Development of synthetic seed and its evaluation under controlled conditions

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Abstract— **The artificial seed technology provides an alternative method of micro-propagation for a wide range of medicinal plants, especially desirable elite genotypes. In the present study Rose (*Rosa indica*), Boganvalia (*Bougainvillea glabra*) and Sweet neem (*Murraya koenigii*) nodal part (2-5mm) were collected for the callus induction. The callus was encapsulated in 4% sodium alginate using MS media. The culture condition of the MS media was optimized at different pH to study the germination of artificial seed. The germination of artificial seed was found to be best at pH 6.6. The in vitro encapsulated nodal segments technology facilitates conservation of genetic diversity.**

Keywords— Rosa indica, Bougainvillea glabra, Murraya koenigii **and Encapsulation.**

Abbreviations: MS media: Murashige and Skoog (1962) media, IAA: Indole-3-Acetic acid, IBA: Indole-3-butyric acid and Kn: kinetin.

Introduction

In recent years, the alginate-encapsulated in vitro-derived shoot tips and nodal segments have been employed as an alternative to somatic embryos method of artificial seed production. Encapsulation of non-embryogenic propagules offers an efficient technique for clonal propagation of elite genotypes e.g. plants of medicinal importance and also enables the establishment of basal collection for gene banks. The artificial seeds (also called somatic seeds, synthetic seeds, clonal seeds, syn-seeds, some seeds) are defined as an alternative to botanic seeds analogue consisting of somatic embryos surrounded by artificial coats. This definition, also popular in these days, is based on the similarity of somatic embryos with zygotic embryos in morphology, physiology and biochemistry (Redenbaugh *et al.,* 1986; Redenbaugh *et al.,* 1988). Artificial seeds have been proposed as a low cost propagation system of uniform plants. For this reason, it has been suggested as a powerful tool for mass propagation of elite species with high economic value (cereals, vegetables, fruit plants, ornamentals, aromatic and conifers) and rare or endangered taxons (Ara *et al.,* 2000). Efficient propagation, artificial seed production and storage protocols of threatened plants allow the continuous supply of plant material of medicinal importance (Srivastava *et al.,* 2009) and enable the establishment of basal collection with representative genetic diversity for gene banks (Bach *et al.,* 2004). Alginate-encapsulated of shoot tips and nodal segments in micro propagation of medicinal plants trees, non-seed producing species, poly-ploid plants with elite traits and male or female sterile ornamental hybrids. The synthetic seed technology can be also considered as an important tool for micropropagation either the cross pollinated crops since the allogamous nature makes it impossible to obtain elite clones by natural seeds (Singh *et al.,* 2009). In natural environment where various infections can threat the organisms, it is important to produce virus-free plants protected against pathogens (Lata *et al.,* 2009). The significant advantage includes their designation as 'genetically identical materials'. The increasing utilization of synthetic seeds for clonal propagation necessitates assessment of genetic stability of conserved seeds and recovered plants (Patel *et al.,* 2000; Thiem *et al.,* 2008). In simple words synthetic seed contains an embryo produced by somatic embryogenesis enclosed within an artificial medium that supplies nutrients and is encased in an artificial seed covering. The technology designed to combine the advantages of clonal propagation with those of seed propagation and storage (Nikhil *et al.,* 2013).

Materials and Methods

1. In-vitro culture of stock plant and callus induction

Nodal part of the plant (*Rosa indica, Bougainvillea glabra and Murraya koenigii*) were excised (5mm) from the plant then sterilized in 0.1% Mercuric chloride (Central drug house (P) Ltd, New Delhi) for 5 minutes and resin 3-4 times with sterile tap water. The explant was cultured on MS media (1962) for callus induction supplemented with IAA (25mg/l), Nicotinic acid (0.5mg/l), Kn (10mg/l) and IBA (20mg/l).

The media was solidified with 0.8% agar-agar (Hi-media Laboratories, Mumbai) and the pH of the media was adjusted to 5.6 prior to autoclaving at 121oC for 15min. The media was at 25±1oC under a16-h photoperiod (12.1 mol m-2 s-1 of light intensity provided by cool white light fluorescent tubes: Philips).

2. Encapsulation of Callus

For the encapsulation procedure, callus induced from the nodal portion of *Rosa indica, Bougainvillea glabra and Murraya koenigii* were mixed in 4% sodium alginate matrix (s d fine-chem limited, Mumbai). The encapsulated callus then picked up by a pair of forceps and gently dropped into

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Calcium chloride (CaCl2: 1.4%) (E. Merck limited, Mumbai) solution in which they were allowed to stand for 20-25minutes for complexing. At the end of 25min the CaCl2 solution was carefully decanted off and the encapsulation callus were washed with 3-4 times sterile water, blotted dry on sterile filter paper and cultured on MS basal media supplemented with 2mg/l Glycine, 0.5mg/l Nicotinic acid, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA. The culture media was optimized at different pH to study their germination.

Table 1
Studies on Alginate encapsulated explant of selected plant

S.N	Plant Species	Encapsula -ted Plant material	Seed Coat
1.	*Rosa indica*	Nodal segment	4% SA, 1.4% CA, 2mg/l Gy, 0.5mg/l NA, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA
2.	*Bougainvillea glabra*	Shoot tip	4% SA, 1.4% CA, 2mg/l G, 0.5mg/l NA, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA
3.	*Murraya koenigii*	Apical meristem	4% SA, 1.4% CA, 2mg/l G, 0.5mg/l NA, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA

(SA- Sodium alginate, CA- calcium chloride, Gy- Glycine, NA- Nicotinic acid, Kn- Kinetin IAA- Indole-3- acetic acid and IBA- Indole-3-butyric acid)

Results and Discussion

The present study deals with the callus induction and synthetic seed production which has been successfully achieved in all explants of *Rosa indica, Bougainvillea glabra and Murraya koenigii.* Swelling and expansion in the different explant (Rosa indica- Nodal segment, Bougainvillea glabra- Shoot tip and Murraya koenigii- Apical meristem) were observed with in a weak after culture initiation. Callus formed after culturing was found to be soft, smooth and white in colour (Fig. 1, 2 & 3).

Figure 1: Callus inductions in *Rosa indica* from Nodal segment. (a) White coloured callus developed onto the MS media supplemented with 2mg/l Glycine, 0.5mg/l Nicotinic acid, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA at pH 6.6 after 4 weeks of culture (0.2cm).

Figure 2: Callus inductions in *Bougainvillea glabra* Shoot tip. (a) White coloured callus developed onto the MS media supplemented with 2mg/l Glycine, 0.5mg/l Nicotinic acid, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA at pH 6.6 after 4 weeks of culture (0.25cm).

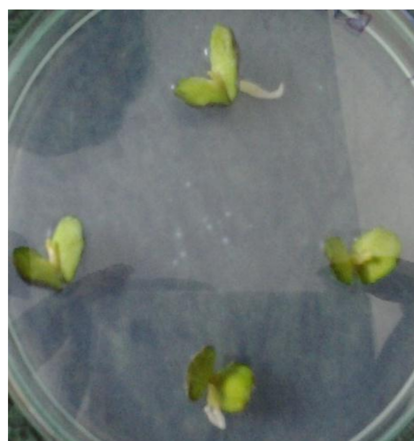

Figure 3: Callus inductions in *Murraya koenigii* from Apical meristem. (a) White coloured callus developed onto the MS media supplemented with 2mg/l Glycine, 0.5mg/l Nicotinic acid, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA at pH 6.6 after 4 weeks of culture (0.3cm).

After callus induction the callus was encapsulated in 4% sodium alginate (s d fine-chem limited, Mumbai) using hardening solution 1.4% calcium chloride ($CaCl_2$) (e. merck limited, Mumbai) (Fig. 5, 6 & 7).

Figure 5: Encapsulated calluses in 4% Sodium alginate and 1.4% Calcium chloride solution (a) encapsulated callus of *Rosa indica* (0.5cm).

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Figure 6: Encapsulated calluses in 4% Sodium alginate and 1.4% Calcium chloride solution (a) encapsulated callus of *Murraya koenigii* (0.55cm)

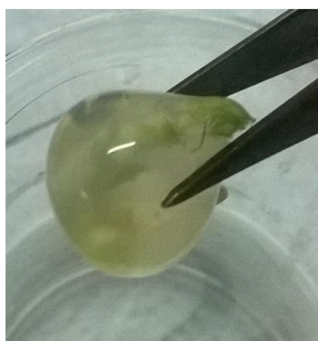


Figure 7: Encapsulated calluses in 4% Sodium alginate and 1.4% Calcium chloride solution (a) encapsulated callus of *Bougainvillea glabra* (0.55cm)

Likewise in the current study, the callus condition was optimized at different pH (4.6, 5.6, 7.6, 8.6 and 9.6) to study the germination of encapsulated callus. The encapsulated callus was maintained in MS media supplemented with 2mg/l Glycine, 0.5mg/l Nicotinic acid, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA. Maximum germination was observed in Bougainvillea glabra- Shoot tip and Murraya koenigii- Apical meristem) at pH 6.6 after 6 weeks of culture while minimum germination was observed at pH 4.6 and 8.6. No growth was observed at pH 9.6 (Fig. 8 & 9).

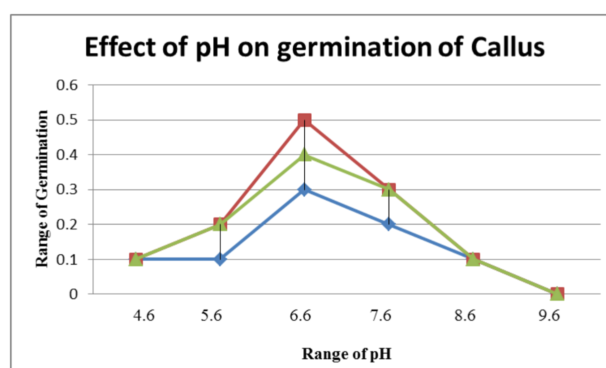
Figure 8: Germination of callus onto MS (Murashige and Skoog (1962) media) media supplemented with 2mg/l Glycine, 0.5mg/l Nicotinic acid, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA (a) Germination of callus of *Bougainvillea glabra* at pH 6.6 after 6 weeks of culture (0.4cm).

Figure 9: Germination of callus onto MS (Murashige and Skoog (1962) media) media supplemented with 2mg/l Glycine, 0.5mg/l Nicotinic acid, 25mg/l IAA, 10mg/l Kn and 20mg/l IBA (a) Germination of callus of *Murraya koenigii* at pH 6.6 after 6 weeks of culture (0.3cm).

Table 2:
Effect of different pH on germination of encapsulated callus

S. No.	pH Range	Name of Plant Species		
		Rosa indica	*Bougainvillea glabra*	*Murraya koenigii*
1.	4.6 (Highly acidic)	*	*	*
2.	5.6 (Acidic)	*	**	**
3.	6.6 (Slightly acidic)	***	*****	****
4.	7.6 (Neutral)	**	***	***
5.	8.6 (Alkaline)	*	*	*
6.	9.6 (Highly alkaline)	-	-	-

*, Poor growth; **, Slightly better; ***, Fine; ****, Good; *****, Very Good.



Graph 1: Effect of different pH on germination of encapsulated calli of *Rosa indica, Bougainvillea glabra and Murraya koenigii* after 6 weeks of culture onto the MS (Murashige and Skoog (1962)) media.

(● *Rosa indica,* ● *Baugainvillea glabra,* ● *Murraya koenigii)*

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Conclusion

In conclusion, the present work reports the successful production of artificial seeds of Rosa indica. Bougainvillea glabra and Murraya koenigii and callus induction were initiated from in-vivo in the different explants (*Rosa indica*- Nodal segment, *Bougainvillea glabra*- Shoot tip and *Murraya koenigii*- Apical meristem). Our study also demonstrated that the slightly acidic pH showed the best germination of encapsulated callus while highly alkaline pH showed no germination.

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