A Viable Approach for a Rapid *In Vitro* Multiplication of the Medicinal Plant *Aloe vera*

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Rapid shoot bud regeneration, directly from the rhizome portion of the medicinally important plant, *Aloe vera,* was obtained without formation of callus in MS medium supplemented with BAP at a high concentration (3.0-5.0 mg/l). At the initiation stage, a low concentration of NAA (0.1 mg/l) was also applied with BAP. In the subsequent subcultures, NAA was not required. Direct rooting of the shoot buds was achieved either by the application of charcoal (0.2%) or on transfer of the regenerants to sand: soil mixture (2:1) at high humidity. Since cytological study of the root tip of the regenerants upto 10th passage (one passage = 45 days) did not show any irregularity, it is assumed that this protocol would produce stable clones for *en masse* propagation of this important medicinal plant.

Key Words: *Aloe vera*, In vitro multiplication, Micropropagation

The *Aloe* plant (*A. vera* Tourn. ex Linn. Syn. *A. barbadensis* Mill) is an important medicinal plant (Anonymous, 1948; Kirtikar and Basu, 1973). The plant is often called a "Doctor in a pot" in USA and Mexico. It is xerophytic in nature with thick, fleshy, strongly cutinised, spiny margined leaves that are arranged in a rosette on a very short stem and its rhizome bears adventitious buds that sprout during rainy season. Use of the *Aloe* leaves in herbal cosmetics and medicines needs widespread cultivation of this plant.

Main practice of its propagation is by suckers. Owing to the widespread male-sterility (Keizer and Cresti, 1987), irregular pollen meiosis, high pollen sterility and failure in fruit set (Sapre, 1975), seed formation is very poor in this plant. Its vegetative propagation is slow and limited for a brief period of time in each season. Micropropagation could be an important tool for its mass scale propagation. Prior to this work, regeneration from callus tissue was reported (Roy and Sarkar, 1991). Present report is perhaps the first one for a direct *in vitro* regeneration of *Aloe* plant from rhizome explants and its successful transplantation and growth in the field.

Materials and Methods

Rhizomes of the *Aloe vera* were harvested from randomly selected plant materials grown in the experimental garden of the Department of Botany, University of Calcutta. The young rhizomes with vegetative buds (4-10 mm thick cubes) were washed thoroughly in running tap water and a 5% liquid detergent (Teepol, Reckitt & Colman, India Ltd.). These were then surface disinfested with 0.1% (w/v) mercuric chloride for five min, subsequently washed thoroughly thrice with sterile distilled water and placed in culture tubes (25mm x 150mm) with cotton plug containing 20ml freshly prepared media. Media consisted of salts medium Murashige and Skoog (1962), supplemented with 3% (w/v) sucrose, different concentrations of BAP (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg/l) alone and in combination with NAA (0.1 and 0.2mg/l). The pH of the media was adjusted to 5.7 and solidified with 0.7% (w/v) agar (Hi-Media, India). The media after dispensing in culture tubes were sterilized at 1.05 Kg/cm^2 for 15 mins at 121°C.

Cultures were incubated at 25° ± 2°C with an illumination of 30 μmoles sec^{-1}m^{-2} from Phillips white fluorescent tubes for 16 hrs/day and at a relative humidity of 60%. Plants were subcultured at an intervel of 45 days. All the sets were repeated thrice and each set was replicated 10 times. For rooting, the regenerated shoots were transferred to MS, MS with activated charcoal (0.2%), and directly to the sand: soil (2:1) mixture at 75-80% humidity. Cytological analysis of the root tips of the regenerated plants was carried out following acetoorcein squash technique (Sharma and Sharma, 1980). Data on shoot proliferation, root initiation, survival rate in the field and chromosome study (up to 10th passage) were recorded. Each experiment was repeated twice; data are scored from ten replicates and the average values were reported.

Results and Discussion

Regeneration of Shoot Buds in the Medium

Shoot regeneration was obtained (Fig. 1) directly from the young rhizome bud explants in MS medium

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supplemented with low concentration (0.1 and 0.2 mg/l) of NAA and high concentration (1.0-5.0 mg/l) of BAP (Table-1). In the following subcultures, NAA was not required and BAP only was used (Table 2) for multiplication of shoot buds (Fig. 2). In higher concentrations of BAP (5.0 mg/1) shoot buds regenerated from the explants in high number but were difficult to separate. Superiority of BAP for shoot-induction was reported by many authors (Balachandran *et al.* 1999; Agretion *et al.* 1996). It is assumed that BAP is converted to other phytohormones in the tissues under cultural conditions (Sharma and Wakhlu, 2003; Zaerr and Mapes, 1982). However, effective concentration of BAP differs in different materials. For example, high BAP concentration reduced shoot bud number in *Acorus* (Harikrishnan *et al.*, 1999; Sabita Ram *et al.*, 2000) while a high BAP concentration was necessary in *Musa* (Krikorian

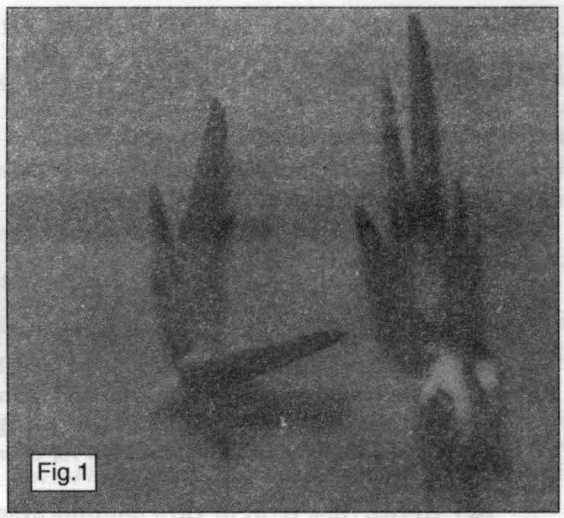

Fig. 1. Regeneration of shoot buds in MS+BAP (3.0 mg/1)+NAA (0.1mg/l)

Table 1. Direct regeneration of shoot buds in MS medium with BAP and NAA

BAP mg/1	NAA mg/1	Observation after 30 days of inoculation
1.0	-	Very few shoot buds (1-2/explant) in 20% cultures.
"	0.1	Very few shoot buds (1-2/explant) with callus formation at the cut ends
"	0.2	More callus formation, shoot buds very small and few
2.0	-	3-4 shoot buds/explants in 60% cultures
"	0.1	3-4 shoot buds/explants in 60% cultures & also callus formation
"	0.2	3-4 shoot buds/explants in 60% cultures, and rooting from the callus
3.0	-	7-8 shoot buds/explant in 90% cultures
"	0.1	6-7 shoot buds/explant and callus at the cut ends
"	0.2	Rooting from the explant, callus formation and 5-7 shoot buds/explant
4.0	-	Very small shoot buds, 8-9/explant in 90% cultures
"	0.1	6-7 shoot buds along with callus formation, leaves deformed
"	0.2	Deformed shoot buds and rooting from the explant in all the cultures
5.0	-	Very small 10-12 shoot buds, difficult to separate, along with callus
"	0.1	Deformed 8-10 shoot buds developed from the callus
"	0.2	Callus formation more pronounced and deformed & albino shoot buds

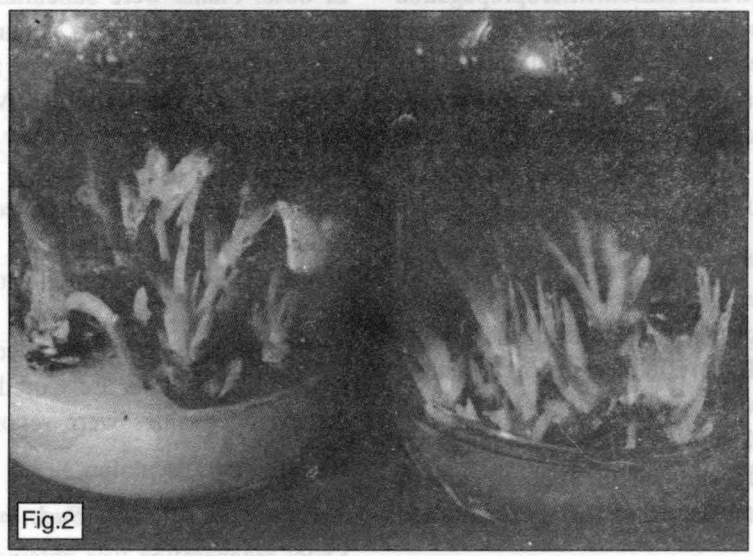

Fig. 2. Multiplication of shoot buds in MS containing BAP 3.0 mg/l at 3rd passage (one passage = 45 days)

and Cronauer, 1983) as well as in the present material. Occasionally, deformed leaves and albino shoots (2-3/ culture) at later stages were observed in MS medium containing high concentration (5.0 mg/1) of BAP. The deformed shoots when shifted to lower concentration of BAP (1.0 mg/1), immediately after detection, reverted to normal ones. However, if maintained there for more than seven days, they appeared to be permanently deformed. So for further studies, immediately after detection, these deformed shoots were discarded.

Rhizogenesis and Acclimatization

The regenerated shoot buds (2-3cm) were transferred to the basal MS medium, MS medium containing 0.2% activated charcoal or directly to sand: soil mixture (2:1) with high humidity (75-80%) at green house for induction of roots (Fig. 3). If the shoots after induction of roots were kept in the same medium (MS or MS + charcoal) for more than 7 days then the basal portion of the roots developed pigmentation (Fig. 3, arrow marked) due to accumulation of phenolics, which did not interfere in the survival of the regenerated plants in the field. Rooting and field survival was the best (100%), when the regenerated shoots were placed directly in the sand: soil mix (Table-3). The rooted plants are growing profusely in the field (Fig. 4). The plants rooted directly in the sand: soil showed better survival in the field, could skip one step in the process of acclimatization and so the process is cost effective. Therefore to make the process cost effective, direct rooting in the sand: soil mixtures were used regularly for mass scale rooting and transplantation to the field. Such *ex vitro* rooting has also been reported in *Agave* (Das, 1992).

Table 2. Effect of different concentrations of BAP on shoot bud multiplication

BAP Concentration (mg/1)	Percent of culture showing multiplication of shoot buds	Mean no. of shoots/culture ± SD (at 3rd passage)	Observation at 3rd passage
0.5	10	1.0+0.58	Poor growth of regenerated shoot buds, necrosis of lower leaves and no further outgrowth formation in the following passages.
1.0	30	3.0+0.33	Pale green leaves, poor growth, non increase in regeneration rate in the following passages.
2.0	60	7.0+0.58	Good growth of shoot buds and further regeneration of 7-10 buds/bud occurred in the later passages if separated form the clump at the end of the passage.
3.0	90	10.0+0.88	Healthy shoot buds develop forming a clump. Separation of each bud induces further regeneration of 8-10 shoot buds and rooting in the later passage.
4.0	90	20.0+0.88	Very small shoot buds, forming clump, difficult to separate, poor growth; shoot buds on transfer to low BAP (1.0mg/1) induced growth, without further regeneration of shoot buds.
5.0	90	25 + 1.20	Very small shoot buds, very poor growth, leaves deformed, callus formation at the base, no root formation on further subculture.

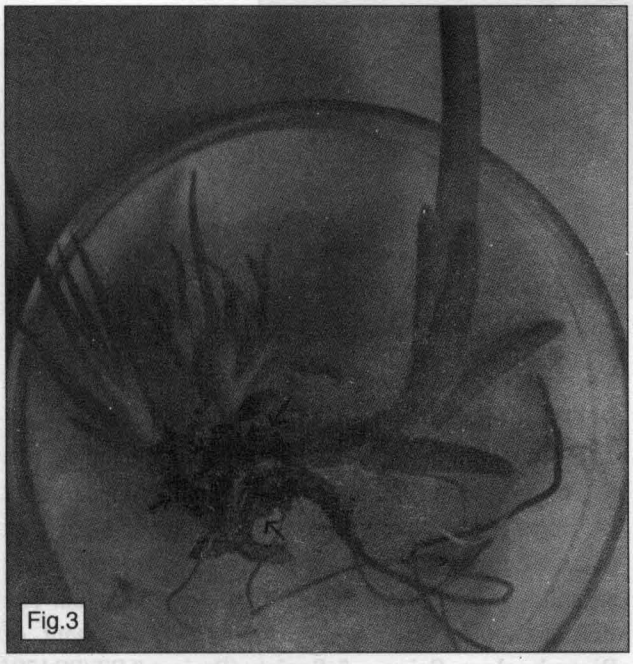

Fig. 3. Rooting in the MS + charcoal (0.1%)

Table 3. Rooting of the regenerated shoots, field trails and chromo some study

Treatment	Observation on rooting (%)	Field survival (%)	Chromosome study
MS	80%	70%	2n = 14
MS + activated Charcoal (0.2%)	100%	80%	2n =14
Sand: soil (2:1)	100%	100%	2n = 14

Fig. 4. Regenerated shoots after transplantation under field condition (plants are one year three months old)

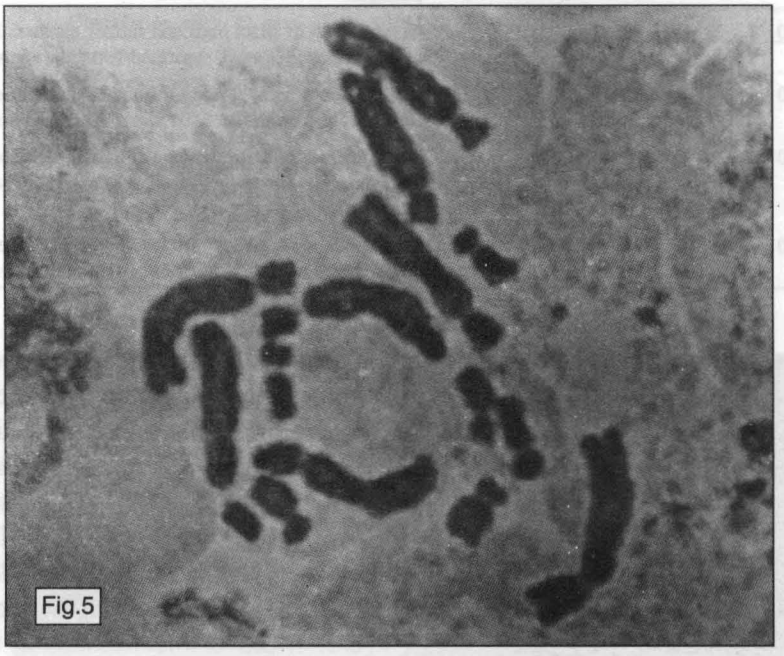

Fig. 5. Aceto-orcein squash of the root tip of the regenerated shoot showing normal chromosome complement (2n = 14)

Data of cytological analysis of the root tips (Fig. 5) of the regenerated shoots upto 10th passage under cultural conditions showed normal chromosome complement (2n=14), with no irregularity in number as well as apparent variation in chromosome morphology. Similar stable regenerants have also been reported earlier in *Asparagus* (Ghosh and Sen, 1992). Thus regenerated plants growing in the field were stable and free from any noticeable phenotypic variability.

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