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Rapid *in vitro* micropropagation of non-bitter vegetable type *Aloe vera* L. (IC333202)

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Non bitter vegetable type *Aloe vera* (IC333202) is an elite medicinal plant grown in different parts of the world. A protocol for large scale *Aloe vera* production was established using micropropagation of axillary shoots. The explants were placed on semi solid MS medium with the addition of various concentrations of BAP and kinetin. After 8 weeks, the best proliferation of shoots per explant (16.7) and the best rooting was observed in IBA free medium. The rooted plant lets were gradually acclimatized in mud pots containing mixture of sand and soil.

Key words: *Aloe vera*, axillary shoot, micro propagation

Aloe vera belongs to the family Xanthorrhoeaceae grows well in a climate similar to that of the Mediterranean area with mild wet winters and hot dry summers. The gel in the leaves provides an excellent medicine for treatment of wounds, burns and other skin disorders, by placing a protective coat over the affected area, hence speeding up the rate of healing and reducing the risk of infection. Its therapeutic use was reported earlier by several workers (Cera *et al.*, 1980; Davis and Leiter, 1988; Afzal *et al.*, 1991). Though the plant can tolerate drought, no juice will be produced if the weather is too dry. *Aloe vera* flourishes well in drained fertile soil and requires about one square meter of field space per plant for growth and development. Though the demand for *Aloe* is increasing, cultivation is slow since the seed set is poor in the field and when available, it takes more time to germinate. Although *Aloe vera* propagates vegetatively in its natural state, but the process is too slow (Meyer and Staden, 1991). Attempts have been made to enhance the production through *in vitro* propagation using synthetic hormones. Several studies have reported the rapid *in vitro* propagation of *Aloe vera* (Meyer Staden, 1991; Aggarwal and Barna 2004; Bhaksha *et al.*, 2005; Gantait 2010; Mukesh Kumar *et al.*, 2011 and Dwivedi *et al.*, 2014a). Scientists had obtained different results by applying formulation of plant growth regulators with Murashige and Skoog (MS) media. The objective of this investigation was to develop a rapid, less expensive, efficient and easy method of micropropagation of non bitter vegetable type *Aloe vera* (IC333202). A new composition of growth regulators was standardized for rapid and efficient micropropagation of *Aloe vera* (non bitter vegetable type) using young axillary shoot.

Materials and Methods

Axillary shoots of non bitter vegetable type *Aloe vera* (IC333202), augmented and maintained in the field gene bank of NBPGR Regional Station, Thrissur (Dwivedi *et al.*, 2014b), served as the explant source for the present *in vitro*

regeneration experiment. The outer scale leaves were removed asceptically and shoots were trimmed to 1- 2cm length and washed with 0.15% Bavistin for 5 minutes and wiped with ethyl alcohol (70%). They were surface sterilised with 0.1% Mercuric chloride solution for 5 minutes followed by three rinses in sterile water. *A. vera was* cultured on MS (Murashige and Skooge, 1962) semi solid medium containing sucrose and agar at 3% and 0.6% respectively. The cultures were maintained at 22 ± 2°C under 16/8 hours photo period, provided by cool white fluorescent tubes. In order to study the effect of different concentrations on shoot multiplication and regeneration, 50, 100, 200, 300 and 400µg/l of Benzyl amino purine (BAP) and Kinetin (Kn) were added separately to basal media. Data on percentage of regeneration, number of shoots and number of leaves were recorded after 15, 30, 45 and 60 days of inoculation. The pH of the medium was adjusted to 5.75 using 0.1 N NaOH or 0.1N HCl before autoclaving. After 8 weeks, the culture developed new proliferated plantlets with well developed leaves and root systems, which were further maintained to hardening. Up on hardening, plant lets were transferred to plastic cups containing sterilised sand. Every day hardened material was moistened with 10 time's diluted MS broth. After 15 days, they were transferred to earthen pots containing soil and sand under green house conditions for 3-4 weeks for acclimatisation.

Results

Experiments were conducted with a view to find out the optimum concentration of BAP and Kinetin for maximum shoot multiplication by using axillary shoots as explants in non-bitter vegetable type *A.vera*. The explants were inoculated on MS semi solid medium supplemented with different concentrations of BAP and Kinetin Data on number of multiple shoots developed were recorded after 8 weeks of incubation. The response of the explants to different concentrations of BAP was inconsistent as shown in Table 1. Among different combinations, the best response was obtained when 50µg/l of BAP was added to basal medium. In this combination all the explants showed shoot regeneration with in 6 weeks of inoculation and the average number of shoots per explants was 16.67 (Fig.1). The second highest response was observed in the concentration of BAP at 200µg/l where an average of 10.5 shoot buds was regenerated from each of the explant (Fig.2). The average number of shoot buds developed for treatment with 300µg/l and 400µg/l of BAP were 10.33 and 10.20 respectively (Fig. 3&4). Minimum shoot buds development of 8.20 was obtained in MS media treated with 100µg/l of BAP (Fig.5).

Enhancement of shoot bud development was observed on medium containing kinetin also. However, regeneration of shoot buds was low in medium treated with Kinetin as compared to BAP in the medium. Maximum shoot bud regeneration of 6.50 was observed when the basal medium was treated with 100µg/l of kinetin (fig-6). Average shoots of 4 was observed in the concentration of 200µg/l and 400µg/l of kinetin (fig.7& 8). Root growth elongation was also observed and data on rooting percentage was recorded in all concentration of BAP and kinetin in MS media (Table.1). All the treatments, except control, showed rooting in the range of 60-80%. However, maximum rooting (80%) was observed when the medium was enriched with 50 µg/l of BAP and the minimum with 400 µg/l of Kinetin. The treatments differ significantly for the average number of leaves developed per explants inoculated. Plantlets were hardened after 8 weeks (fig.10) and transferred to potting mixture for acclimatization (fig.11).

Discussion

Growth regulators, mainly cytokinins are the most important factors affecting shoot proliferation. A range of cytokinins, BAP, kinetin, 2-ip, and zeatin have been used in

micropropagation research work (Bhojwani and Razdan 1992). A wider survey of existing literature suggests that BAP is the most reliable and useful cytokinin, which was in line with our results.

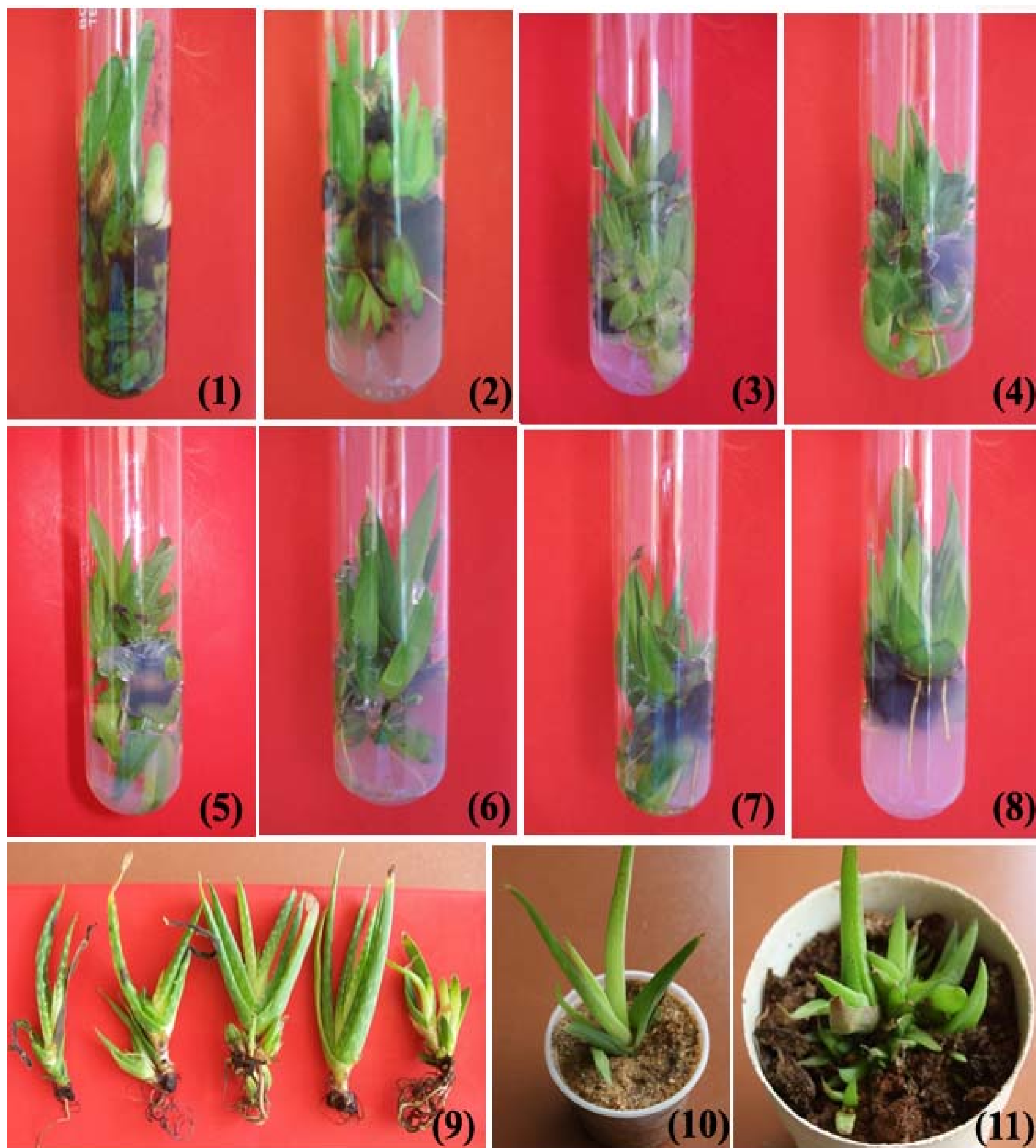

Figure 1- 11. 1. Multiple shoot induction (16.67) from the explants in MS medium with 50µg/l BAP. 2. Multiple shoot induction (10.50) from the explants in MS medium with 200µg/l BAP. 3. Multiple shoot induction (10.33) from the explants in MS medium with 300µg/l BAP. 4. Average shoots of (10.20) from MS medium with 400µg/l BAP. 5. Multiple shoot induction (8.20) from the explants in MS medium with 100µg/l BAP. 6. Induction of (6.50) shoots in MS medium with 300µg/l kinetin. 7. Induction of (4) shoots in MS medium with 200µg/l kinetin. 8. Induction of (4) shoots in MS medium with 300µg/l kinetin. 9. Rooting of shoots in MS medium with cytokinin. 10. Shoots transferred to plastic cups with sterile sand for hardening. 11. Hardened plants in pots in shade house

Table 1: Effect of different concentrations of BAP (50µg/l - 400µg/l) and Kinetin (50µg/l - 400µg/l) on regeneration, shoot proliferation, rooting and leaf development in non bitter vegetable type *Aloe vera*.

Treatments	% of regeneration	Average No. of shoots/ explants	Rooting percentage (%)	Average no. of leaves/explant
MS basal	100	1.1	-	3
MS+ BAP 50µg/l	100	16.67	80	41
MS+ BAP 100µg/l	83	8.2	70	24.6
MS+ BAP 200µg/l	100	10.5	68	30
MS+ BAP 300µg/l	100	10.33	65	27.5
MS+ BAP 400µg/l	100	10.2	72	32.67
MS+Kn (50µg/l)	100	2	70	6
MS+Kn (100µg/l)	100	2.33	75	11.33
MS+Kn (200µg/l)	100	4	65	16.67
MS+Kn (300µg/l)	100	6.5	62	22
MS+Kn (400µg/l)	100	4	60	16.5

In the present study, 100% regeneration was observed in all combinations, except with 100µg/l of BAP and signs of shoot proliferation were observed within a week of culturing. Multiplication of shoot was best observed on MS medium treated with 50µg/l of BAP (Table.1, Fig.1) where the percentage of shoot proliferation was 100 and the total number of shoots was 14. These findings are supported by those of Bhandari *et al.*, (2010), Gantait *et al.*, (2010) and Mangal Singh *et al.*, (2009) where BAP variation affecting shoot proliferation was reported. Abrie and Staden (2001) and Chaudhuri and Mukundan (2001) had also reported the use of BA in shoot proliferation of *Aloe polyphilla* and *Aloe vera* respectively. It was also reported that highest shoot proliferation in *Aloe vera* was found in MS medium containing BA and Indole-3-butyric acid (IBA) (Aggarwal and Barna 2004; Mukesh Kumar 2011 and Meyer and Stayden 1991). This is in contrast to earlier reports in *Aloe vera* by Natali *et al.*, (1990), where better proliferation occurred on medium containing Kinetin instead of BA. Baksha *et al.* (2005) also reported that the enhancement of shoots was observed by using BA and NAA. Rooting response of micro-shoots was also reported with the use of growth regulators such as NAA and IBA in medium (Bhogwani and Razdan 1992). In the present study, healthy roots were obtained in all combinations of cytokinin with MS medium in 8 weeks of time (Fig. 9). Highest root response in *A. vera* was reported in hormone free medium (Bhandari *et al.*, 2010; Aggarwal and Barna 2004). Highest shoot proliferation in *Aloe vera* was also reported in MS medium containing BA at 1 mg l^{-1} and IBA 0.2 mg l^{-1} (Mukesh Kumar *et al.*, 2011). The highest percentage of root induction (80) was observed in MS medium supplemented with 50µg/l of BAP, which also showed maximum shoot proliferation (16.67). This is contrary to the report by Abrie and Staden (2001) in *Aloe vera* where highest root induction was observed in MS medium supplemented with IBA (0.5 mg l^{-1}). Up on hardening, explants were transferred to plastic cups containing sterilised sand and everyday moistened with 10 times diluted MS broth. After two weeks, they were transferred to earthen pots containing soil and sand under green house conditions for 3-4 week for acclimatisation. The survival rate was 80% and the plants established well in 4-6 weeks of growth. Dwivedi *et al.* (2014a) have already reported a protocol for micropropagation on bitter type *A. vera*.

The present experiment demonstrated a very simple one step protocol for the rapid propagation of non-bitter vegetable type *A. vera* (IC333202) with

medicinal importance, which can be applied as a part of *in vitro* conservation of germplasm and its rapid multiplication to fulfil the demand of users and farmers.

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