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Synthetic Seeds: A Novel Approach of *In Vitro* Plantlet Formation in Vasaka (*Adhatoda vasica* Nees.)

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Abstract

Shoot buds of *Adhatoda vasica* Nees. isolated from multiple shoot cultures were encapsulated in 3% sodium-alginate with different gel matrices. Maximum conversion of the encapsulated shoot buds into plantlets was achieved on Gamborg's B5 medium containing 4.65 μM Kinetin and 50 mg l^{-1} Phloroglucinol and the plantlets were successfully grown in soil.

Keywords: Adhatoda vacsica Nees, B5 medium, Calcium chloride, Encapsulation, Plantlet, Shoot bud, Sodium alginate, Synthetic seed, Tissue culture, Vasaka.

Abbreviations

DW, Distilled Water; KIN, kinetin; PG, phloroglucinol; B5M, basal B5 medium; $B5M_1$, B5M+KIN; $B5M_2$, B5M+KIN+PG.

Introduction

Heavy exploitation, low propagation response and meagre systematic cultivation has resulted in the important medicinal plants becoming extinct and endangered. Very limited scientific studies have been carried out on *in vitro* conservation of the medicinal plants (Handa and Kaul, 1996). Vasaka (*Adhatoda vasica* Nees. syn. *Justicia adhatoda* L.), owing to the presence of alkaloids is considered to be a valuable medicinal plant (Atal, 1981;Soomro *et al.*, 1997). The plant possesses alkaloids, vasicine and vasicinone which are responsible for bronchodilatory activity of *Adhatoda* (De and Parikh, 1983). Besides its pharmacological activities, the leaf extract of the plant has insecticidal activity on grain insects (Ambasta, 1986). It acts as an antiseptic to destroy germs of diseases in drinking water and for removing noxious insects (Pal and Jain 1998).

Despite the immense significance, no systematic cultivation of *Adhatoda vasica* has been undertaken so far. Vasaka seldom sets seeds and is mainly vegetatively propagated by cutting which is season dependent. Effective rooting fails to take place in shoot cuttings (Uppal and Khosla, 1993; Anuraaga, 1999). Consequently, its natural resources are fast diminishing. The application of micropropagation techniques for *Adhatoda* are expected to bring about rapid multiplication and to ensure availability of desirable planting material throughout the year (Srivastava and Pande, 1998). The present study reports an efficient regeneration schedule of *Adhatoda vasica* Nees. through synthetic seeds.

Materials and Methods

Plant materials and culture conditions

Multiple shoot buds obtained *in vitro* (Anand and Bansal, 1998) were used as the starting material for synthetic seed production. Shoot buds measuring 2–4 mm in length with 1–2 primordial leaves were selected for encapsulation. In order to test the viability of synthetic seeds formed encapsulation studies were carried out with hydrogel [sodium alginate (4% w/v)] and complexing agents [$CaCl_2 \cdot 2H_2O$ (1.1% w/v)]. Sodium alginate was prepared by dissolving in (a) distilled water at room temperature (DW), (b) Gamborg's medium (B5M) [Basal B5M] (Gamborg *et al.*, 1968), (c) $B5M_1$ [B5M fortified with cytokinin, kinetin (KIN) (4.65 μM)], and (d) $B5M_2$ [B5M having KIN (4.65 μM) and Phloroglucinol (PG) (50 mg l^{-1})]. The complexing agent ($CaCl_2 \cdot 2H_2O$) was prepared in distilled water.

Both the solutions were autoclaved separately for 15 min at a pressure of 1.1 kg cm^{-2} and temperature of 121 ℃. In order to assess their germination potential the synthetic seeds prepared in each of the

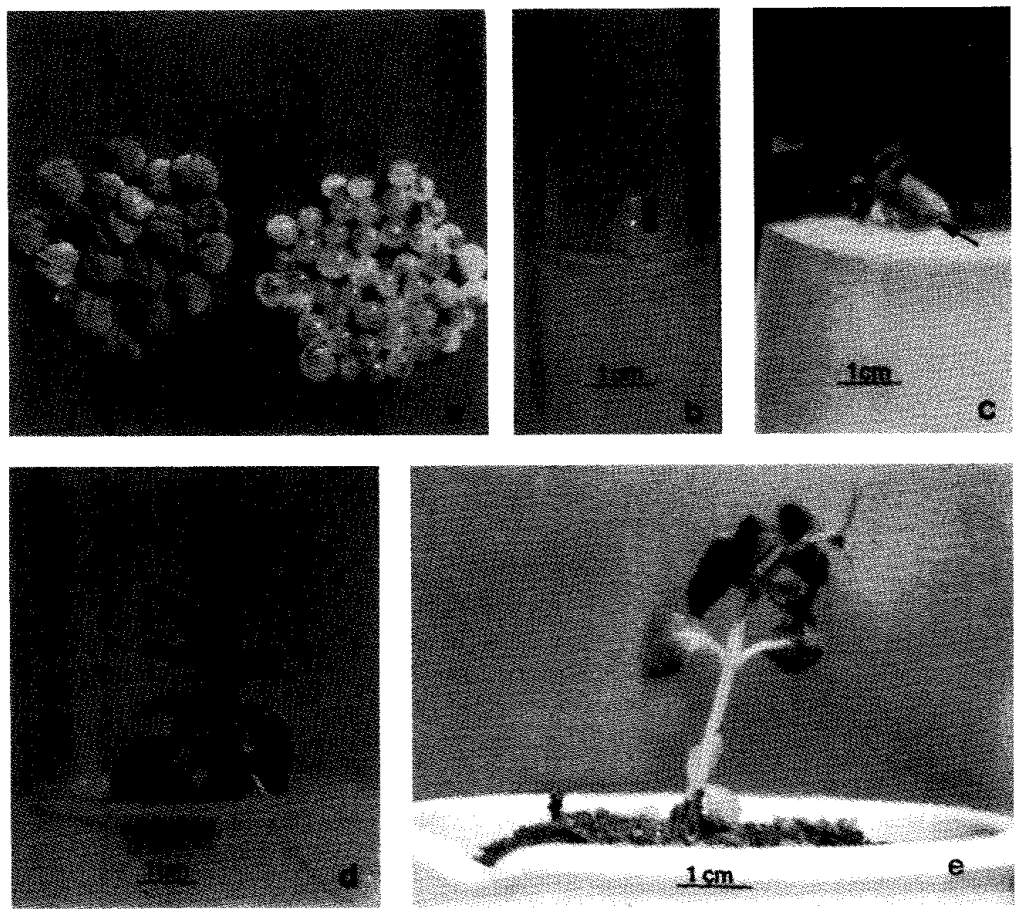

Fig. 1 Gemination and growth of synthetic seeds of *A. vasica*.
(a)Natural (n) and synthetic (s) seeds of *A. vasica*.
(b)Synthetic seed inoculated on $B5M_2$.
(c)Synthetic seed germination showing root formation (arrow).
(d)Synthetic seed derived plantlet (4 weeks).
(e)A soil transferred plant (8 weeks).

various matrices, viz. DW, B5M, $B5M_1$ and $B5M_2$ were separately placed on these media, viz. B5M, $B5M_1$ and $B5M_2$. For each treatment, 15 replicates were maintained and each experiment was repeated at least three times. Shoot buds were immersed in sodium alginate gel and with a sterile forcep slowly dropped in the beaker containing calcium chloride solution carefully so as to encapsulate the whole bud. The flask containing these encapsulated buds was then shaken in an orbital shaker at 60-80 rpm for 20 min to complete the complexation process. The calcium chloride was removed from the flask by decanting the solution. The traces of calcium chloride was removed from the encapsulated buds by washing with sterilized double distilled water twice. The encapsulated buds were subsequently placed on sterilized filter paper to remove excess water. The encapsulated shoot buds now called as synthetic seeds (**Fig. 1a**) were inoculated on a suitable medium (**Fig. 1b**) to achieve germination.

The cultures were maintained in culture tubes and kept in the culture room at a temperature of 25 ± 2 ℃, relative humidity (RH) of 60-70% and a light intensity of 40 μmol m^{-2} s^{-1} (white fluorescent tube) at a 16/8 h (light/dark) photoperiodic regime.

Results and Discussion

Synthetic seed germination

In all cases synthetic seed germination occurred within 8-10 days of incubation primarily by breaking open the calcium alginate matrix resulting in the emergence of shoot. This was followed by the gradual emergence of root after a week (**Fig. 1c**). The development of shoots and roots was simultaneous and resulted in rapid growth of plantlets within six-seven weeks. Some of the synthetic seeds failed to germinate and shoot buds turned brown within the matrix 10-15 days after culture. As shown in **Table 1**, the encapsulated shoot buds prepared in DW exhibited no germination response when cultured on B5M, $B5M_1$ and $B5M_2$. Synthetic

Table 1. Percentage of plantlet development from encapsulated shoot buds of *A. vasica on different matrices and media*

Serial No.	Encapsulation matrix (Na Alg 4%)	Inoculation media		
		B5M	$B5M_1$	$B5M_2$
1.	DW	–	–	–
2.	B5M	18.89 ± 2.14	27.78 ± 2.78	54.58 ± 1.37
3.	$B5M_1$	42.36 ± 2.22	21.03 ± 1.51	24.36 ± 2.22
4.	$B5M_2$	66.28 ± 2.22	48.48 ± 1.51	33.33 ± 2.72

Incubation period:12weeks
Values are Mean ± SE
B5M BasalB5M
$B5M_1$ B5M+KIN (4.65 μM)
$B5B_2$ B5M+KIN (4.65 μM)+PG (50 mgl^{-1})
DW Distilled water
Na Alg Sodium alginate
– No response

seeds prepared with B5M matrix and inoculated on the three media resulted in a high rate of plantlet formation (54.58%) on $B5M_2$. Similarly for seeds prepared in $B5M_1$ gel matrix B5M media was found suitable for plantlet formation (42.36%) while that in $B5M_1$ and $B5M_2$ produced fewer plants (21% and 24% respectively). The $B5M_2$ encapsulated synthetic seeds proved to be most effective when inoculated on B5M and registered the highest frequency of synthetic seed germination (66.28%) resulting in complete plantlet formation within 3-4 weeks. Although the encapsulated (in $B5M_2$) shoot buds placed on $B5M_1$ and $B5M_2$ resulted in plantlet formation (**Fig. 1d**) but their frequencies varied (48.48% and 33.33% respectively). These studies indicate that the presence of nutrients and plant growth regulators (PGRs) in the encapsulation matrix is more important than in the inoculation medium. Consequently, synthetic seeds formed in $B5M_2$ encapsulation matrix germinated best on B5M inoculation medium. However, in some cases the synthetic seeds failed to exhibit proper germination and resulted in the formation of slender leaves with complete failure of induction of root. Therefore, in order to increase the germination percentage, further improvement in culture conditions might be required. The plantlets thus obtained were maintained on B5M for a period of six weeks. After which the developing plantlets were transferred to soil. The plantlets were gradually naturalized to the environment and sucessfuly grown in soil (**Fig. 1e**). Synthetic seeds encapsulation has been reported as a powerful tool for mass production of elite plant species. And such seeds have been known to contain mostly tissue culture derived somatic embryos (Redenbaugh *et al.*, 1986; Rao *et al.*, 1997). However, in recent years the encapsulation of shoot buds for the formation of synthetic seeds has received considerable attention (Bapat *et al.*, 1987; Ganapathi *et al.*, 1994). Shoot bud encapsulated synthetic seeds of medicinally important vasaka (*Adhatoda vasica* Nees.) are expected to be superior to the conventional synthetic seeds as it would obviate the need of somatic embryos which are not induced with ease in several species. In the present investigation, a large number of shoot buds obtained by mulitple shoot induction from shoot bud cultures *in vitro* were used for the preparation of numerous synthetic seeds. Although a number of plantlets can be procured from multiple shoot cultures, the process is tedious. Besides, the multiple shoots have to be separated individually at the time of rooting. By employing the procedure of encapsulation of shoot buds, the intermediary phases of elongation and rooting can be eliminated.

Since the encapsulated shoot buds are useful in germplasm storage (Fabre and Dereuddre, 1990), they can be treated like a seed having the additional advantages for handling, transportation, efficient delivery of plants and would minimise the cost of production.

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