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Micropropagation of vegetable type aloe vera (*Aloe barbadensis* Mill.)

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Abstract

Micropropagation protocol for vegetable type Aloe vera (*Aloe barbadensis* Mill.) has been developed using shoot tip explants. The protocol standardized for multiple micro-sucker proliferation, induction of *in vitro* adventitious roots and acclimatization of rooted micro-sucker from *in vitro* environment to greenhouse condition. Maximum proliferation of micro-suckers was obtained with combined supplementation of Benzyl adenine (BA) 2 mg + Thidiazuran (TDZ) 0.5 mg + 0.1 mg Naphthalene acetic acid (NAA) to per liter basal Murashige & Skoog (MS) medium containing 0.8% agar and 3 % sucrose. The maximum rootable micro-sucker (~ 2 m) long were achieved after transfer of culture to hormone free medium for two weeks. The elongated micro-suckers were rooted successfully under *in vitro* condition. The better quality adventitious roots formation observed with NAA 0.5 mg/l supplemented to ½ MS medium. The regenerated plants were successfully acclimatized under Hi- Tech greenhouse and maintained in protrays for further growth and development

Keywords.: Aloe vera, micropropagation, micro-suckers.

Introduction

Aloe vera (*Aloe barbadensis* Mill.) is an important plant which belongs to family Liliaceae and extensively used in therapeutic uses (Cera *et al.*, 1980 ; Davis and Leiter, 1988, Hart *et al.*, 1998 ; Anshoo *et al.*, 2005). Recently, it is widely used in several cosmetic preparations and culinary purpose particularly in many districts of Rajasthan. The leaf pads and flower stalks are mainly used for culinary and pickle preparation. Among several genotypes only sweet type Aloe vera is used for culinary preparation (Saroj *et al.*, 2004).

Aloe vera can be grown in arid and semi-arid regions as ground story crop in orchards or agro-forestry models. Its gel is very effective for treatment of wounds, burns and other skin disorders, placing a protective coat over the affected area, speeding up the rate of healing and reducing the risk of infection. Quality Aloe vera pads produce can be harvested when grown under partial shade conditions.

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Its commercial cultivation for vegetable farming requires large quantity of planting material of sweet type genotype which cannot be obtained through vegetative sucker production since the *in vivo* sucker production in Aloe vera is rather slow and insufficient to meet the demand for commercial vegetable farming. *In vitro* propagation offer a possibility to improve the regeneration efficiency of Aloe vera. Several attempts have been made for in vitro propagation of Aloe vera with varied success with different combination of plant growth regulators (Meyer and Staden, 1991., Aggarwal and Barna 2004, Fattachi *et al.*, 2004, Wenping *et al.*, 2004). The hormonal requirements for *in vitro* regeneration are varied for different genotypes (Ray and Sarkar 1991, Chaudhuri and Mukundan, 2001., Liao *et al.*, 2004, Wenping *et al.*, 2004, Saroha *et al.*, 2005). Under present investigation regeneration efficiency of sweet type genotype of Aloe vera was evaluated with different combination of plant growth regulators using shoot tip explants.

Materials and Methods

Preparation of mother stock plants

The mother plant of Aloe vera was planted in greenhouse at a spacing of 60 x 60 cm in well prepared

beds. The suckers of 10-12 cm height were collected from the mother plants and washed thoroughly in running tap water for excision of shoot tip explant.

Preparation and sterilization of explant

The expanded leaves of sucker and basal portion below 2.0 cm to the shoot tip were washed with distill water containing 0.1% Tween-20 for about 10-15 minutes. The explants rinsed with water and soaked for 30 minutes in 0.2% Bavistin solution. The shoot tip after through rinsing with distill water, were then subjected for surface sterilization with 3-4 minutes under laminar flow hood. Explants were dissected and trimmed to the size (~ 1.0 cm) for inoculation onto different medium for micro-sucker proliferation.

Culture media and conditions for microsucker proliferation

Surface sterilized shoot tip explants were inoculated on MS medium supplemented with 30 g sucrose, 8.0 g agar and various concentration of BA (1, 2, and 4 mg/l) alone or in combination of TDZ 0.5 mg/l and NAA 0.1 mg/l. The pH of the medium was adjusted to 5.7 and sterilized by autoclaving at 121°C 15 lbs for 15 minutes. The details of the proliferation medium are given in Table 1. The cultures were incubated at 26±2°C, 16 hrs photoperiod provided by cool fluroscent tube with 1000 lux intensity. Observation with respect to multiple sucker production were recorded after I and III subculture. The period of each subculture was four weeks.

Culture medium for rooting

After passing proliferated cultures onto elongation phase for two weeks (i.e. MS medium without hormone) the elongated micro-suckers of about 2 cm long were used for rooting experiment. The micro-sucker transferred to rooting media containing ½ MS medium supplemented by 3.0% of sucrose, 0.8% agar and varied levels of auxin such as IAA and NAA (0.1, 0.5, 1.0 and 2.0 mg/l).

Acclimatization of plantlets

For hardening of the plantlets in arid ecosystem, a three step hardening procedure was adopted by transferring rooted micro-suckers into protrays containing potting mixtures of vermiculite and cocopeat/vermicompost in the ratio of (3:1) and the plantlets were kept under acclimatization hood (25±2°C temperature and 80-90% RH), 5000 lux intensity for 10 days. Thereafter, transferred to green house with 28±5°C temperature 60-70% RH) under 150000 lux light intensity. The acclimatized plantlet were further transferred to environmental controlled glass-house covered with shade net of 50% light intensity for further growth and development. Statistical analysis: The observations recorded with respect to different parameters were analysed by calculating the mean value of 10-15 replicates (cultures) in each treatments. The experiment were arranged in completely randomized design for statistical analysis.

Results and discussion

Surface sterilization of Aloe vera is extremely important because the suckers normally emerged from underground stem and axillary buds, which host a large variety of microorganism. In this experiment, the expanded leaves of sucker and basal portion below 2.0 cm to the shoot tip was removed and wash with distill water containing 0.1% Tween-20 for about 10-15 minutes was important. The treatment with mercuric chloride was not sufficient for *in vitro* establishment of culture. Thus, the

Table 1. Effect of BA and NAA on the percentage of responsive explants and multiple sucker production

	Treatments MS + PGR (mg/l)			% of responsive explants	No. of microsuckers explants after 4 weeks of inoculation	No. of microsuckers explants after third sub culture	Average length of microshoots (cm)
	BA	+ TDZ	+ NAA				
T_1	0.0	+ 0.0	+0.0	0	1.0	1	1.7
T_2	1.0	+0.0	+0.0	100	2.0	3.4	2.2
T_3	2.0	+0.0	+0.0	100	2.0	4.0	1.9
T_4	4.0	+0.0	+0.0	60	2.0	3.2	1.6
T_5	1.0	+ 0.5	+0.0	100	2.2	4.5	1.9
T_6	2.0	+ 0.5	.+0.0	100	2.0	3.2	1.6
T_7	4.0	+ 0.5	+0.0	60	2.0	2.6	1.3
T_8	1.0	+ 0.5	+ 0.1	100	3.2	8.0	2.3
T_9	2.0	+ 0.5	+ 0.1	100	3.6	12	1.9
T_{10}	4.0	+ 0.5	+ 0.1	60	2.0	4	1.7
	CD				0.17	0.71	0.30

Table 2. Effect of different auxins and their concentration on the root formation in microsucker

Auxin	Concentration mg/l	% of microsucker with roots	No. of root/microsucker	Average length roots (cm.)
Control	0.0	40	1.8	1.2
IAA	0.1	45	1.9	1.5
	0.5	50	2.0	2.1
	1.0	50	2.1	1.5
	2.0	60	2.5	1.9
NAA	0.1	100	3.0	2.8
	0.5	100	4.8	2.3
	1.0	70	3.1	1.6
	2.0	40	2.1	1.4
CD			0.60	0.41

Table 3. Influence of hardening procedure on survival percentage of plantlets

Hardening steps	Survival % of plantlets	
	Vermiculite : Cocopeat (3:1)	Vermiculite : Vermicompost (3:1)
Two step procedure: In greenhouse for 10-12 days and subsequent transfer in shade house .	70	40
Three step procedure: In acclimatization hood kept in culture room for 10-12 days and subsequent transfer to Environment control greenhouse for 8-10 days, thereafter to shade house.	100	60

explants rinsing with water and soaking for 30 minutes in 0.2% Bavistin solution were also necessary steps of decontaminant procedures. The shoot tip after thorough rinsing with distil water, were then subjected for surface sterilization with 3-4 minutes with 0.1% $HgCl_2$ solution under laminar flow hood. Explants were dissected and trimmed to the size (~ 1.0 cm) for inoculation onto different medium for micro-sucker proliferation.

In the present study, shoot tip explants were assessed for *in vitro* morphogenic response. The concentration and combination of plant growth regulator greatly influenced the responsive explants in terms of percentage of responsive culture and rate of micro-sucker proliferation. The highest percentage (100%) of explants were found morphogenic responsive when cultured on several medium composition as compared to medium containing higher concentration of BA alone or in combination of TDZ or NAA (as indicated in Table 1). Medium devoid of plant growth regulators could not show response for multiple sucker proliferation. After passing to three sub-cultures, the proliferation of micro-sucker was remarkably high (12 micro-shoots) in T_9 treatment (Table 1). The maximum mean length (2.3 cm) of micro-sucker was recorded in BA 1.0 mg + 0.5 mg TDZ + 0.1 mg NAA per litre combination.

Further, increase in concentration of cytokinin (BA and TDZ) alone or in combination of NAA reduced micro-sucker proliferation. The variation in proliferation of shoots and responsive culture was also reported by Saroha *et al.* (2005), Aggarwal and Barna (2004) and Chaudhari and Mukundan (2001). The results of these workers indicated that the combination of two cytokinins along with auxin either IAA, NAA or BA was superior as compared to addition of cytokinin alone. The study of Saroha *et al.* (2005) further confirmed that TDZ 1 mg/l was superior to BA in combination of Adenine Sulphate or NAA.

The culture with proliferated micro-suckers were subjected to hormone free medium for 2-3 weeks for elongation to get rootable size of micro-sucker. The cent per cent rooting was observed in micro-suckers cultured on 0.5 mg/l NAA. The longest root was achieved with 0.1 mg/l NAA . The response to all the concentrations of IAA was poor for root formation in terms of rooting percentage and number of roots and root length. *In vitro* rooting in aloeveira has been obtained by Liao *et al.* (2004), Ray and Sarkar (1991), Natali *et al.* (1990) with varied response of different auxins.

Hardening of plantlets

The success of any *in vitro* regeneration protocol largely depends on the survival and growth performance of propagated plantlets *ex vitro*. Moreover, under hot arid

agro-ecosystem, hardening and acclimatization of plantlets are very challenging component of micropropagation. (Singh *et al.*, 2005) In the present study a three step hardening facility (Table 4) was devised and maximum 100 per cent of plantlets were acclimatized successfully as compared to low survival rate (40%) under the two step procedure. This was achieved probably due to integration of acclimatization hood as one of the important hardening steps, which favorably supported the process and facilitated the desired temperature (25-30°C) and humidity (80-90%) suited to hardening during first stage of hardening which favourably supported the *in vitro* produced delicate roots in autotrophic media. In Aloe vera, Ahmed *et al.* (2007) recorded 82 per cent survival in tissue culture Aloe vera plantlets during acclimatization with mixture of garden soil, compost and sand in proportion 2:2:1. Similar results were also noted by Agarwal and Barna (2004). Preece and Sutter (1991) also emphasized that certain environmental conditions are necessary for acclimatization of plantlets using controlled environmental facilities.

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