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The use of encapsulation dehydration for the first time in *Bunium persicum* to converse this important endangered medicinal plant

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ABSTRACT

India is known as "The Home of Spices". Seed spices are an important group of agricultural commodities and play_ a significant role in our national economy. Bunium persicum (Boiss.) Fedtsch, a member of family Apiaceae, is one of the medicinally important spices but it is critically endangered. Plant tissue culture plays a very significant role in production and conservation of an endangered medicinal plant. Hence encapsulation dehydration was carried out for the first time in Bunium persicum to converse this important endangered medicinal plant. For encapsulation shoot buds and somatic embryos were excised from in vitro stock cultures, and these explants were encapsulated into beads. The beads were dehydrated by air drying in laminar flow chamber to reduce the water content, followed by direct immersion in liquid nitrogen in cryovials for one month. Frozen beads were quickly thawed in water bath at 40°C for 3 minutes and cultured on MS medium fortified with 0.25 mg/l TDZ and 200 mg/l activated charcoal for shoot retrieval. A maximum of 45.8 % shoot retrieval was recorded. The technique of encapsulation dehydration would help in making the germplasm available on sustainable basis and save it from the verge of extinction

Key words: *Bunium persicum, In vitro conservation, Encapsulation dehydration, Synthetic seeds*

Introduction

In recent times, medicinal and aromatic plants constitute the most viable alternative cash crops for the growers and the pharmaceutical industries. *Bunium persicum* commonly known as kalazeera is a perennial endangered medicinal herb, which is a native of Europe and Western Asia. It has been found growing wild at altitudes ranging between 2000 to 3000m above sea level in the Western Himalayas region extending upto Baluchistan and Afghanistan [1]. In India, it grows wild in some parts of Himachal Pradesh (Lahaul-Spiti, Chamba, and Kinnaur), Jammu and Kashmir (adjoining areas of Kishtwar) and in some parts of Uttarakhand (Garhwal and Kamoan) [2].

Bunium persicum, kalazeera, is an expensive spice, extensively used for culinary, flavouring and carminative purposes. Its fruits commonly called as seeds are used as prized spices in cooking for flavouring. Kalazeera is however one of the over exploited economically important spice plants without planned cultivation [3]. It has also found place in indigenous system of medicine as it is considered as a stimulant and has found to be useful in diarrhoea [4], dyspepsia, fever, stomach-ache, obstinate hiccup [5]. The seeds have, however, been replaced by their essential oil which is now widely used for seasoning, pickles, sauces, candies etc.

In nature this plant is under constant threat of biotic and abiotic factors *viz*. grazing, indiscriminate collection by human beings and trampling. Gradual decline in size and number of population has reduced yield and consequently there is an increase in market price. These factors can be checked by standardization of agro practices. Efforts have been directed for its domestication. The spice in nature propagates through seed and takes 4-6 months for germination and crop can be harvested after three years of sowing. On maturity, it yields 5-10g mature fruits/plant during June-July and average yield vary around 5-6 quintal per hectare in a well-established crop.

A long juvenile period to get mature tubers for yield, has hampered the conventional breeding programs and inspite of high material costs, systematic cultivation is not undertaken anywhere. Unabated the plant extraction and habitat

destruction continues to be, far are not days when these herbal gems will become extinct from globe. So far, this plant has not got the required attention from the researchers. Hence the present study was carried out to conserve the species using plant tissue culture, as it plays a very significant role in production and conservation of endangered medicinal plants. Encapsulation dehydration is an *in vitro* conservation technique, which can be applied for conservation of this important spice for short and long term basis. The encapsulation dehydration procedure is based on the technology developed for the production of artificial seeds and it involves encapsulation of explants in alginate beads, followed by pregrowth treatment in a medium containing a level of sucrose, then dehydrated under laminar air flow, followed by rapid cooling in liquid nitrogen. This procedure provides much greater flexibility and ease of handling of explants to be cryopreserved.

The method of encapsulation dehydration has become an important asset and has a practical application for conservation of endangered medicinal plants [6,7]. Many earlier workers have reported encapsulation dehydration in various plant species e.g. [8] Azalea; [9] 2006 in *Rauvolfia tetraphylla*; [10] *Psidium guajava*; [11] *Inula racemosa*, [12] *Gentiana kurroo*.

Materials and Methodologies

Shoot buds and somatic embryos were excised aseptically in a laminar air flow chamber from *in vitro* stock cultures of *Bunium persicum* and the explants excised were then kept overnight in preculture medium named EN1 solution (MS + 0.5 M Sucrose) (Table 1). The explants were then placed in 10 ml of sodium alginate solution of different concentrations (1-5%) and swirled gently. Sodium alginate beads were formed by dropping procedure as follows, 5 ml of sodium alginate solution containing the shoot bud/ somatic embryo inside was drawn out with the help of a dropper [11]. Then the explants encapsulated with sodium alginate were dropped one by one into calcium chloride solution of different concentrations (0.25-2.00%). Encapsulated beads formed thus were placed in EN3 solution (MS + 0.8 M Sucrose) (Table 1) and kept on orbital shaker for different durations for proper formation of shape. The beads were then kept for dehydration inside laminar cabinet on filter papers for 5-6 hours. 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 for different durations. After cryopreservation the cryovials were taken out and thawed quickly in a water bath at 40°C with constant agitation. Henceforth the manipulations were carried out inside laminar air flow chamber. To test the retrieval after cryopreservation the beads were given different treatments and some were kept directly on solidified MS medium under dark as well as light conditions and others were kept on liquid MS media in dark as well as light at 22°C. The experiment with each explant was repeated thrice so as to be sure about the per cent conservation.

Results

Preculturing of explants in EN1 had a beneficial effect on survival of cryopreserved explants (Table 2). Table 2 clearly depicts that majority of explants encapsulated and germinated when preculturing treatment was given prior to conservation treatment.

In the subsequent bead formation the results of both the explants were at par, beads of desired shape, texture and quality were obtained when 3.00% sodium alginate solution was used to make complex with 0.50 % $CaCl_2$ solution. When

Table 1: Composition of various solutions used for encapsulation of *in vitro* explants of *Bunium persicum.*

Solution for Encapsulation	Composition
Preculture medium (EN1)	MS + 0.5 M Sucrose
Sodium alginate solution	MS + 0.4 M Sucrose + Sodium alginate
Calcium chloride solution	MS + 0.4 M Sucrose + Calcium chloride
EN3 solution	MS + 0.8 M Sucrose
Medium for regeneration	MS medium + 3.00 % sucrose + 0.25 mg/l TDZ + 200 mg/l activated charcoal + 8.00% agar

Table 2: Effect of preculturing on germination of explants after cryopreservation.

S.No.	Explant	Preculturing	Germination percentage of buds
1.	Shoot buds	+	72.8
		-	2.65
2.	Somatic embryos	+	75.5
		-	3.80

Table 3: Effect of different durations of thawing on germination percentage.

S.No.	Explant	Duration of liquid nitrogen treatment	Thawing Temperature	Germination percentage
1.	Encapsulated Shoot bud	1 hr	40°C	72.8
		2hr	40°C	72.2
		4 hr	40°C	72.8
		1Day	40°C	71.9
		3 days	40°C	71.6
		1 week	40°C	72.4
2.	Encapsulated Somatic embryo	1 hr	40°C	75.5
		2hr	40°C	74.7
		4 hr	40°C	75.1
		1Day	40°C	75.5
		3 days	40°C	75.7
		1 week	40°C	74.9

sodium alginate was used at a concentration above 3.00 %, beads were although of desired oval shape but they turned out to be very hard and failed to germinate on either of the medium (liquid as well as solid MS supplemented with growth regulators) and when below 3.00% concentration was used distorted beads were obtained and 1.00% sodium alginate failed to produce beads. While when $CaCl_2$ was used above 0.5 per cent very hard beads were obtained.

Hence beads formed with 3.00% sodium alginate and 0.50% $CaCl_2$ were considered as the best type of beads formed as they had perfect oval shape and they were not very hard. The beads thus formed were kept on orbital shaker inside a flask containing EN3 solution for 1-2 hours for pre conditioning and the 1- 2 hours preconditioning maintained the uniform shape and texture of beads. Here the duration of pre conditioning was recorded to an important factor because if the pre conditioning was increased from 2 hours then the beads turned brown and hard and again faced problem with germination. Then dehydration of beads was carried out aseptically and it was observed that five to six hours dehydration was must.

Then the beads were transferred to pre cooled cryovials and kept for one hour to one week in a cryocan containing liquid nitrogen. After cryopreservation the cryovials were taken out and thawed quickly in a water bath at 40°C with constant agitation. Thawing of the cryovials proved to be an effective measure of retrieval of encapsulated beads (Table 3).

Then the beads or the synthetic seeds so produced were placed on solid as well as liquid regeneration medium that had earlier been standardized in our laboratory earlier and germination was observed in liquid medium as well as solid medium though it was poor on solid medium and best on liquid medium. The germinated encapsulated beads when cultured on MS medium fortified with 0.25 mg/l TDZ and 200 mg/l activated charcoal for shoot retrieval a maximum of 45.8 % shoot retrieval was recorded. After that rooting of these shoots was attempted on already standardized medium in our laboratory and then these *in vitro* plantlets shifted to field conditions.

Discussion

Bunium persicum (Boiss.) Fedtsch is a prized medicinal spice and condiment as well as aromatic plant, yielding the seed spice “Kalazeera”. The overexploitation of this plant species has drastically reduced its population in nature. The domestication/cultivation on a large scale will check the ruthless extraction of seeds from its wild plants which has otherwise threatened the species into an endangered category. In the present investigation a protocol has been devised for conservation of *Bunium persicum* by encapsulation dehydration and the results clearly show that this method has potential for long term storage.

To the best of our knowledge no such work has been done earlier in *Bunium persicum* so we will discuss here some other medicinal plants. Preculturing prior to encapsulation has been found beneficial for the explants, which were cryopreserved, as it enhanced survival after immersion into liquid nitrogen and it hardened them for their treatment with cryoprotectants as well as to tolerate the freezing stress. These results are in agreement with the similar work carried out by [8,11-14].

The encapsulation of beads was attempted with different concentrations of sodium alginate and $CaCl_2$ and it was observed that the beads differed morphologically with respect to shape, texture and transparency with different concentrations. Hence the assessment of various concentrations was pre requisite in order to standardize the preparation of characteristic beads. An encapsulated matrix of 3.00% sodium alginate with 0.50% $CaCl_2$ was found most suitable for the formation of ideal beads and earlier many workers have also reported the use of 3.00% sodium alginate [8-12,15,16].

In the present investigation preculturing was done before as well as after encapsulation of the explants which might have prevented stress due to desiccation as a result a good percentage of retrieval was obtained after thawing. Rapid thawing at 40°C for 3 minutes after treatment ensured retrieval, these results are in agreement with work done by [8,11,12]. The efficacy of solid and liquid medium was tested for the germination of encapsulate beads or synthetic seeds and it was found that liquid medium gave better results, as it has been earlier reported by many workers [10,11].

Conclusion

The present study demonstrates that the combined encapsulation dehydration method can be efficiently utilized to cryopreserve *Bunium persicum* – an endangered medicinal plant and to promote its subsequent regeneration. This method could be applicable to the long-term preservation in liquid nitrogen. Moreover, the procedure is easy to perform and there is no need for sophisticated equipment. No morphological abnormalities were observed in the plants developed after encapsulation dehydration procedure.

References

1. Bhartiya S P. Black Zira is a good bet. *Ind Farming*, 1967, 13(1): 30-32.
2. Panwar KS, Sagwal JC, Sharma SK, et al. Economic viability of kalazeera cultivation in high altitude dry temperate region of Himachal Pradesh. *Agri Situation Ind*. 1993,48:151-154.
3. Raina AK, Jamwal PS. 1989. Conservation of Bunium persicum (Boiss) Fedtsch. kalazira in North West Himalaya. Proceedings of the 10th Annual Session of Academy of Environmental Biology. Symposium on Environmental Impact on Biosystems.
4. Kataria HC, Ahmed, Gupta SS. Indigenous medicinal plants and important synthetic drugs used in Diarrhoea: A review. *Orient J Chem*. 1994,10(1): 41-44.
5. Chauhan N S. 1999. Bunium persicum (Boiss) Fedtsch. Medicinal and Aromatic Plants of Himachal Pradesh. Indus Publishing Company, New Delhi, India, p 429.
6. Fabre J, Dereuddre. Encapsulation dehydration: a new approach to cryopreservation of Solanum shoot tips. *Cryoletters*.1990,11:413-426.
7. Hasan SMZ, Takagi H. Alginate coated nodal segments of yam (Dioscorea spp.) germplasm exchange and distribution. *Plant Genet Resour Newslett*.1995,103:32-35.
8. Verleysen H, Samyn G, Bckstaele EV, et al. Cryopreservation of Azalea by encapsulation dehydration. *Acta Hortic*. 2003,612:139-145.
9. Faisal M, Naseem A, Anis M. In vitro plant regeneration from alginate encapsulated micro cuttings of Rauvolfia tetraphylla L. *American-Eurasian J Agric Environ Sci*. 2006,1(1):1-6.
10. Rai K M, Jaiswal V S, Jaiswal U. Encapsulation of shoot tips of guava (Psidium guajava L.) for short term storage and germplasm exchange. *Scientia Horticulturae*. 2008, 118(2):33-38.
11. Kaur R, Kashyap A, Majeed S, et al. In vitro propagation and conservation of Inula racemosa Hook F. an Endangered Medicinal Plant of Temperate Origin. *J Adv Lab Res Biol*. 2009, 1(1):67-70.
12. Sharma A. 2014. Studies on in vitro morphogenesis of Gentiana kurroo Royle – an endangered medicinal plant. Ph.D. Thesis Dr Y S Parmar University of Horticulture and Forestry. 123p.
13. Paques M, Poisonnier M, Dumas E, et al. 1997. In: Cryopreservation of dormant and non dormant broad leaf trees. Altman A and Ziv M (eds.) p. 491-497.
14. Paul H, Gaigny G, Sangwan NBS. Cryopreservation of apple (Malus x Domestica Borkh.) shoot tips following encapsulation dehydration or encapsulation vitrification. *Plant Cell Rep*. 19:768-774.
15. Halmagyi A, Deliu C. Cryopreservation of strawberry shoot tips by encapsulation dehydration. *Not Bot Horti Agrobo*. 2006,34:28-33.
16. Singh AK, Sharma M, Varshney R, et al. Plant regeneration from alginate encapsulated shoot tips of Phylianthus amarus schum and thonn, a medicinally important plant species. *In Vitro Cell Dev Biol Plant*. 2007,42(2):109-113.