The results reveal that frequency of leaf structure variation was highest in somatic embryo derived somaclones. Flower cluster as well as fruit size variation was highest in the plants regenerated from the 12th subculture. Overall, maximum variation was observed *in vitro* plants generated from the 12th subculture.

Based on the field performance initially 25 putative somaclones from the six populations (Table 3) were selected for evaluation of their performance under field conditions. Those somaclones were selected considering the important agronomical traits such as number of flower cluster per plant, number of flower per cluster, number of fruit per cluster, average fruit weight, number of runner production, fruit shape. We decided to evaluate these somaclones for three clonal generations. The putative clonal lines were propagated through rooted runners in the nursery. The results of the field performance of selected lines revealed that 20% (5 out of 25) putative somaclonal lines did not survive in the field under varying climatic conditions. Also, 32% (8 out of 25) clonal lines had poor horticultural performance in subsequent generations and 36% (9 out of 25) reverted back to the original phenotype. On the basis of field performance three distinct somaclonal lines were selected. These selected somaclones were micropropagated to evaluate their genetic stability and subsequently re-evaluated for field performance. To examine the extent of stability of variation of the selected three clonal lines, these lines were propagated by rooted runners and evaluated for a further two successive clonal generations. Data were collected for the three important agronomic traits from the two successive clonal generations. Mean performance and analysis of variance were estimated for three agronomic important traits of three

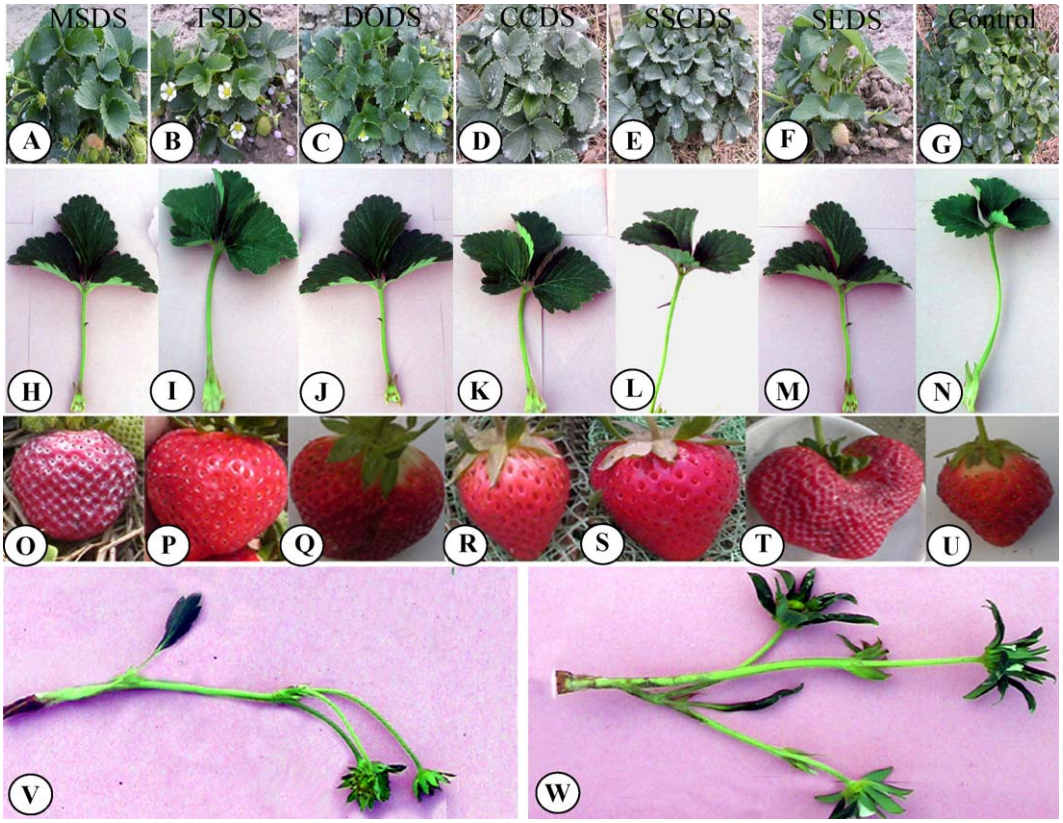

Fig. 4. Somaclonal variation in quantitative traits. MSDS-meristem derived somaclone, TSDS-12th subculture derived somaclone, DODS-direct organogenesis derived somaclone, CCDS-callus culture derived somaclone, SSCDS-2nd subculture derived somaclone and SEDS-somatic embryo derived somaclone; canopy size A–G, leaves morphology H–N, fruit morphology O–U and flower cluster variant V–W.

Table 3
Frequency of variant phenotypes in regenerated plants population.

Methods to obtain somaclone	Total number of regenerated plants	Leaves structure variant	Flower cluster variant	Fruit size variant	Total variant	Number of selected putative somaclones
Meristem culture	568	15(2.64)	4(0.70)	33(5.81)	52(9.15)	4
Second subculture	943	9(0.95)	4(0.42)	23(2.44)	36(3.82)	1
Twelfth subculture	1154	22(1.91)	19(1.65)	79(6.85)	120(10.40)	10
Direct regeneration	543	8(1.47)	7(1.29)	25(4.60)	40(7.37)	2
Callus culture	673	11(1.63)	7(1.04)	34(5.05)	52(7.73)	2
Somatic embryogenesis	745	19(2.55)	11(1.48)	49(6.58)	79(10.60)	6
Total	4626	84(1.82)	62(1.34)	243(5.25)	389(8.41)	25

Numbers in parenthesis are percentage of somaclonal variants.

Table 4
Field performance of selected three somaclones from six populations.

Clone	Clone derived from	No. of flower per plant	No. of fruit per plant	Average fruit weight	Summer survival
Variant 1	Meristem culture	18^{a}	11^{b}	12.18^{c}	79%
Variant 2	12th subculture	16^{b}	10^{b}	18.63^{b}	82%
Variant 3	Somatic embryogenesis	12^{c}	8^{c}	21.29^{a}	81%
Control		20^{a}	13^{a}	10.87^{c}	–
Analysis of variance					
Somaclonal variant (S)		**	**	***	
Clonal generation (C)		NS	NS	NS	
S × C		**	**	**	

Means with same letter in a column are not significantly different at $P \geq 0.05$ based on DMRT test. *** and ** significant at 0.1% and 1.0% levels, respectively; NS = non-significant.

somaclonal varints and results are presented in Table 4. Significant differences were found among the studied colons in regards to yield contributing traits such as number of flower per plant, number of fruits per plant and average fruit weight. Mean was significantly different in the traits number of flower per plant, number of fruits per plant and average fruit weight among the studied somaclonal lines. Analysis of variance revealed that the somaclonal lines (S) showed highly significant difference in all the traits (Table 4). The clonal generations (C) had no significant difference for the studied traits. On the other hand interaction of S × C showed significant differences among all the study traits. Stable phenotypic variation was clearly observed in the leaf morphology, flower cluster branching and fruit shape (Fig. 5). We were able to co-relate these phenotypic changes with changes in the DNA banding pattern following RAPD as differences were observed in the selected three clonal lines (Fig. 6). We examined somaclonal variation among the selected three somaclonal lines by RAPD using 15 random primers. Out of 15 random primers tested only eight primers successfully produced scoreable RAPD bands for all three clonal lines. Five primers produced polymorphic RAPD profile. Although not all clonal line gave unique products with every primer. Some primers e.g. AL-04 and O-05 revealed more polymorphic than others.

4. Discussion

In our present investigation, somaclonal variation was induced via different tissue culture methods in strawberry. Our objective was to develop new cultivars adaptive to the semi arid regions in Bangladesh. *In vitro* tissue culture techniques have been known to be an important tool in the induction of variation leading to the development of new plant genotype (Larkin and Scowcroft, 1988; Kuksova et al., 1997). This source of variability is considered as a useful tool for geneticists and plant breeders (Amzad et al., 2003). In several crop species somaclonal variation was documented based on evaluation of phenotypic variation and RAPD profiles (Watanabe et al., 1998; Jayanthi and Mandal, 2001; Martins et al., 2004; Saker et al., 2005). In strawberry, earlier studies demonstrated that somaclones could be regenerated from leaf derived callus culture (Popescu et al., 1997), somatic embryogenesis (Donnoli et al., 2001) and callus culture of leaves and petiole irradiated with gamma rays (Kaushal et al., 2004). In the present investigation, we induced somaclonal variation using different tissue culture techniques, i.e. meristem culture, direct organogenesis from leaves, callus culture and somatic embryogenesis. We identified three somaclones on the basis of the variation of phenotypic polymorphic characters when compared to control. The genetic variation was also observed in these somaclones using RAPD.

For the induction of somaclonal variation, a high concentration of BAP was applied in order to regenerate adventitious buds from explants. The high requirement of BAP for efficient shoot regeneration has also been observed by other researchers. For example, Popescu et al. (1997) reported that 4.4 mg l^{-1} BAP was best for direct shoot regeneration from strawberry leaf explants while Kaushal et al. (2004) observed 2 mg l^{-1} BAP with 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} kinetin as suitable for plant regeneration from leaf and petiole derived calli. In higher amounts, BAP induces variation and has been extensively used to induce somaclonal variation in different crops (Chatterjee and Gupta, 1998; Manjula et al., 2000; Ahmed et al., 2002; Amzad et al., 2003; Kaushal et al., 2004). In our studies, culturing explants in a BAP rich medium resulted in successful induction of variation among the regenerated plants. Our results also revealed that regenerated plants were more vigorous than control plants, and which could prove to be useful in order to survive the semi arid conditions in certain parts of Bangladesh. Single fruit weight, fruit size and in many of the cases percent of summer survival was higher than control. The highest percent of somaclonal variation was found in plants that had been derived through somatic embryogenesis. Several variants reverted back to the parental phenotype in subsequent generations indicating their epigenetic nature.

Random amplification of polymorphic DNA (RAPD) is an important tool which has been extensively used to identify

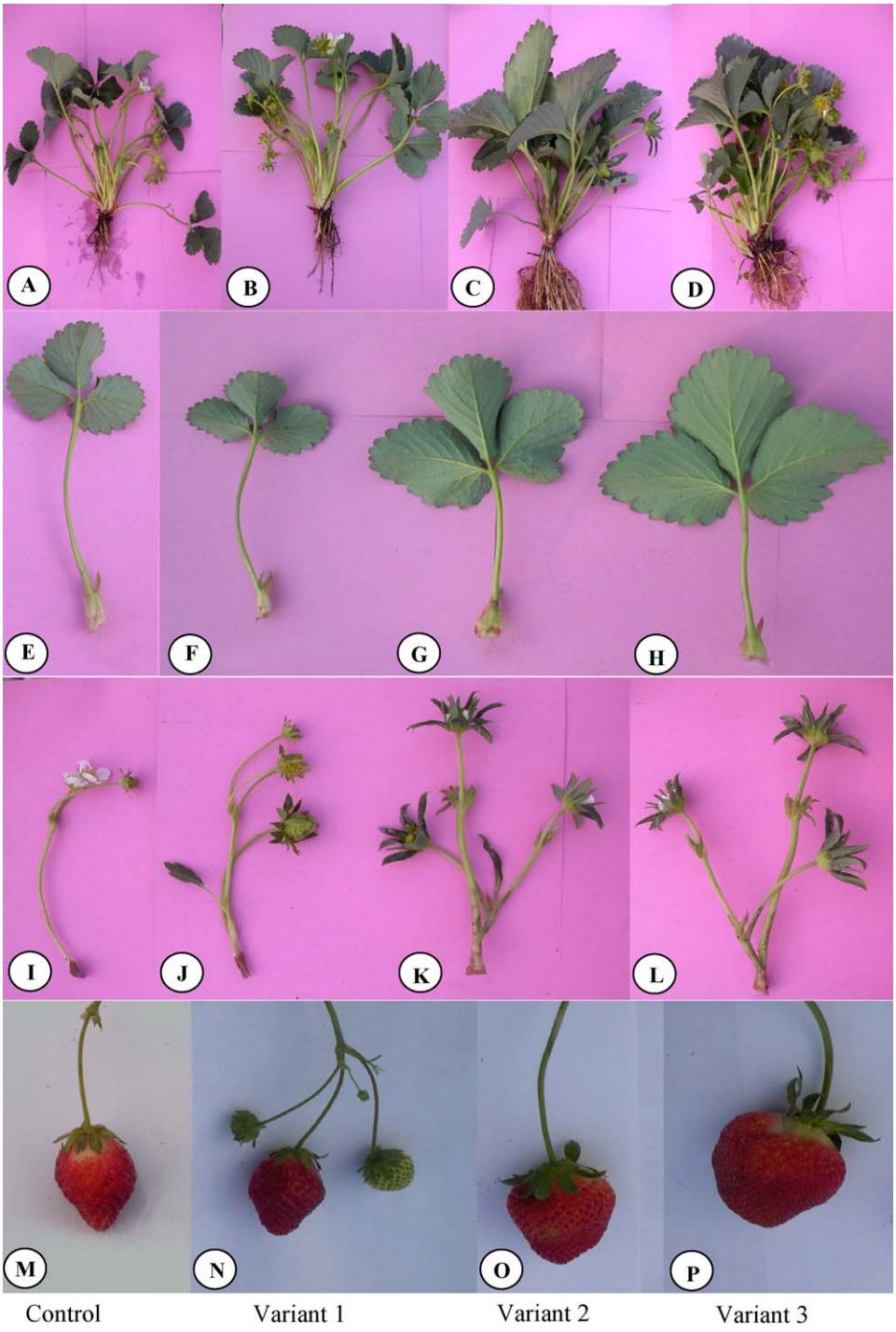

Fig. 5. Comparison of phenotypic changes of the three selected somaclonal variant with the control. A–D: plant size variant, E–H: leaf shape variant, I–L: flower cluster variant, M–P: fruit shape variant.

polymorphism among the genotypes. It has also been used for characterization of different strawberry cultivars (Gaafar and Saker, 2006). RAPD offers great potential for generating large number of markers representing a random sample of genome and has efficiently been used to give reliable and reproducible results for estimating the genetic variation (Chen et al., 1998). Brown et al. (1993) found major differences in wheat plants derived from protoplast using RAPD while Piccioni et al. (1997) found new RAPD markers in somatic embryos derived from alfalfa callus. We used RAPD to characterize our polymorphic phenotypic plants. Polymorphic banding pattern was observed with five primer and was most prominent with the primers AL-04 and O-05, as indicated in Fig. 6. This confirmed that variation observed in three selected somaclones were genetic. These somaclones were distinct from each other in terms of fruit and other horticultural characters, and have potential for commercial cultivation in Bangladesh. Our results also demonstrated that the variation which was observed in tissue culture derived clones were both genetic and epigenetic. Such genetic variants, merit detailed horticultural investigation for evaluation of their potential as a new cultivar for Bangladesh.

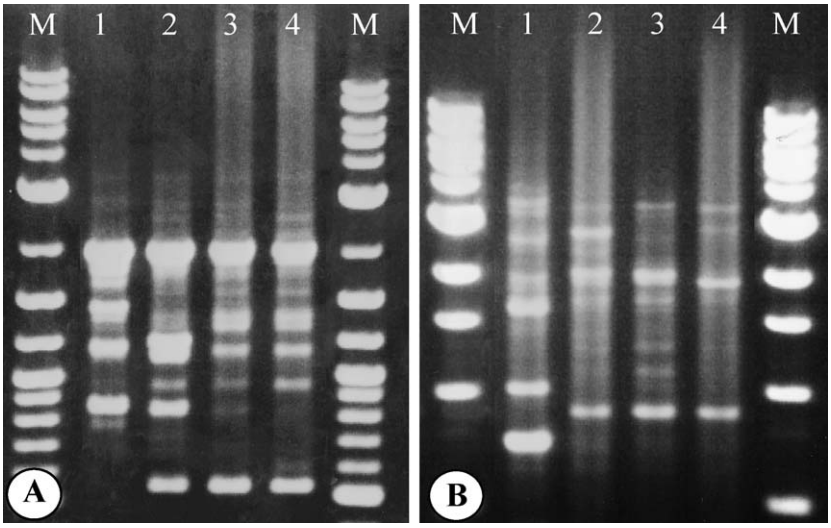

Fig. 6. RAPD profile of primer AL-04 (A) and O-05 (B). M: marker, 1: variant 3, 2: variant 2, 3: variant 1 and 4: control.

References

Ahmed, J., Nedra, E., Claire, K., Jacques, D., 2002. Morphological and hormonal characterization of strawberry *in vitro* plants raised through axillary or stipular adventitious shooting. Plant Growth Regul. 38, 273–278.

Amzad, H., Kunihiko, K., Mineo, M., Kazuhiro, N., 2003. Somaclonal variation of regenerated plants in chili pepper (*Capsicum annuum* L.). Euphytica 130, 233–239.

Battistini, C., Rosati, P., 1991. In vitro evaluation of somaclonal strawberry (*Fragaria* × *Ananassa* "Brighton") variants for susceptibility to *Phytophthora cactorum*. In: Dale, A., Lubby, W.W. (Eds.), The strawberry into the 21st Century. Timber Press, Portland, Or., pp. 121–123.

Brown, D.C.W., Watson, E.M., Pechan, P.M., 1993. Induction of desiccation tolerance in microspore-derived embryos of *Brassica napus*. In Vitro Cell Dev. Biol. 29, 113–118.

Chatterjee, B., Gupta, P.D., 1998. Induction of somaclonal variation by tissue culture and cytogenetic analysis in *Oryza sativa* L. Biol. Plant 40 (1), 25–32.

Chen, W.H., Chen, T.M., Fu, Y.M., Hsieh, R.M., Chen, W.S., 1998. Studies on somaclonal variation in *Phalaenopsis*. Plant Cell Rep. 18, 7–13.

Chevreau, E., Brisset, M.N., Paulin, J.P., James, D.J., 1998. Fire blight resistance and genetic trueness-to-type of four somaclonal variants from the apple cultivar. Euphytica 104 (3), 199–205.

Donnoli, R., Sunseri, F., Martelli, G., Greco, I., 2001. Somatic embryogenesis, plant regeneration and genetic transformation in *Fragaria* spp. Acta Hortic. 560, 235–240.

Evans, D.A., Sharp, W.R., Medina-Filho, H.P., 1984. Somaclonal and gametoclonal variation. Am. J. Bot. 71 (6), 759–774.

Gaafar, R.M., Saker, M.M., 2006. Monitoring of cultivars identity and genetic stability in strawberry varieties grown in Egypt. World J. Agric. Sci. 2 (1), 29–36.

Hammerschlag, F.A., Ognianov, V., 1990. Somaclonal variation in peach: screening for resistance to *Xanthomonas campestris* pv. *pruni* and *Pseudomonas syringae* pv *syringae*. Acta Hortic. 280, 403–408.

Hirochika, H., Sugimoto, K., Otsuki, Y., Tsugawa, H., Kanda, M., 1996. Retrotransposons of rice involved in mutations induced by tissue culture. Proc. Natl. Acad. Sci. U.S.A. 93 (15), 7783–7788.

Hwang, S.C., Ko, W.H., 1987. Somaclonal variation of bananas and screening for resistance to Fusarium wilt. In: Persley, G.J., De Langhe, E.A. (Eds.), Banana and Plantain Breeding Strategies. ACIAR Proceedings, No. 21, Canberra, pp. 151–156.

Jayanthi, M., Mandal, P.K., 2001. Plant regeneration through somatic embryogenesis and RAPD analysis of regenerated plants in *Tylophora indica* (Burm. F. Merrill.). In Vitro Cell Dev. Biol. Plant 37, 576–580.

Kaeppler, S.M., Phillips, R.L., 1993. Tissue culture-induced DNA methylation variation in Maize. Proc. Natl. Acad. Sci. U.S.A. 90 (19), 8773–8776.

Kaeppler, S.M., Kaepller, H.F., Rhee, Y., 2000. Epigenetic aspects of somaclonal variation in plants. Plant Mol. Biol. 43, 179–188.

Kaushal, K., Nath, A.K., Kaundal, P., Sharma, D.R., 2004. Studies on somaclonal variation in strawberry (*Fragaria* × *ananassa* duch.) cultivars. Acta Hortic. 662, 269–275.

Kuksova, V.B., Piven, N.M., Gleba, Y.Y., 1997. Somaclonal variation and invitro induced mutagenesis in grapevine. Plant Cell Tissue Org. Cult. 49, 17–27.

Larkin, P.J., Scowcroft, W.R., 1988. Somaclonal variation—a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60, 197–214.

Manjula, S.M., Kuruvinashetti, M.S., Chandrashekhar, C.H., 2000. Regeneration, establishment and evaluation of somaclones in *Sorghum bicolor* (L.) Moench. Euphytica 115, 173–180.

Martins, M., Sarmento, D., Oliveira, M.M., 2004. Genetic stability of micropropagated almond plantlets, as assessed by RAPD and ISSR markers. Plant Cell Rep. 23, 492–496.

McPheeters, K.D., Skirvin, R.M., 1989. Somaclonal variation among ex vitro 'Thornless Evergreen' trailing blackberries. Euphytica 42, 155–162.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Plant Physiol. 15, 473–497.

Peschke, V.M., Phillips, R.L., 1992. Genetic implications of somaclonal variation in plants. Adv. Genet. 30, 41–75.

Phillips, R.L., Kaeppler, S.M., Olhoft, P., 1994. Genetic instability of plant tissue cultures: breakdown of normal controls. Proc. Natl. Acad. Sci. U.S.A. 91 (12), 5222–5226.

Piccioni, E., Barcaccia, G., Falcinelli, M., Standardi, A., 1997. Estimating somaclonal variation in axillary branching propagation and indirect somatic embryogenesis by RAPD fingerprinting. Int. J. Plant Sci. 158, 556–562.

Popescu, A.N., Isac, V.S., Coman, M.S., Radulescu, M.S., 1997. Somaclonal variation in plants regenerated by organogenesis from callus culture of strawberry (*Fragaria* × *Ananassa*). Acta Hortic. 439, 89–96.

Sahijram, L., Soneji, J.R., Bollama, K.T., 2003. Analyzing somaclonal variation in micropropagated bananas (Musa spp.). In Vitro Cell Dev. Biol. Plant 39 (6), 551–556.

Saker, M.M., Adawy, S.S., Mohamed, A.A., El-Itriby, H.A., 2005. Monitoring of cultivar identity in tissue culture-derived date palms using RAPD and AFLP analysis. Biol. Plant 50, 198–204.

Skirvin, R.M., Mcpheeters, K.D., Norton, M., 1994. Sources and frequency of somaclonal variation. Hortic. Sci. 29 (11), 1232–1237.

Skirvin, R.M., Norton, M., Mcpheeters, K.D., 1993. Somaclonal variation: has it proved useful for plant improvement? Acta Hortic. 336, 333–340.

Sue, P., Bailey, L.G., Baum, B.R., 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Reptr. 15 (1), 8–15.

Watanabe, A., Araki, S., Kobari, S., Sudo, H., Tsuchida, T., Uno, T., Kosaka, N., Shimomura, K., Yamazaki, M., Saito, K., 1998. *In vitro* propagation, restriction fragment length polymorphism and random amplified polymorphic DNA analyses of *Angelica* plants. Plant Cell Rep. 18, 187–192.