Research in Plant Biology, 1(3): 48-52, 2011
ISSN : 2231-5101
www.resplantbiol.com

Regular Article

Induction of somatic embryogenesis and genetic fidelity of endangered medicinal herb *Curculigo orchioides* Gaertn

Swati Patel[2], Yogesh T Jasrai[1*] and Roshni Adiyecha[1]

[1]Department of Botany, University School of Science, Gujarat University, Ahmedabad-380009, Gujarat, India
[2]C.G.Bhakta Institute of biotechnology, Gopal Vidhyanagar, Bardoli, Surat-394350
*yjasrai@yahoo.com

An efficient regeneration system, through somatic embryogenesis was developed for *Curculigo orchioides* Gaertn - an endangered medicinal herb. Somatic embryos were developed on MS medium containing 8 - 15µM BA from leaf explants. The highest, 69 % leaf explants responded in terms of embryogenic calli with average 8 embryos on MS medium containing 8µM BA. Regenerated plantlets were transferred to autoclaved mixture of soil: sand: compost (1:1:1; v/v/v) for hardening. Genetic fidelity of somatic embryogenesis derived regenerant was assessed using random amplified polymorphic DNA (RAPD).

Key words: Somatic, embryogenesis, fidelity

Curculigo orchioides Gaertn (family-hypoxidaceae) commonly known as **kali musli**, is an endangered medicinal herb. In india it is distributed in sub-tropical Himalayas from Kumaon eastward and in the western ghats from Gujarat-Maharastra southwards. It appears first with onset of monsoon and last to disappear on completion of monsoon (Francis *et al.* 2007). The leaves, roots and rhizome of *Curculigo* are medicinally useful (Bhamare, 1998). The plant possesses uterine stimulant (Dhar *et al.* 1968), hypoglycaemic, spasmolytic and anticancer, (Dhar *et al.* 1968; Aruna and Sivarama-krishnan, 1990), phagocytic (Kubo *et al.* 1983) immuno-adjuvant (Oru and Kogyo, 1983), antineoplastic, immuno-stimulant and hepatoprotective activities (Latha *et al.* 1999; Rajesh *et al.* 2000).

Removal of plants for medicinal and edible, tuberous roots as a substitute for safed musli, coupled with extensive denudation of forests floor caused by cattle grazing (Jasrai and Wala, 2000), poor seed setting and germination are some of the major causes that contribute to the herb being categorized as a threatened plant (Augustine and D'sousa, 1997). Hence the methods for large scale *in vitro* propagation are needed to meet the commercial demand and to conserve this valuable plant.

Somatic embryogenesis has a tremendous potential for large scale production of plant material (Garcia and Martinez, 1995) and is considered as an effective aid in genetic transformation studies. It represents an alternative tool for massive clonal propagation. This appears to be a potential solution to the problem of field propagation, especially in area with frequent disease transmission and maintenance of cultivars that have been selected for important genetic characteristics (Amirato and Styer, 1985).

In this study an efficient, rapid and reproducible plant regeneration system was established for *Curculigo* through somatic embryogenesis. Direct regeneration of somatic embryos is important for the conservation of this endangered species, as rare somaclonal variants are likely to arise from indirect regeneration through callus.

Materials and method

Young leaves of *Curculigo orchioides* shoots were used as an explant. Leaves were surface sterilized with 0.1% of $HgCl_2$ (2 min). Treated leaves were thoroughly washed with sterilized distilled water (4 times) and inoculated aseptically on MS medium (Murashige and Skoog, 1962).

***Culture media and growth condition*: ** Leaf pieces (0.5 cm long) were inoculated on MS medium with 4.4µM , 8µM and 15µM BA with 3 % sucrose (w/v). The pH of media was adjusted to 5.8 and autoclaved at 121 ^{o}C (20 min). For all media, 0.8 % agar-agar was added as gelling agent for semi-solid medium. The cultures were incubated at 25^{o}C under 16 h photo-period with 55 µ mol $m^{-2}s^{-1}$ photon flux density. Seventy replicates each were studied for somatic embryogenesis.

DNA extraction and RAPD analysis: Young leaves from regenerants were subjected to DNA extraction and purification (Doyle & Doyle, 1987). The modified PCR conditions were optimized (Akhare *et al.* 2008). The amplification products were revealed using agarose gel (1.2 %, w/v) electrophoresis system. All the reactions were repeated at least twice, and only the consistently reproducible bands were considered.

Results and Discussion

The present study was conducted for *in vitro* propagation of *Curculigo orchioides* through indirect somatic embryogenesis. The leaf-age of explants plays a major role in somatic embryogenesis (Thomas and Jacob, 2004). Low frequency of embryogenesis and embryogenic induction related to the age of explants suggest that the intrinsic physiological stage of explant plays decisive role in the induction of embryogenesis. Such observations were reported in somatic embryogenesis from zygotic embryo culture (Mathur, 2000; Gogate and Nadgauda, 2003). The middle lamina of the *in vitro* derived leaf explants (0.5 cm long) inoculated on MS media with 8µM and 15 µM BA showed callus formation. Callus formation was initiated within 2 weeks from the cut ends of the leaf explants. High frequency of callus formation was obtained in next 15 days after initiation (Fig -1 a). The callus was compact and pale-white in colour. Eventually, translucent patches of embryogenic cells were differentiated from the well developed callus. On the MS media with 8 µM BA, about 69 % cultures responded in terms of embryogenic calli, while about 40 % cultures developed embryogenic calli on MS media with 15 µM BA. Earlier, similar (89 %) response was obtained with 8 µM BA for direct embryogenesis (Thomas and Jacob, 2004). The translucent emebryogenic calli when subcultured to the fresh medium (Dodeman *et al.* 1997) transformed into green embryoids (Fig-1b). The growth of embryoids was slow and occasionally get detached from the other cells and continued their growth.

Average 8 embryos were formed on the MS medium with 8 µM BA while 4 embryos were developed on MS medium with 15 µM BA. It is very difficult to observe the different stages of emryogenesis in monocots (Thomas and Jacob, 2004). Moreover, the heart shaped stage is absent in monocots as they have only one cotyledon (Sivakumar *et al.* 2003). The histological analysis of the embryogenic calli clearly showed globular shaped embryo (Fig-

1c). When these embryoids were transferred to MS medium with ½ strength nitrogen salts and supplemented with lower concentration of BA (0.44µM), developed shoots and roots (Augustine *et al.* 2008), giving rise to complete plantlets. Plantlets were then placed in thermocol cups containing mixture of soil, sand and compost (1:1:1; v/v/v) for hardening (Fig-1d). The plantlets were irrigated with ¼ strength MS medium (without sucrose) for one week to recover the shock of changes in its new environment (Kar and Sen, 1985). For an initial period of about 2 weeks humidity was maintained by spraying intermittent water mist (Jasrai *et al.* 1999; Rolf and Ricardo, 1995). On establishment, the plantlets showed tremendous growth of rhizome, roots and lush green shoots without any morphological variation with 95% survival.

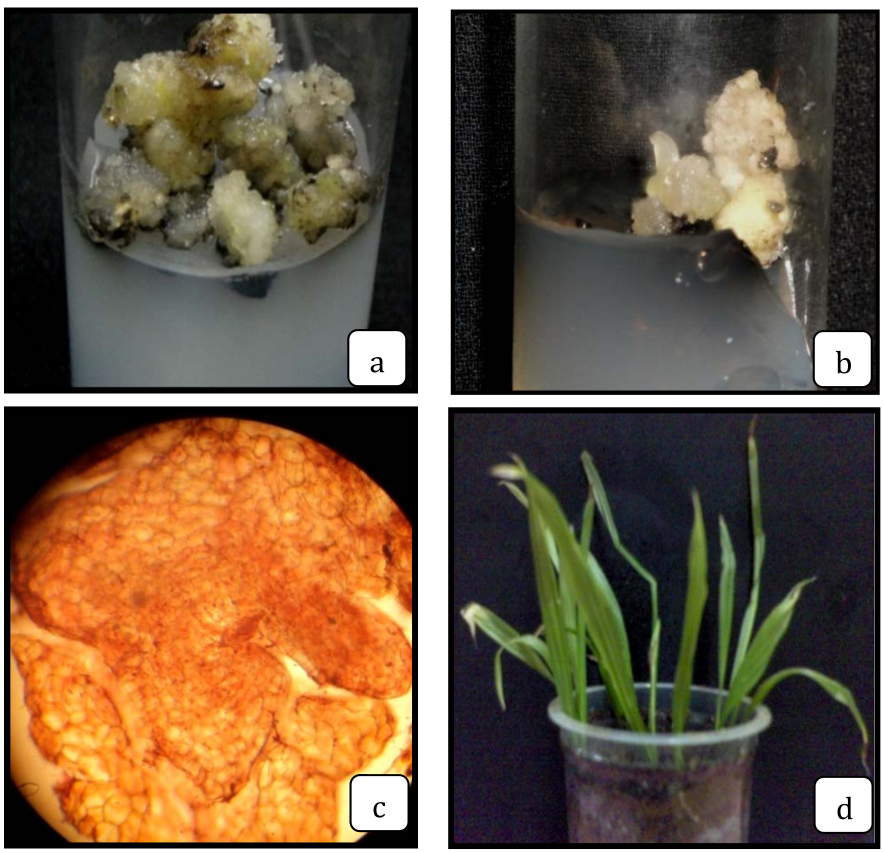

Fig 1: Somatic embryogenesis in *Curculigo orchioides* Gaertn., a- embryogenic calli, b-translucent somatic embryo, c- embryoids in section d- Plantlets regenerated being hardened.

In plants regenerated via somatic embryogenesis, the quality of somatic embryos determines the production of true-to-type plants. Molecular tools are more reliable than phenotypic observation for evaluating tissue culture induced variations (Leroy *et al.* 2000). The genetic fidelity of regenerated plants, derived through somatic embryogenesis was analyzed through RAPD analysis. Of the 10 primers tested, OPE 18 amplified products that were monomorphic across all the micropropagated plants (Fig-2). No variation was observed among the regenerants.

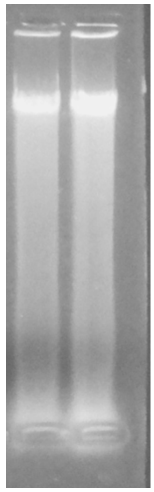

Fig 2: RAPD profile of *Curculigo orchioides* regenerants derived through somatic embryogenesis.

Earlier uniformity in somatic embryo derived plants was noticed in *Tylophora indica* (Jayanthi and Mandal, 2001), *Panax notoginseng* (Shoyama *et al.* 1997), *Picea mariana* (Isabel *et al.* 1993). Similarly, genetic integrity among the somatic embryo-derived regenerants within the cultivar of *Brassica oleracia* was confirmed by ISSR analysis. The lack of variation in somatic embryo derived regenerants could be due to the stringent internal genetic controls throughout embryo formation, may be through selection against abnormal types (Leroy *et al.* 2000).

The present study established an effective protocol of plantlet regeneration through somatic embryogenesis in *Curculigo orchioides* with genetic fidelity evaluation. The optimum concentration of BA (8 μM) was found to be effective on callus and embryo formation. This would help large scale propagation of *C. orchioides* through *in vitro* regeneration.

References

Akhare, A.A., Sakhare, S.B., Kulwal, P.L., Dhumale, D.B. and Kharkar, A., 2008. RAPD profile studies in Sorghum for identification of hybrids and their parents. *International J. of Integrat. Biol.*, **3:**18-24.

Amirato, P.V. and Styer, D.J., 1985**.** Strategies for large scale manipulation of somatic embryos in suspension cultures, Biotechnology in plant Science. Academic Press: New York, pp.161-178.

Aruna, M. and Sivaramakrishnan, V.M., 1990. Plant products as protective agents against cancer. *Ind. J. Expe. Biol.,* **28:**1008-1011.

Augstine, A.C., Nivas, S. and D`Souza, L., 2008. Induction of embryos and plantlets from anthers of *Curculigo orchioides* Gaertn-An endangered medicinal herb. *Ind. J. Biotech.*, **7:** 536-540.

Augustine, A.C. and D`souza, L.D., 1997. Regeneration of an anticarcinogenic herb, *Curculigo orchioides* (Gaertn). *In vitro Cell Dev. Biol-plant*, **33:**111-113.

Bhamare, P.B., 1998. Traditional knowledge of plants for skin ailments of Dhule and Nandubar districts, Maharatra (India). *J. Phyto. Res.*, **11:**195-196.

Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N. and Ray, C., 1968. Screening of Indian Plants for biological activity, Part I. *Indian J. Exp. Biol.*, **6:**234-247.

Doyle, J.J. and Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.,* **19:**11-15.

Dodeman, V.L., Ducreux, G. and Kreis, M., 1997. Zygotic embryogenesis versus somatic embryogenesis. *J. Exp. Bot.,* **48(8):**1493-1503.

Francis, S.V., Senapati, S. and Rout, G.R., 2007. Rapid clonal propagation of *Curculigo orchioides* Gaertn., an endangered medicinal plant. *In vitro Cell Dev. Biol-Plant*, **43:**140-143.

Garcia, E. and Martinez, S., 1995. Somatic embryogenesis in S*olanum tuberosum* L. cv. Desiree from stem nodal sections. *J. Plant Physiol*, **145:** 526-530.

Gogate, S.S. and Nadgauda, R., 2003. Direct induction of somatic embryogenesis from immature zygotic embryo of cashewnut (*Ancardium occidentale* L.). *Sci Hort*., **97:**75-82.

Isabel, N., Tremblay, L., Michaud, M., Tremblay, F.M. and Bousquet, J., 1993. RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis-derived populations of *Picea mariana* (Mill.) B.S.P. *Theor. and Appl. Gen*., **86:** 81-87.

Jasrai, Y.T., Kannan, V.R., Ramakanthan, A. and George, M.M., 1999. Ex vitro survival of *In vitro* derived Banana plants without greenhouse facilities. *Plant Tiss. Cul*., **9:**127-132**.**

Jasrai, Y.T. and Wala, B.B., 2000. *Curculigo orchioides* Gaertn. (Kali musli) : An endangered medicinal herb, In *Role of Biotechnology in Medicinal and Aromatic Plants*, Vol. IV, Eds. Khan I.A. and Khanum A., Hyderabad, India: Ukaaz Publications, pp. 89-95.

Jayanthi, M. and Mandal, P.K., 2001. Plant regeneration through somatic embryogenesis and RAPD analysis of regenerated plants in *Tylophora indica. In vitro Cell Dev. Biol-Plant,* **37:** 576-580.

Kar, D.K. and Sen, S., 1985. Propagation of *Asparagus racemosus* through tissue culture. *Plant Cell Tiss. Org. Cul*., **5:**89-95.

Kub**o,** M., Namba, K., Nagamoto, N., Nagao, T., Nakanishi J., Uno, H. and Nishimura, H., 1983. A new phenolic glucoside, curculigoside from rhizomes of *Curculigo orchioides*. *Plant. Med*., **47:**52-55.

Latha, U., Rajesh, M.G. and Latha, M.S., 1999. Hepatoprotective effect of an Ayurvedic medicine. *Ind. Dru*., **36:**470-473.

Leroy, X.J., Leon, K., Charles, G. and Branchard, M., 2000. Cauliflower somatic embryogenesis and analysis of regenerant stability by ISSRs. *Plant Cell Rep.,* **19:**1102-1107.

Mathur, G.,Von Arnold, S., Nadgauda, R., 2000. Studies on somatic embryogenesis from immature zygotic embryos from chir pine (*Pinus roxburghii*). *Curr.Sci*., **79:** 999-1004.

Murashige, T. and Skoog, T.A., 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*., **15:** 473–497.

Oru, Y. and Kogyo, K.K., 1983. Immune adjuvant from plant root. *Japan Kokai Tokkyo Koho,* **58:** 594.

Rajesh, M.G., Paul, B. and Latha, M.S., 2000. Efficacy of kamilari in alcoholic liver cirrhosis. *Antisep*., **97:** 320-321.

Rolf, D.I. and Ricardo, T.F., 1995. Micropropagation of *Alpinia purpurata* from inflorescence buds. *Plant Cell Tiss. Org. Cult*., **40:**183-185.

Shoyama, Y., Zhu, X.X., Nakai, R., Shiraishi, S. and Kohda, H., 1997. Micropropagation of *Panax notoginseng* by somatic embryogenesis and RAPD analysis of regenerated plantlets. *Plant Cell Reports*, **16:** 450-453.

Sivakumar, G., Krishnamurti, K.V., Rajendra, T.D., 2003. Embryoidogenesis and plant regeneration from leaf tissue of *Gloriosa superb*. *Planta Med*., **69:** 479-481.

Thomas, T.D. and Jacob, A., 2004. Direct somatic embryogenesis of *Curculigo orchioides* Gaertn. an Endangered medicinal herb. *J. Plant Biotech*., **6 :**193-197.