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Conservation of Potent Anti-Cancerous Medicinal Plants of India by Micropropagation – A review

Sunita George[1], Neeti Yadav[2], M. Leleeka Devi[3] and Aradhana Yadav[4]

[1,2,3,4]Department of Botany Daulat Ram College University of Delhi Delhi-110007
E-mail: [1]sunitageorge2012@yahoo.com

Abstract—*Humans have been using many ethno-medicinal plants as a source of medicine for different ailments and curing diseases since time immemorial. The demand for plant medicine derived from plants is increasing in both developing and developed countries since they have few or no side effects. The rich resource of medicinal plants is disappearing at an alarming rate as a result of over-exploitation. Therefore, the management of traditional medicinal plant resources has become a matter of urgency. The demand of uniform medicinal plant- based medicines warrants their mass propagation through plant tissue culture strategy. Tissue culture technology is potent and has opened extensive areas of research for biodiversity conservation. Plant in vitro regeneration is a biotechnological tool that offers a tremendous potential solution for the propagation of endangered and superior genotypes of medicinal plants which could be released to their natural habitat or cultivated on a large scale for the pharmaceutical product of interest.*

1. INTRODUCTION

The emphasis has been made to conserve some ethnomedicinal plants which are used for the treatment of cancer e.g *Podophyllum peltatum* (Berberidaceae), *Camptotheca acuminata* (Nyssaceae), *Swertia chirayita* (Gentianaceae) *Clerodendrum serratum* (Verbenaceae). Due to over-exploitation these species have been declared endangered and are on the list of vulnerable and rare (IUCN status, International Union for the Conservation of Nature and Natural resources). Therefore they need to be conserved to prevent their extinction in the wild while allowing commercial production of the herbal formulation. The increasing high usage of these plants for various drug purposes as well as the illegal overharvesting combined with habitat destruction resulted in a drastic reduction of its populations and has brought these plants to the verge of extinction. The increasing national and international demand has led to unscrupulous collection from the wild and adulteration of supplies. The potent use of these plants in the treatment of cancer has pressurised the need for the conservation of plants. Micropropagation can play a significant role in a holistic conservation strategy by providing high-quality genetically uniform clones for sustainable use and thereby saving the genetic diversity of these species in nature. Superior quality disease free plants can easily be availed on mass scale by tissue culture techniques. This would enhance the availability plant material of those species. Tissue culture protocols have been developed for a wide range of medicinal plants.

The micropropagation of these plants was studied extensively and the successful protocols were developed.

***Podophyllum peltatum* L.** (May apple) is a species belonging to Berberidaceae that grows as a branched rhizome system [24]. Each branch has a terminal bud. The drugs *viz.* etopside and teniposide are semi synthetic derivatives of lignan. Podophyllotoxin extracted from *Podophyllum emodi* Wall ex Royale native to India [23]. Due to its overexploitation this species has been declared endangered [17]. *Podophyllum peltatum* has been used as alternative source for commercial production of podophyllotoxin [15]. Podophyllotoxin has antimitotic effect thus it is a possible treatment for cancer especially for treatment of ovarian cancer. Micropropagation of this species has not been described in the literature. The present study by Moraes-Cerdeira et al. (1998) was undertaken to develop *in vitro* propagation protocol that would provide high yielding *P. peltatum* clones.

Apical, axillary and adventitious buds were initiated *in vitro* from rhizome tips that was collected from the campus after 45 days of cultivation on Murashige and Skoog's (1962) medium with 3% sucrose, 0.8% agar, 0.025% activated charcoal and 4.4 μM 6-benzyladenine (BA), medium was adjusted to 5.7 [23]. All the three different buds were inoculated in the same above medium with similar composition but with a different BA concentration. The number of induced buds derived from axillary (3.1) and adventitious buds (3.4) were significantly higher than those induced from apical buds (1.3). The bud induction was more on Murashige and Skoog's (1962) medium with no BA for all types of buds. Increase in concentration of BA fro6m 0 to 17.7μM decreased proliferation of bud and led to induction of leaf in the same cultures. Axillary and adventitious buds proliferated more newly induced buds than apical buds. Moreover apical buds produced significantly more roots than adventitious and axillary buds. Plantlets derived from adventitious buds were smaller with more juvenile appearance than plantlets induced from apical buds. Every induced bud had a single leaf and it

senesced in culture, an apical bud was present at the leaf base. This *in vitro* pattern of *Podophyllum peltatum* growth was similar to the description of wild plant growth.

Bud cultures cultivated *in vitro* for six months with buds and fully developed leaves with no roots and plantlets were able to produce lignans at reasonable concentrations. The lignan extraction and analysis of these cultures used the same procedure as used by Bastos et al.(1996). There was no difference between cultures 1 and 2 in terms of tissue and plant structures in whole plantlet cultures. Podophyllotoxin concentrations of plants collected from wild population varied between population and tissue type. The amount in leaf blades varied from 0 to 542mg/100g of dry weight. In roots and rhizomes the amount varied from 74mg to 260mg/100g, while petioles had 6.3mg to 31.3mg/100g of dry weight. The lignan content in *in vitro* cultures varied in cultures 1 and 2 and podophyllotoxins concentration was 175.8mg and /100g dry weight. The quantity of lignan in culture 2 were as high as found in wild populations. But it was low in culture 2 compared to culture 1.

Cultured explants served as propagules for commercial cultivation of *Podophyllum.* Rooted buds acclimatized better than plantlets under both environmental conditions. In conclusion different types of buds *viz.* apical, axillary and adventitious were induced from terminal bud derived from rhizome tips of *P. peltatum*. Buds, leaves and plantlets induced *in vitro* were also able to produce lignans.

Camptotheca acuminata Decaisne (family Nyssaceae) native to China produces camptothecin an anticancer indole alkaloid identified by Wall et al. (1966). Camptothecin prevents tumor by inhibiting activity of DNA topoisomerases I [20]. Camptothecin inhibits human immunodeficiency virus [26] and another retrovirus, equine infectitious anemia virus [27] in cultured cells. Because of potential clinical uses, it is important to increase the camptothecin yield so that it does not become limiting as recently occurred with taxol, another anticancer drug [16]. *C. acuminata* trees are commonly raised from seed but there are no reports on clonal propagation of this species. Jain and Nessler (1996) developed methods for clonal propagation of juvenile *C. acuminata* through *in vitro* shoot proliferation.

Shoot tip explants excised from 25-30 days old seedlings were cultured on three different basal media *i.e*., Linsmaier and Skoog (LS) 1965; Gamborg (B_5) et al. 1968 and Lloyd and McCown (LM) 1981. Preliminary experiments showed that cytokinin *viz*. 6-benzyladenine (BA) or Kinetin (Kn) was required for growth of *C. acuminata* shoot buds *in vitro*. Each media was tested supplemented with BA or Kinetin. The best shoot growth was seen on cytokinin supplemented Gamborg's medium. Shoot buds grown on Gamborg's medium produced single dark green fast growing shoots whereas buds cultured on LS and LM [2] media produced single slow growing shoots with callus at the base of explants. Excised buds transferred directly to semi solid media without pretreatment produced single shoot per explants. Preliminary tests showed that pre-soaking for 48hrs and 17.7μM BA (cytokinin) increased the frequency of shoot bud induction *i.e*., 11-18 shoots. For optimum rooting the excised shoots were transferred to B_5 medium with 4.9 μM IBA 90% plantlets survived in the soil and resumed normal growth.

Swertia chirayita (Roxb. ex Fleming) H. Karst. *Swertia chirayita* (Gentianaceae), a popular medicinal herb indigenous to the temperate to Himalayas is used in traditional medicine to treat numerous ailments such as liver disorders, malaria and diabetes and are reported to have a wide spectrum of pharmacological properties [12,11]. The anticancerous properties of this plant has also been extensively studied [10]. The seeds of the species collected from Garhwal district were soaked overnight in 400ppm gibberellic acid solution. The seeds were rinsed and inoculated on semi solid MS ½ medium. The approached resulted in 80% germination. Nodal explants derived from 4 week old seedlings were inoculated on MS medium supplemented with BAP or Kinetin or 2-iP at different concentration ranging from 0.5-10μM. The maximum multiplication rate of 4-5 folds every 4-weeks and shoot elongation of 2.8cm was obtained on MS+ 4 μM BAP+1.5 μM 2iP. Among 3 auxins tested and naphthalene acetic acid, MS medium supplemented with 1 μM NAA was most optimal for the root induction. The shoots inoculated on medium without activated charcoal developed calluses at the base of the shoot and roots remained thick and tuberous. Thereafter they were taken out for successful hardening in green house conditions under control temperature 27±2°C and humidity gradient upto 70%. Survival rate percentage of 95% was achieved [13].

Clerodendrum serratum **L.** is a flowering plant which belongs to the family Verbenaceae. The leaf and root of *Clerodendrum serratum* have much medicinal value. Saponins (terpenoids and steroids), flavonoids and phenolics isolated from the roots of this plant have been the focus of phytochemical investigations ascribed to saponins, which are known to possess anti-cancer activity. The root extracts (aqueous and methanolic extract) of *Clerodendrum serratum* were studied for in vivo anticancer activity using Dalton's Lymphoma Ascites (DLA) cell in mice at the dose of 100 mg and 200 mg/kg body weight. This study revealed that methanolic extract exhibit significant anticancer activity as compared to aqueous extract of the root [6].

Clerodendrum serratum is one of the endangered species and therefore needs to be protected and conserved through various approaches. A protocol was developed for in vitro cloning of *Clerodendrum serratum* on MS [3], media by using shoot nodal explants by Sharma et al., 2009.

For micropropagation, young shoots were cut into small pieces (about 1.5cm long) having a nodal portion in between with auxiliary bud. These shoot pieces were used as explants and were inoculated into four different basal media viz. Murashige and Skoog's medium (MS), Schenk and Hildebrandt medium

(SH) [8], Woody Plant Medium (WPM) [9] and Gamborg's medium (B5) [1]. All the four different media were supplemented with different concentration 0.5mg/L, 1.0mg/L and 2.0mg/L of cytokinin 6-Benzyl amino purine (BAP), Kinetin (KN) and Thia Dia Zuron (TDZ). Highest percentage of shoot induction (90%) was achieved in MS medium supplemented with 0.5mg/L BAP (3.3 ± 0.04) followed by MS medium supplemented with 0.5mg/L KN (2.0 ± 0.04). WPM medium supplemented with 2.0mg/L BAP showed less number of shoots. Shoot length was also highest (4.52 ± 0.32 cm) in MS + 0.5mg/L medium and least in WPM + 2.0mg/L BAP medium. Effects of all three medium viz. WAP, SH and B5 were almost similar when supplemented with lower concentration of hormones but varies greatly at higher concentration [4]. BAP at concentration of 0.5mg/ L showed maximum shoot bud induction, shoot number and shoot length [5].

For optimal rooting, auxins (NAA-0.5mg/l) and cytokinin (BAP) alone showed highest number of root induction (85%) when supplemented with half strength of MS medium. Healthy plants with well developed roots were kept for hardening at 25-32°C temperature, light (2000 lux) and humidity (70 - 80%) in green house under diffused sunlight and their survival rate was 73% .

2. CONCLUSION

Destruction of plant resources is a normal occurrence. The current speed of extinction through human interferences is estimated to be approximately 100-1000 times faster than the natural speed of extinction. The above plants have been used mostly as traditional drugs. Due to its multiple uses the demand of the above plants is on the rise by both national and international trading leading to increasing over harvesting of wild populations which has resulted in its drastic reduction. The implication of losing the above plant species due to extinction lies not only in the loss of genes useful for plant development or in the biosynthesis of new compounds but also the loss of potentially novel compounds of pharmaceutical or nutraceutical benefit. The development of efficient micropropagation protocols, can guarantee an adequate supply of the plants (devoid of environmental-imposed constraints) with subsequent reduction in uncontrolled harvesting pressure on wild population. Micropropagation protocols have been successfully established for ***Podophyllum peltatum* , *Camptotheca acuminata* , *Swertia chirayita, Clerodendrum serratum*** using different explants. The micropogation method would help to save the endangered status of the above plants. It would also encourage the farmers for commercial cultivation of these plants. This approach will help farmers in securing better economic returns and also reduce over-exploitation of plants from wild there by complementing the conservation process.

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