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Artificial seeds production of *Inula racemosa* Hook. F.

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ABSTRACT

The aim of this research paper was to develop an efficient protocol for the production of artificial seeds of Inula racemosa, an important medicinal plant of temperate alpine region. The plant has been listed as critically endangered by Red Data book criteria of International Union of Conservation of Nature and Natural Resources (IUCN). Artificial seeds offer an attractive and quick method for rapid propagation and further conservation of the plant species which are at the verge of extinction. In the present investigation, 32 weeks old callus culture was used as an explant for artificial seed production. 2% and 3% sodium alginate solution was used for encapsulation of the explant which was further sealed by keeping them in $CaCl_2.2H_2O$ solution for 30 minutes. Beads of desired shape, texture and quality were obtained at 3 % sodium alginate whereas at 2% sodium alginate seeds were malformed, too soft to handle and very fragile. To maintain the viability of synthetic seeds, seeds were kept in sterilized cryovials. The cryovials were then cryoconserved at -196^{o}C temperature of liquid nitrogen for future propagation and conservation of the plant.

Introduction

Medicinal plants constitute a very important bioresource in India because it has one of the richest plant based traditions in the world. Home to around 20,000 medicinal species (Dev, 1997), India is the largest producer of the medicinal herbs and appropriately called the *"botanical garden of the world"* (Ahmedullah and Nayar, 1999). In Indian system, Ayurveda has a plenty of references of plants used for medicinal purposes as for example, in Charak Samhita (1000 BC) 595 plants are referred for their therapeutic use. The reference of medicinal plants and herbal medicine is estimated to be worth 80 billion US dollars a year. International export trade in medicinal plants from India is 32,600 tonnes a year (Jabeen *et. al.,* 2007). The demand for medicinal plants has increased globally due to resurgence of interest in herbal medicines, culinary herbs, natural therapeutic essential oil and pharmaceuticals (Shawl and Qazi, 2004). Most of the demand is being met through collection of large quantities of these plants and their parts from wild populations. Due to the crude and unscientific extraction methods and absence of any effort to cultivate them, the rate of exploitation may exceed the rate of natural regeneration.

Thus, there is an urgent need to develop propagation as well as conservation strategies for the medicinal plants; otherwise most of the medicinal plants will become extinct while many of them at present fall in the category of endangered species (Kaur *et. al.,* 2007).*Inula racemosa* is one such medicinal species of the Himalayan range which is witnessing a speedy decline in density, dwindling both in size and number (Shabir *et. al.,* 2010).

Inula racemosa Hook. F.:

Inula racemosa is commonly called Pushkarmool belongs to family Asteraceae. The plant is distributed in temperate alpine Himalayas at an altitude of 1,500- 4,200m from Kashmir to Kumaon, Afghanistan to Central Nepal (Firdous *et. al.,* 2018). Pushkarmool is an important medicinal plant of the North Western Himalayas (Anonymous, 1998; Wani *et. al.,* 2006). The plant is used in Ayurveda as an expectorant and resolvent in indurations. It is considered as a *"Rasayana"* (rejuvenator, immunomodulator) by Ayurvedic physicians. According to Bhavaprakasha, the drug is bitter pungent in taste (Bhavaprakasha, 1961). The aqueous extract of the fresh or dry root is given orally in rheumatic pains and liver problems. Externally a paste or liniment is used for relieving pain. The root is also used in veterinary medicine as a tonic. The root forms important ingredients of several polyherbal formulations for heart diseases and inflammatory conditions of spleen and liver.

Inula racemosa Hook. F. is listed as critically endangered by Red Data book criteria of International Union of Conservation of Nature and Natural Resources (IUCN) due to fragile nature of its habitat, habitat destruction, illegal and destructive harvesting from the wild source and great market demand (Parvaiz *et al.,* 2006). So far, this plant has not got the required attention from researchers hence except for a few efforts not

much work has been done for its cultivation and conservation.Cryopreservation techniques offeran attractive and quick method for rapid multiplication and further conservation also. Among the cryopreservation techniques, encapsulation method for producing artificial seeds or synthetic seedshas become an important asset in micropropagation as the alginate coat protects micropropagules and thus has practical application for germplasm conservation of an elite/endangered plant species and exchange of axenic plant materials between laboratories (Fabre and Derenuddre, 1990; Hasan and Takagi, 1995). Synthetic seeds are artificially encapsulated somatic embryos or other vegetative parts such as shoot buds, cell aggregates, axillary bud, or any other plant parts which can be sown as a seed and converted into plant under *in vitro* or *in vivo* conditions.

In addition, alginate coated propagules are relatively inexpensive to produce and easy to handle, transport and plant. This method also offers several advantages such as therisk of contamination and human errors is lower, less expensive and storage time can be prolonged. Cryopreservation of the plant species will help in making the germplasm available on sustainable basis and save it from the verge of extinction. The production of synthetic seeds in *Inula racemosa* is useful since the plant has propagated through natural seeds and rootstocks. However, propagation through conventional method is a season dependent process and required lengthy cultivation cycle. Review of literature suggested that little work have been published on the production of artificial seeds of *Inula racemosa* Hook F. So, the present study was focused to develop an efficient protocol which can solve the purpose for its commercial exploitation and could be an answer to remove the endangered status of the species.

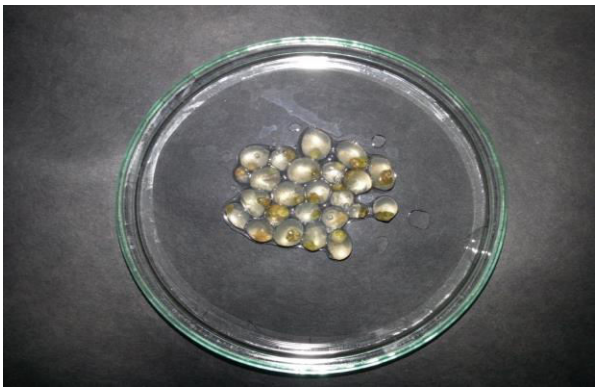

Fig- 1: Artificial seeds encapsulated by sodium alginate (3% sodium alginate)

Material and Methods

Explant

Somatic embryos initiated from the callus of shoot tips taken*in vitro* grown seedlings when media was supplemented with various growth hormoneswas used as a source of explant.

Encapsulation Techniques and Bead Formation

Explants were excised and kept in sodium alginate solution of two different concentrations (2% and 3%) and swirled gently. Sodium alginate beads were formed by dropping procedure. After that callus culture encapsulated with sodium alginate rinsed drop wise in the $CaCl_2.2H_2O$ solution.In order to seal the beads the encapsulated callus culture were kept in this solution for 30 minutes. Then, the beads were taken out and transferred into sterile distilled water to wash out the excess $CaCl_2.2H_2O$ solution.After that, the beads were kept for dehydration inside on filter papers for 5-6 hrs. All the manipulations were carried out aseptically in a laminar air flow chamber. For cryopreservation the beads were transferred to the pre-cooled cryovials which were placed in small slender muslin cloth bags and these bags were quickly plunged into a container of liquid nitrogen and kept at different durations.

Results and discussion

Small gel beads were obtained when small pieces of callus culture were coated with 3% and 2% sodium alginate and then treated with $CaCl_2.2H_2O$ solution. Freshly encapsulated seeds obtained appeared elastic to the touch and showed a transparent gel layer of 2-4 mm at 3% sodium alginate (Figure 1) whereas at 2% sodium alginate seeds were malformed, too soft to handle and very fragile (Figure 2). To maintain the viability of synthetic seeds, seeds were kept in sterilized cryovials. The cryovials were then cryoconserved at -196^{o}C temperature of liquid nitrogen for future propagation and conservation of the plant.

Fig- 2: Artificial seeds encapsulated by sodium alginate (2 % sodium alginate)

Conclusion

The encapsulated beads formed at two different combinations of sodium alginate solutions (2 and 3 percent) and $CaCl_2.2H_2O$ solution differed morphologically with respect to texture, shape and transparency. An encapsulated matrix of 3 percent sodium alginate was found most suitable for the formation of ideal beads (Figure 1). Sodium alginate concentration at 2 percent was not suitable for encapsulation because the resulting beads were without defined shape and were too soft to handle (Figure 2).

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