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Somaclonal variation in plants regenerated from cultures of two morphologically distinct accessions of *Aloe vera* Linn

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Abstract

Aloe vera (Linn.), an important medicinal plant is cultivated throughout the world. Due to absence of sexual reproduction it lacks genetic variation generated through genetic recombination. The present study was aimed to compare the morphological and biochemical characters of tissue culture derived and field grown clones of two different accessions of *Aloe vera* with a view to exploit somaclonal variations for plant improvement. The stem disc explants obtained from two morphologically distinct accessions of *Aloe vera* (HPM1 and PBL3) were cultured on MS medium supplemented with 2, 4 - D (1.0 mg/l) and Kinetin (0.2 mg/l). The callii obtained were sub-cultured on shoot proliferation medium and then on rooting medium. Assessments were made on nearly one year old plants. Plants regenerated by tissue culture techniques exhibited various morphological and biochemical variations. Comparison of somaclones with the parental clones showed variation in size of plants, size of leaves, spines, etc. The callus regenerated plants of HPM1 were bigger in size than the parental clones and showed marginal increase in the amount of carbohydrate, protein, chlorophyll and phenol contents over the control plants. There was decrease in aloin content and juice quantity but increase in gel content in the somaclones. The tissue culture raised plants of PBL3 were smaller in size and exhibited decreased amount of carbohydrate, protein, chlorophyll, aloin, juice and gel contents than the parental clones but have increased amount of phenols.

Keywords: *Aloe vera*, Somaclonal variation, Callus**.**

Introduction

Aloe vera (Linn.), a member of family Liliaceae is an important medicinal plant with many cosmetic properties. This plant is cultivated throughout the world for its thick flesh from which many medicinal and cosmetic products are prepared. It has been shown that the products of this plant are anti-bacterial [1], anti-viral [2], anti-fungal [3] and have properties like anti-septic, anti-tumoral, anti-inflammatory, anti-oxidant and immune-stimulant [4]. The juice of this plant is considered useful in stomach disorders [5].

The conventional plant breeding relies mainly on the natural genetic variation. Plant breeders combine the desired genes from different crop varieties and related species by sexual hybridization and develop new varieties with the desirable traits. In *Aloe vera,* the situation is entirely different. Due to absence of sexual reproduction, the germplasm lacks genetic variation generated through genetic recombination. The plant breeders are in a tight situation as hybridization is not possible. There is great potential of plant tissue culture techniques in improvement of this vegetatively propagated crop. The present study was aimed to compare the morphological and biochemical characters of tissue culture derived and field grown clones of two different accessions of *Aloe vera* with a view to exploit somaclonal variations for plant improvement.

Materials and Methods

Plant Material

The explants for tissue culture were obtained from two morphologically distinct accessions of *Aloe vera* (HPM1 and PBL3). Accession HPM1 was originally collected from town Sundar Nagar in Himachal Pradesh while accession PBL3 was obtained from fields at village Sehaura, Ludhiana in Punjab. Both these clones were maintained and multiplied at Botanic Gardens, Punjabi University, Patiala (India).

In vitro culture

Shoot tip explants obtained from clones of above accessions were washed thoroughly in running tap water for 20 min and then with 5% (v/v) teepol solution (10 min). The explants were washed again and treated with 1% Bavistin and 0.25% Dithane M-45 for 20 min. The explants were surface disinfected with 0.1% $HgCl_2$ for 4-5 min and washed again 3-4 times with sterile double distilled water under laminar flow. The explants were trimmed to remove extra outer portion of stem discs and carefully inoculated in culture vessels containing 40 ml of MS medium [6] supplemented with 2, 4-D (1.0 mg/l) and Kinetin (0.2 mg/l). The cultures were kept in the culture room at 27±1ºC under fluorescent (Philips) light with a 16 h photoperiod.

Five callii of each clone were sub-cultured on the basal MS medium containing BA (1.0 mg/l) and IBA (0.2 mg/l). The callii on sub-culturing showed signs of proliferation after two weeks. On almost all callii bud appeared and developed into shoots by 20th day of sub-culturing. For rooting, the shoots (1-2) were excised and placed vertically in culture tubes containing MS medium supplemented with NAA (0.3 mg/l). Rooted plantlets were transferred to pots containing garden soil and rice husk (1:1 w/w) acclimatized in a culture room and afterwards transferred to the green house and finally to the open field.

Once the plantlets were obtained, the experiment was executed at the experimental plots located at the Botanic Gardens, Punjabi University, Patiala in July, 2008. Both type of plants, the vegetatively propagated plants from the mother clones (control) and the regenerants obtained via tissue culture were raised in field keeping 60×60 cm spacing. The control plants were randomly distributed among micro-propagated plants.

Assessments were made on nearly one year old plants for comparison of morphological and biochemical parameters. The biochemical parameters studied were Carbohydrate content [7], Chlorophyll content [8], Protein content [9], Phenol content [10], Aloin content [11] and value aided products - Juice and Gel [12].

Results and Discussion

Plants of two morphologically distinct accessions of *Aloe* germplasm were selected for tissue culture study. The plants of PBL3 (Fig. 1) have bigger leaves (32x4.5 cm) which are borne in spiral orientation. On the other hand the plants of accession HPM1 (Fig. 2) are small sized and have small (9.6 x 1.9 cm), parallelly oriented, dark green leaves with green marginal spines. The baby plants of these parental clones and the tissue culture regenerated plants were grown in the experimental fields (Figs. 3-7). Observations were made on nearly one year old plants of both kinds clonal as well as micro- propagated plants

Figs 1-7. *In vitro* studies in *Aloe vera*

1. PBL3 clonal (mother) plant; **2.** HPM1 clonal (mother) plant; **3** Callus on MS+2, 4-D (1mg/l) + Kinetin (0.2mg/l); **4**. Plants regenerated on callus with MS+ IBA (0.2mg/l) + BA (1.0mg/l); **5.** Plants established on soil: rice husk (1:1); **6**. PBL3 micro-propagated plant; **7**. HPM1 micro-propagated plant

Comparison of somaclones with the parental clones showed variation in size of plants, size of leaves, spines, etc. (Table 1, 2). The tissue culture regenerated plants of HPM1 are bigger in size than the parental clones whereas in PBL3 the trend is otherwise. The micro-propagated plants of HPM1 showed change in leaf orientation as well.

The results obtained in our study revealed the differences in biochemical parameters among somaclones and the parental clones (Tables 1 and 2). The callus regenerants of HPM1 showed marginal increase in the amount of carbohydrate, protein, chlorophyll and phenols over the control plants. The aloin content was reduced from 8.9% in parental clones to 6.85% in the micro-propagated plants. There was decrease in juice quantity but increase in gel content in the somaclones. The tissue culture raised plants of PBL3 showed a different trend. These plants showed decreased amount of carbohydrate, protein, chlorophyll, aloin, juice and gel contents than the parental clones but have increased phenol content.

Table 1: **Morphological and biochemical comparison of clonal and regenerated plants of accession HPM1**

Character	Clonal Plants	Somaclonal plants
Plant Height (cm)	13	18
Leaves:		
Colour	Dark Green	Dark Green
Striations	Present	Present
Orientation	Parallel	Spiral
Length (cm)	9.6	10.2
Breadth (cm)	1.9	1.6
Spines:		
Colour	Green	Green
Size (mm)	1.0	0.5
Carbohydrate content(mg/g)	1.08±0.003	1.13±0.014
Chlorophyll content (µg/mg)	3.03±0.006	3.08±0.019
Phenol content (mg/100g)	111.8±5.70	118.75±20.05
Protein content (mg/g)	0.34±0.005	0.45±0.006
Aloin content (%)	8.9	6.85
Juice content (ml/100g)	64.76	63.14
Gel content (%)	0.23	0.29

Table 2: Morphological and biochemical comparison of clonal and regenerated plants of accession PBL3

Character	Clonal plants	Somaclonal plants
Plant Height (cm)	38	36
Leaves:		
Colour	Light Green	Light Green
Striations	Present	Present
Orientation	Spiral	Parallel, Spiral
Length (cm)	32	28
Breadth (cm)	4.5	3.2
Spines:		
Colour	Green	Green
Size (mm)	2.0	0.5
Carbohydrate content(mg/g)	2.01±0.004	1.74±0.007
Chlorophyll content (µg/mg)	5.34±0.078	4.06±0.016
Phenol content (mg/100g)	120.0±10.08	125.03±12.06
Protein content (mg/g)	1.28±0.002	1.26±0.004
Aloin content (%)	13.75	10.4
Juice content(ml/100g)	82.64	76.7
Gel content (%)	0.45	0.39

Plants regenerated by tissue culture techniques exhibited various morphological and biochemical variations due to mutations believed as somaclonal variations [13]. Among callus regenerated plants mutations at different stages like single gene, multigene and cytoplasmic mutations have been described [14, 15]. Chromosomal aberrations ranging from changes in ploidy to laggards, bridges in meiosis I and II, micronuclei, etc have been observed in micro-propagated plants [16, 17]. The high variability observed in micro-propagated plants might be triggered by the cytokinin during micro-propagation [18]. The frequency of somaclonal variation would depend upon culture protocol applied during *in vitro* process particularly hormone composition and number of subcultures [19].

Conclusion

The results obtained in present study demonstrated the induction of variation in two accessions of *Aloe.* The genetic variability in the culture regenerated plants has great potential to be used for improvement of *Aloe* germplasm.

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