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Morphological and Genetic Characterization of Micropropagated Field Grown Plants of *Aloe vera* L.

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Abstract

A large scale shoot multiplication from apical meristem in *Aloe vera* L. was obtained on MS with 35.5 μM BAP, 9.8 μM IBA and 81.4 μM adenine sulphate. Fifty micropropagated plants were successfully transferred to the field and maintained to attain reproductive phase. Field evaluation of micropropagated plants is important to assess predicted clonal fidelity. Exo-morphological evaluation of *Aloe* plants, identified three seed setting plants, designated as somaclones. Seeds were viable and germinated (70.58%) *in vitro*. Although chromosome number and morphology of somaclones were identical with the donor plants their RAPD profiles and ITS-1 sequences were different from donor plant. This study reports Seed setting somaclones in *Aloe vera,* for the first time which may serve as new genetic resource for biotechnological improvement.

Introduction

Aloe vera L. is an ancient perennial medicinal herb of Asphodelaceae. The plant is renowned for its leaf gel which has proven its efficacy against external burns, skin disorders (Nia et al. 2004), gastrointestinal malfunction (Grindlay et al. 1986, Shelton 1991) and prevents UV-B induced immune suppression of the skin (Strickland et al. 1994). This medicinal plant is also used as anti-cancer (Winters et al. 1981), anti-oxidant (Hu et al. 2003), anti-inflammatory (Davis et al. 1994), anti-diabetic agent (Davis et al. 1989). The leaf gel of *A. vera* is composed of a large number of nutritionally enriched compounds of which aloin, an anthraquinone glycoside, is identified as the most important bioactive compound.

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Although *A. vera* produces a large number of healthy bisexual flowers, it reproduces via vegetative propagation. Naturally producing viable seeds has been reported in a few species of *Aloe*, such as *A. arborescens* (Amoo et al. 2012), *A. saponaria* (Velasquez-Arenas and Imery-Buiza 2008). Conventionally, plant breeders recombine the desired genes from plant varieties and related species by sexual hybridization and develop new cultivars with the desirable traits. However, in case of *A. vera* sexual hybridization is not possible due to lack of fertile seed production.

Micropropagation technique offers great potential for plant improvement, if genetically uniform, quality plants can be regenerated in large numbers independent of seasonal and other environmental variables. Generation of micropropagated plants of *A. vera* through *in vitro* culture and their field transfer has been reported by many workers (Wenping et al. 2004, Liao 2004, Aggarwal and Barna 2004, Rathore et al. 2011), but very few of them have studied the long term evaluation of field grown micropropagated plants in this species, even though it is essential for commercial production. Tissue culture induces variation which can result in a range of genetically stable variation, useful in crop improvement (Larkin and Scowcroft 1981). The occurrence of somaclones has been reported in many *in vitro* regenerated plants (Jaljai et al. 2006, Minano 2009). Evaluation of clonal fidelity is vital parameter to test the *in vitro* grown plants for commercial use and trade. Chromosomal analysis coupled with DNA fingerprinting has emerged as most desirable genetic tools to study genetic variation among plants (Garcia-Mas et al. 2000, Chaudhuri et al. 2009).

We have focused on one certified cultivar of *A. vera* having high aloin content. We have standardized *in vitro* multiplication protocol of *A. vera* and transferred the micropropagated plants to the field (Das et al. 2010a). This report aims to study the exo-morphological characters, floral traits, cytological analysis and analysis of DNA fingerprinting profiles with RAPD marker and sequencing data of ITS-1 and 2 of nuclear ribosomal DNA of *in vitro* field grown and donor plants of *Aloe vera*.

Material and Methods

Plants of bitter cultivar of *Aloe vera* (AvS1) were collected from National Bureau of Plant Genetic Resources (NBPGR), Central Arid Zone Research Institute (CAZRI), Jodhpur, India and were maintained in the experimental garden. An efficient micropropagation protocol using shoot apical meristem was established involving the induction, multiplication and *in vitro* rooting of the regenerated shoots and their acclimatization under *ex vitro* conditions (Das et al. 2010a). The plantlets were successfully hardened and the plants were transferred to a larger

field in the Ramkrishna Mission Vivekananda Centenary College, Rahara and maintained for further study.

Fifty tissue culture regenerated plants were selected randomly. Quantitative and qualitative morphological traits of those plants were studied in detail. Inflorescence characteristics were carefully recorded. Morphological traits of fruit and seeds were studied and recorded accordingly. Mature and immature seeds were placed in full and half strength MS and water soaked filter paper for *in vivo* and *in vitro* germination, respectively and percentages of seed germination under the different conditions were recorded.

Somatic chromosome analysis was carried out from the root tips of the tissue culture raised plants following EMA method and Giemsa staining according to the basic protocol of Fukui (1996) with modifications (Jha and Yamamoto 2012). Chromosome analysis was carried out following the standard protocol of (Sharma and Sharma 1980). Pollen viability was tested following Singh and Dhuria 1960 and the percentage of viable pollen grains was recorded.

Genomic DNA was isolated from leaf tissue following the method of Doyle and Doyle (1990) with minor modifications. One gm of fresh leaf tissue was grounded in liquid nitrogen and then transferred to 6 ml of freshly prepared extraction buffer [100 mM Tris-HCl (pH 8.0), 25 mM EDTA, 1.5 M NaCl, 2% CTAB (W/V), 1% polyvinyl pyrrolidone (PVP), β-mercaptoethanol (0.2%, v/v)]. Protein and cellular debris were removed by treating the homogenate with an equal volume of chloroform: isoamyl alcohol (24 : 1) and then centrifuged at 10,000 rpm for 20 min at room temperature (RT) (25 ± 2ºC). DNA precipitation step was carried out with 3M sodium acetate and chilled ethanol. The pellet obtained was dissolved in TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA). RNA contamination was removed by RNase A (1U/µl) treatment. The quality of DNA was checked through 0.8% agarose gel analysis and the quantity was measured through a spectrophotometer.

Amplification of RAPD fragments were performed with RAPD decamer primers (Operon Technologies Inc., Alameda, CA) following the protocol of Williams *et al.* (1990) with minor modifications (Rathore et al. 2011). 2. The amplified fragments were visualized under UV light and documented using the gel documentation equipment (BioRad). The data were used to calculate similarity coefficient (Nei and Li 1979), and a dendrogram was constructed by UPGMA cluster analysis using the NTSYS program (2.1) to analyse the genetic relationship.

ITS1 and ITS2 regions of ribosomal DNA were independently amplified using primers (5′-TCCGTAGGTGAACCTGCGG-3′) and (5′-GCTGCGTTCTT CATCGATGC-3′) and (5′-GCATCGATGAAGAACGCAGC-3′) and (5′-TCCTCC

GCTTATTGATATGC-3′) of White et al. (1990), respectively. PCR amplification parameters were 1 denaturation cycle of 3 min at 94ºC followed by 35 cycles of 1 min at 94ºC, 1 min at 55ºC and 2.5 min at 72ºC and a final extension at 72ºC for 10 min. The PCR products were resolved in 1.5 % agarose gel. Three hundred bp and 340 bp fragments were obtained for each ITS1 and ITS2 regions, respectively. The PCR products were purified using PCR purification kit (QIAGEN) and sequenced using universal primers. All the sequences were subjected to Nucleotide BLAST and Clustal-Ω software-based analysis (Banerjee and Nandagopal 2009). Completed sequences were submitted to GENEBANK database of NCBI (Table 5).

The experiments were set up in a randomized design. Data were analyzed by ANOVA to detect significances between the mean values. Mean values differing significantly were compared using DMRT at a 5% level of confidence ($p = 0.05$). Variability of data has been expressed as the mean ± standard error (SE).

Results and Discussion

Shoot apical meristems of the donor *Aloe vera* plant (Fig. 1a) were cultured in MS supplemented with 3% sucrose. Shoot bud induction was found best in MS containing 35.5 μM BAP, 9.8 μM IBA and 81.4 μM adenine sulphate (Fig. 1b). Multiplication of newly formed shoot buds was obtained in MS supplemented with 8.87 μM BAP and 2.46 μM IBA and 108.58 μM adenine sulphate (Fig. 1c) (Das et al. 2010a). A large number of proliferated shoot buds were obtained within 4 weeks of the first subculture (Fig. 1d). Rooted plants (Fig. 1e) were hardened in the greenhouse and transferred to a large field for further growth (Fig. 1f). Micropropagated plants were acclimated in the field condition and they started flowering within one and a half year (Fig. 1g).

Regenerated plants attained a height of average 55.16 ± 1.30 ~ 55.16 cm within 2-3 years (Table 1). The fleshy, thick (2.22 ± 0.33 ~ 2.22 cm), lanceolate leaves were dark green in color and grow to 52.91 ± 1.14 cm with 8.11 ± 0.52 cm width at base (Table 1). The leaf margins were armed with conspicuous, stout spines. In contrast to the donor plants, the *in vitro* raised plants possessed red, curved spines showing a higher spine frequency *i.e*., 10 per 10 cm of length of leaf (Table 1) than that of the donor plant. Both the donor and *in vitro* raised plants slowly offset to form a clump. The *in vitro* raised plants produce average 9 suckers per plant per year which is higher than that of mother plants (Table 1).

Both the donor and *in vitro* raised plants flowered nearly at the same time of the year *i.e.,* between September and December. Within one and half years, the field grown *in vitro* raised plants produced inflorescences which at maturity attained an average height of 156.6 ± 9.81 ~ 156.6 cm (Table 2). In donor plants of

AvS1, bright-orange flowers were observed on 160.2 ± 4.30 ~ 160 cm long inflorescence axis bearing 1-4 shafts (Table 2). However, *in vitro* raised plants produced an average of 251.22 ± 0.91~ 251 numbers of orange flowers distributed in 10 lateral shafts of an intact inflorescence (Fig. 2a) (Table 2).

Table 1. Exo-morphological characteristics of *Aloe vera*.

	Characters	Donor plant (Mean ± SE)	Tissue Culture Regenerates (Mean ± SE)
Quantitative traits	Plant height (cm)	70.00 ± 2.20^{a}	55.16 ± 1.30^{b}
	Leaf thickness (cm)	2.48 ± 0.31^{b}	2.22 ± 0.33^{c}
	Leaf width (cm)	10.25 ± 0.44^{b}	8.11 ± 0.52^{d}
	Leaf Length	80.37 ± 1.33^{c}	52.91 ± 1.14^{d}
	Spine frequency/10 cm length of leaf	6.0 ± 0.11^{b}	10.09 ± 0.23^{b}
	Number of suckers/plant	5.01 ± 0.41^{c}	9.00 ± 0.22^{a}
Qualitative traits	Leaf Shape	Lanceolate	Lanceolate
	Leaf colour	Dark green	Dark green
	Leaf Spots	Elliptical, light green, concentrated at the base	Elliptical, Light green concentrated at the base, more in number on adaxial surface of lamina
	Spine Colour	White with red tip	Red
	Spine Nature	Stout, pointed	Curved, stout

Each value represents the mean ± S.E. Mean values having different letters in superscript are significantly different from each other ($P \leq 0.05$) according to Duncan's Multiple Range Test (Wang et al. 2009)

Table 2. Characteristics of inflorescence of *Aloe vera*

Characters	Donor plant	Tissue Culture Regenerates
Number of inflorescence/plant/ flowering season	1.0 ± 0.41^{a}	1.0 ± 0.41^{a}
Height of inflorescence (cm)	160.2 ± 4.30^{b}	156.6 ± 9.81^{c}
Number of shafts/inflorescence	4.96 ± 0.29^{d}	10.26 ± 1.11^{d}
Number of flowers on the main axis	45.69 ± 2.27^{e}	52.23 ± 1.99^{e}
Number of flowers in the lateral shafts	32.33 ± 1.59^{a}	203.11 ± 0.66^{b}
Total number of flowers/inflorescence	89.22 ± 2.10^{a}	251.22 ± 0.91^{c}
Duration(days) of flowering/inflorescence	25.1 ± 1.36^{b}	27.39 ± 2.14^{d}
Duration(days) of the inflorescence	37.58 ± 1.55^{c}	45.01 ± 1.22^{c}

Each value represents the mean ± S.E. Mean values having different letters in superscript are significantly different from each other ($P \leq 0.05$) according to DMRT (Wang et al. 2009).

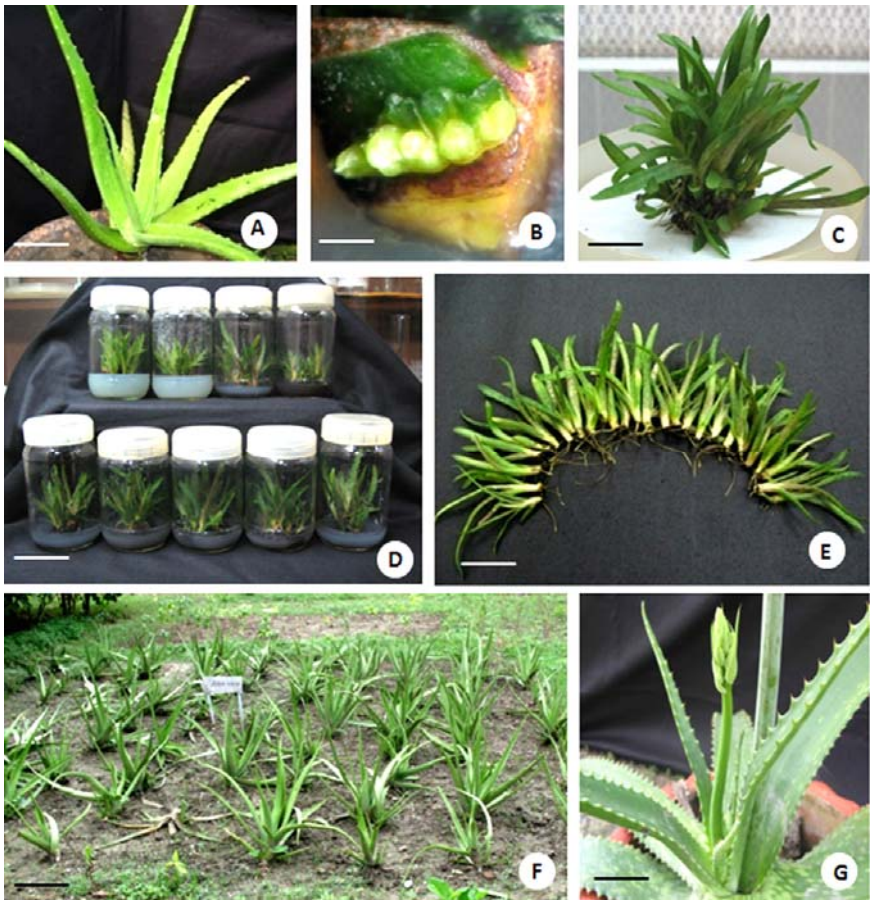

Fig. 1 Micropropagation and field transfer of *in vitro* grown plants of *Aloe vera* (a) The donor plant of *Aloe vera*. Bar = 8.0 cm. (b) Initial induction of shoot buds. Bar = 0.5 cm. (c) Multiplication of shoot buds. Bar = 0.5 cm. (d) Large number of shootlets in the culture medium. Bar = 0.5 cm. (e) Rooted shoots prior to hardening. Bar = 0.5 cm. (f) Micropropagated plants growing in the field conditions. Bar = 0.5 cm (g) Tissue culture raised field grown plant showing the production of inflorescence. Bar = 0.5 cm.

At the end of the flowering season, one important observation was noted in 3 *in vitro* raised plants of AvS1 among the fifty studied. Flowers were found to convert into fruits in those 3 plants for three consecutive years (Fig. 2a,b,c). Simultaneous development of flowers and fruits were noted in the same flowering axis (Fig. 2d,e,f). Full bloom of flower and subsequent conversion of flowers to fruit and consequential maturation occurred within 6-7 weeks (Fig. 3a,b,c). The average number of fruits per plant was 34.2 ± 2.56 ~34 and each pod was found to contain 12.6 ± 1.44 ~12 seeds in the first year (Table 3). In the second and third year, the number of fruits per inflorescence increased to 40.0 ± 1.66 ~40

and 42.00 ± 1.56 ~ 42, respectively (Table 3) but the number of seeds per fruit was found to remain constant. The size and weight of the mature fruit was ~ 21mm x 8mm and ~90 - 95 mg, respectively and individual seeds weighed ~ 0.92 ± 0.23 mg in both the years (Fig. 3d). Field evaluation data of *Aloe vera* (AvS1) revealed

Fig. 2. Flowering and formation of fruits and seeds in the *Aloe vera* (a-c) *Aloe vera* somaclones showing inflorescence in the first, second and third year of flowering, respectively. Bar = 8.0 cm. (d-f) Maturation of inflorescence with flowers and settings of fruits in three successive years. Bar = 8.0 cm.

that the somaclones produced varied number of fruits with viable, fertile seeds through non-transformed method. Somaclonal variation provides a valuable source of genetic variation for the improvement of crops through the selection of novel variants, which may show resistance to disease, improved agronomic quality, or higher yield. Since, propagation by means of sexual reproduction through seeds is very rare in *Aloe vera*, viable seed production in *Aloe* may facilitate undertaking different genetic improvement programs.

Cultivated *Aloe* species produces bisexual flowers and their microspores are produced through regular meiosis but no one has yet reported any form of seed setting (viable or nonviable, mature or immature) in any cultivar of this species. Velásquez-Arenas et al. (2008) reported the floral phenology of *Aloe vera* and *Aloe saponaria,* where they observed ~228 yellow flowers on a long inflorescence axis with 1 - 3 shafts in *Aloe vera*. Although both of them flowered by the end of the flowering period, fruits were observed only in *A. saponaria* with 12% reproductive efficiency. Thus, propagation of *Aloe vera* solely depends on production of limited numbers (4 - 5) of vegetative suckers per plant per year.

The average number of seeds obtained in somaclones was ~400 - 480 per plant per year. The data revealed from the observations during the first year showed that $\underset{\sim}{}$ 61% and $\underset{\sim}{}$ 22% seeds of un-dehisced and dehisced fruits, respectively, germinated under *in vitro* condition within 4 weeks (Table 3) (Fig. 3e-h). In the first year, seed germination in natural environment or *in vivo* condition was not achieved. But, in the second and third year, 25 and 28% seeds of dehisced mature fruits were germinated in *in vivo* condition (Fig. 3i, k). In the same year the percentage of seed germination increased to 70.58% in *in vitro* condition (Fig. 3j). The results point out to the fact that seeds isolated from green un-dehisced fruits have more potential for germination than completely mature seeds. Germinated plants were transferred to potted soil pots and kept in green house (Fig. 3l).

A higher percentage of germination from green pods is beneficial as it will reduce the breeding cycle. Weitbrech et al. (2011) pointed out that early seed germination contributes to better seed and seedling performance and it is important for plant establishment in the natural and agricultural ecosystem. Although *in vivo* seed germination could not occur in the first year, in the second and third year ~25% and ~28% seeds of dehisced mature fruits germinated under *in vivo* condition.

Mitotic chromosomal analysis of donor plant and tissue culture raised plants of this cultivar showed diploid cells having 2n = 14 chromosomes with bimodal karyotype (Fig. 4a). There were no anomalies in gross chromosome structure and organization of any of the regenerated plants. From meiotic chromosome analysis it has been observed that both the mother and tissue culture regenerates had a consistent haploid chromosome number of n = 7 in meiotic metaphase. Different stages of meiosis including metaphase I (Fig. 4b) and II, anaphase I and II and telophase were found and no abnormalities were noted in any of the stages. Pollen mother cells were usually regular with predominant bivalent (II) pairing (Fig. 4c). This confirms the basic number x = 7. Approximately, 96.5% pollen viability was recorded in both the cases.

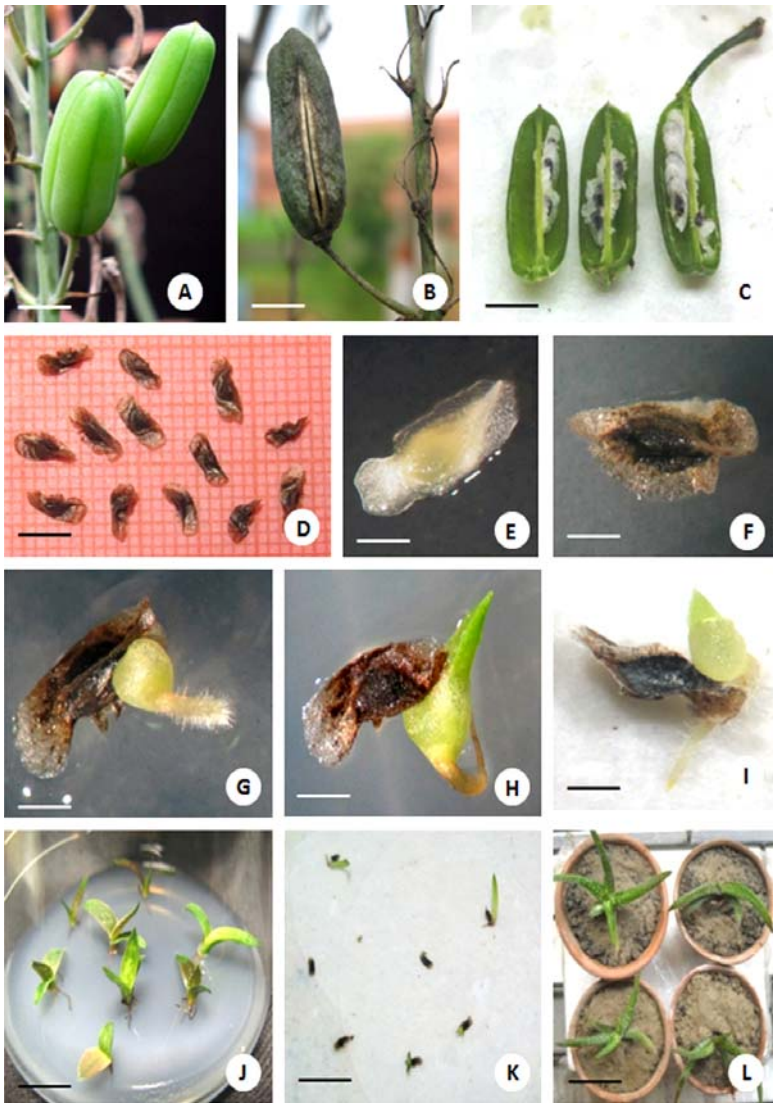

Fig. 3. Morphology of fruits and germination of seeds in *Aloe vera* (a) Green immature fruits of somaclone of *A. vera*. Bar = 2.0 cm. (b) Mature fruit before dehiscence. Bar= 2.0 cm (c) Longitudinal section of fruit showing seeds. Bar = 2.0 cm. (d) Isolated seeds from mature non-dehiscent green fruits of *A. vera* (e)-(h) Stages of seed germination in *in vitro* condition. Bar = 0.5 cm. (i) *In vivo* germination of seeds. Bar = 0.5 cm. (j-k) R_1 seedlings of somaclonal *A. vera* with two leaf stage. Bar = 0.5 cm. (l) Seedlings growing in green house. Bar = 8.0 cm.

Male sterility, a complex phenomenon is controlled either by cytoplasmic or nuclear genes which directly affects self compatibility, pollen viability. These results further cause failure in fertilization followed by lack of formation of fruits and seeds. In *Aloe*, male sterility and self-incompatibility have been reported

earlier (Tie et al. 2004) and hardly any strategy has been reported so far, in the production of fertile seeds. Analysis of meiotic chromosomes in *Aloe* revealed that the mother plants could produce microspores through normal meiosis but they are incompetent to germinate, which is a prerequisite for fertilization and seed setting. While microspores of somaclone executing normal meiosis and pollen mitosis gained competency and participated in seed setting process. The competency factor has entered the reproductive cycle.

Table 3. Morphological characteristics of fruits of somaclone of *Aloe vera*

Characters	Data of 1st year	Data of 2nd year
Colour of immature fruit	Light green	Light green
Colour of mature fruit	Dark green	Dark green
Number of fruits/inflorescence	34.2 ± 2.56^{d}	40.00 ± 1.66^{c}
Number of seeds/fruit	12.00 ± 0.12^{a}	12.00 ± 0.12^{a}
Time (days)needed for maturation of fruits	15.06 ± 1.98^{b}	15.06 ± 1.98^{b}
Seed germination percentage under *in vivo* condition	Nil	25%
Seed germination percentage under *in vitro* condition	61%	70.58%

Each value represents the mean ± S.E. Mean values having different letters in superscript are significantly different from each other ($P \leq 0.05$) according to DMRT (Wang et al. 2009).

RAPD analysis through DNA fingerprinting profiles of the somaclones (AvST1-I, AvST1-II, AvST1-IV) showed different banding patterns relative to the donor plant. As shown in Fig. 4d, the DNA fingerprinting profile generated with primer OPB 07, two bands having molecular weight 700 bp and 2 kb of the donor plant were absent in the profiles of the somaclones and germinating seeds. Again, RAPD profile generated with primer OPB 16 (Fig. 4e) revealed that one parental band of 650 bp was absent in the profiles of somaclones and germinating seeds while three new bands with molecular weight 2 and 1.8 kb and 1.5 kb were generated in the profiles of somaclones, germinating seeds. Moreover, RAPD profile generated with primer OPB 18 (Fig. 4f) showed that two distinct bands of molecular weight 580 bp and 1 kb in the profile of donor plant was absent in the profiles of somaclones and seed germinated plants. Thus, polymorphic bands in the DNA fingerprinting profiles of the donor and somaclones of AvS1 confirmed genetic difference among them which in turn provide important evidence of genetic basis of their morphological differences.

Table 4. RAPD primers utilized for the assessment of *in vitro* raised plants of *Aloe vera*

Primers	Sequence 5/→3/	% of GC	Size range of amplicons (in Kb)	Total no. of amplicons
OPA 07	GAAACGGGTG	60	0.2-1.5	7
OPB 01	GTTTCGCTCC	60	0.55-3.0	6
OPB 02	TGATCCCTGG	60	0.45-2.0	7
OPB 07	GGTGACGCAG	70	0.5-2.3	4
OPB 16	TTTGCCCGGA	60	0.6-2.0	6
OPB 18	CCACAGCAGT	60	0.25-1.5	10
OPD 01	ACCGCGAAGG	70	0.4-2.0	9
OPD 03	GTCGCCGTCA	70	0.48-2.8	11
OPD 05	TGAGCGGACA	60	0.6-3.0	7
OPM 02	ACAACGCCTC	60	0.5-2.0	9
OPM 04	GGCGGTTGTC	70	1.5-3.0	5

Table 5. Features of ITS1 sequences of tissue culture regenerated plants of *Aloe vera*

Code	Description	Accession no. (ITS1)	Average size of ITS1 (bp)	Average GC % of ITS1
AvST1-VI	Clonal plant	KP823453	321	59.2
AvST1-I	Seed setting somaclone	KP823447	321	58.57
AvST1-II	Seed setting somaclone	KP823448	306	61.44
AvST1-IV	Seed setting somaclone	KP823449	322	58.38
AvST1-SdG1	Seed germinated plant	KP823451	301	58.47
AvST1-SdG2	Seed germinated plant	KP823452	321	59.19

The utility of RAPD technology for molecular analysis of *in vitro* regenerated plants has been documented by many workers (Hussain et al. 2008, Xing et al. 2010, Mohanty et al. 2011). RAPD analysis of *Allium cepa* showed a unique band in independent gametoclones which was proposed to have arisen due to a DNA sequence which was highly vulnerable to tissue culture-induced mutation (Bohance et al. 1995). Variations observed in total number of RAPD bands as well as the number of specific bands among the donor plant and somaclones of *Aloe vera* signify genetic differences of the genotypes due to tissue culture and induced somaclonal variation.

Table 6. Details of changes in nucleotide in ITS1 sequence of AvST1-SdG1

Population code	Type of nucleotide change		Nucleotide position in ITS1 Sequence* (5′→3′)
AvST1-SdG1	Single base substitution	Transition	A190G
			G193A
			A194G
		Transversion	C185A
			C186A
			C196G
			C481A

*Reference sequence is ITS sequence of AvS1 (donor plant)

ITS1 sequence data of donor and tissue culture regenerated plants of AvS1 revealed the average size of ~320 bp. The PCR products were purified and sequenced, Fig. 4g represents sequence chromatogram of 18S-ITS1-5.8S. Sequences have been submitted to NCBI database and accession numbers and %GC of those are tabulated in Table 5. The data showed that the length and %GC of ITS1 sequences of all types of tissue culture regenerated plants of AvS1 were similar to that of the donor plant. Multiple sequence alignment of ITS1 sequences indicated that the somaclones and seed germinated AvS1 plants exhibited little divergence in the length and sequence of ITS1 with that of donor plant. In AvST1-SdG1 seed germinated plant, single nucleotide substitution occurred at 8 different nucleotide positions in ITS1 sequence (Table 6). Moreover, nucleotide substitutions occurred at 423rd base of AvST1-I and at 478th base of AvST1-IV. Like the donor plant, the conserved stretch (5′-GGCGCGATGGGCGCCAA GGAA-3′) has also been found in ITS1 regions of all tissue culture regenerated plants of AvS1. ITS2 region showed conserved nature in the length and sequence among all *in vivo* and *in vitro* populations of *Aloe vera.* Conserved nature of ITS2 sequence has been reported earlier by several authors (Liu and Schardl 1994).

ITS1 sequence analysis of tissue culture regenerated plants of AvS1 showed overall similarity in length and sequence with that of the mother plant. Morphologically distinct 3 somaclones of AvS1 (AvST1-I, II, IV) showed very little divergence in ITS1 sequence data among them. We randomly selected germinating seedlings of AvST1-I and II and found ITS1 sequence of AvST1-SdG1 (R1 plant) was slightly different from its R0 plant which is AvST1-I somaclone and also from the donor plant AvS1. Since, *Aloe vera* mainly reproduces by vegetative means and its gene flow is restricted due to male

sterility and self-incompatibility, the seed setting somaclonal plants (R0) and seed germinated plants (R1) of *Aloe vera* in future may open a new window in the breeding program of this commercially important plant. Stable somaclonal ~~variants~~ variation of a specific type may be advantageous for the improvement of certain traits in breeding programs (Karp 1995, Jain 2001).

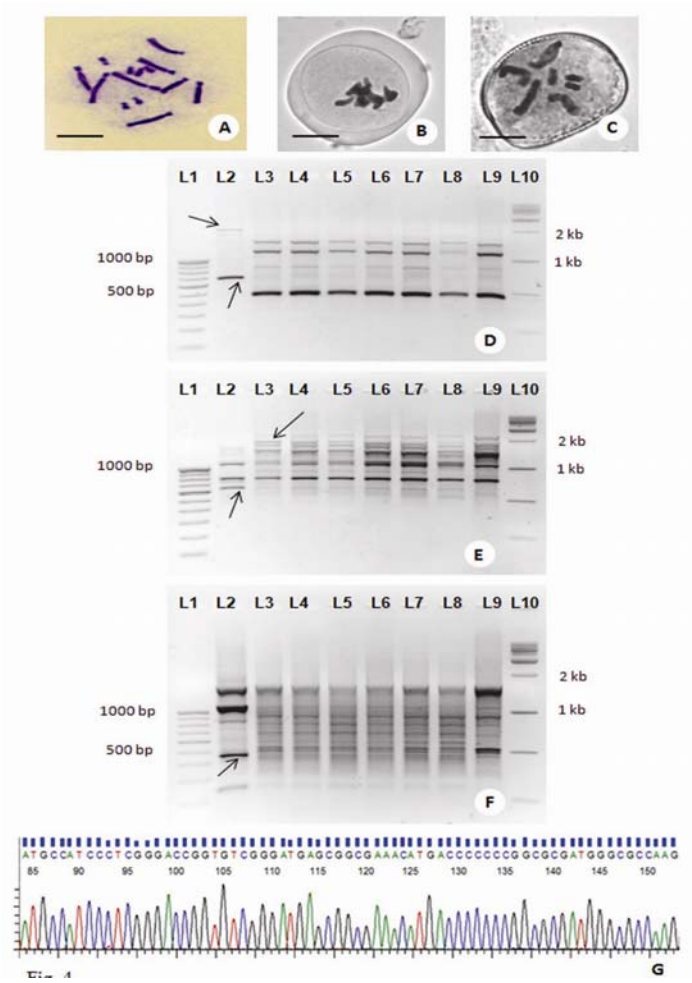

Fig. 4 Genetic studies of somaclonal *Aloe vera* (a) Mitotic metaphase of seed setting somaclones showing 2n = 14 bimodal chromosomes. Bar = 3μM. (b) Meiotic metaphase I showing normal bivalents. Bar = 5 μM. (c) Pollen mitosis of donor *A. vera* plant showing distinct n = 7 bimodal chromosomes. Bar = 5 μM. (d)-(f) DNA fingerprinting profiles of donor and somaclones of *Aloe vera* generated with RAPD primer OPB 07, OPB 16 and OPB 18, respectively. Lane 1: 100 bp DNA ladder; lane 2: Donor plant; lane 3-5: Seed setting somaclones (AvST1-I, AvST1-II, AvST1-IV); lane 6-9: Seed germinated plants (g) Representative chromatogram of 18S-ITS1-5.8S partial nucleotide sequence of tissue culture regenerated plants of *Aloe vera.*

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