Pak. J. Agri. Sci., Vol. 49(1), 41-45; 2012
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

IN VITRO RESPONSE OF *CONVOLVULUS SCINDICUS* TO DIFFERENT GROWTH HORMONES - AN ATTEMPT TO CONSERVE AN ENDANGERED SPECIES

Haider Abbas[1,*], Muhammad Qaiser[2] and Sher Wali Khan[3]

[1]Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan; [2]Federal Urdu University of Arts, Science and Technology, Karachi-75300, Pakistan; [3]Department of Biological Sciences, Karakoram International University, Gilgit-Baltistan-15100, Pakistan
[*]Corresponding author's e.mail: invitro.life@gmail.com

Convolvulus scindicus Stocks is an endangered taxon in the southern region of Pakistan. It is exposed to hostile environmental conditions and subjected to various threats including habitat loss, grazing activities and soil erosion. There is a need to take steps for its protection to avoid its extirpation. In this connection an *in vitro* propagation and conservation protocol was established to study its response to different growth hormones. Fresh seeds were collected from the wild, germinated *in vitro* and then seedlings were grown aseptically to obtain explant. The cultures maintained on MS medium containing various growth hormones for 6-7 months. 2.5 mg/l BAP along with 0.5 mg/l Kin and 0.5 mg/l NAA induced maximum number of 8.20 shoots per explant with 56% shoot regeneration frequency. Well developed shoots were rooted on MS medium containing 2 mg/l IAA with maximum number of roots (1.50) per explant and maximum rooting frequency of 67%.

Key words: Conservation, *Convolvulus scindicus*, environment, growth hormones, propagation

INTRODUCTION

Convolvulus scindicus Stocks (Convolvulaceae), a small branched herb to under shrub (Austin and Ghazanfar, 1979). It is a sub endemic species with a narrow geographic distribution, grows in Thar Desert region that is divided in between Pakistan and India and in adjoining areas of southern Pakistan (Roy and Pandey, 1971; Oudhia, 2003; Abbas and Qaiser, 2011). Habitat destruction and the continuous harvesting of native plants are the main factors affecting nearly all plant species (Malda *et al*., 1999; Beck and Dunlop, 2001) while, species natural rate of recruitment also has a direct influence on its extinction (Martin and Pardeep, 2003). According to Abbas and Qaiser (2011) anthropogenic activities i.e., habitat loss, grazing activities, soil erosion fuel wood cutting, poultry business and its extremely restricted geographic range in the study area (172.82 km^2) is importantly playing a role in habitat loss of the plant.

As the *in situ* environment is becoming unfavorable for its survival along with low and fragmented population size, there is a dire need of an urgent conservation effort for avoiding its extirpation (Abbas and Qaiser, 2011). Although species conservation is achieved most effectively through the management of wild populations and natural habitats (*in situ* conservation) and *ex situ* methods can be used to complement *in situ* methods and in some instances, may be the only option for conservation of some species (Maunder *et al*., 1998; Ramsay *et al*., 2000; Rajwana *et al*., 2011). *Ex situ* conservation has gained the international recognition through its inclusion in the ninth article of Convention on Biological Diversity (CBD).

In vitro techniques and applications are very useful in the conservation of a large number of rare (Holobiuc *et al*., 2009) and threatened (Pence, 2005; Rajasekharan *et al*., 2009) species with poor and uncertain responses to conventional methods of propagation (Sarasan *et al*., 2006). Engelmann (1997) reported that standard culture environment can be effectively utilized for short to medium term *in vitro* conservation of plant germplasm, through increasing intervals between subcultures in slow growing species. According to Martin and Pardeep (2003) it is the only feasible way to maintain a gene bank of plants for their future sustainable utilization.

The scarcity of published methods for *in vitro* culture of wild taxa and the limited amount of experimental plant material make the choice and development of initial culture medium for rare and threatened plants somewhat arbitrary (Krogstrup *et al*., 2005). There are no reports on the *in vitro* culture of *C. scindicus*. The prime focus of the present investigation was to establish a fast *in vitro* micropropagation protocol for the conservation of the plant.

MATERIALS AND METHODS

***a. Plant material*:** Fresh seeds of *C. scindicus* were collected

from the wild population and used as an initiating material for further study.

***b. Sterilization of seeds*:** Seeds were thoroughly washed for 20 minutes under running tap water, followed by quick dip in 95% ethanol for 20 seconds. Thereafter, surface sterilization was performed using 10% commercial bleach (NaOCl) containing 3-6 drops of Tween 20 in 200 ml solution for 15 minutes. Sterilized seeds were rinsed 3 times with autoclaved distilled water before culture on MS media.

***c. Media and culture procedure*:** Different concentrations of growth regulators {i.e. 6-benzylaminopurine (BAP), Kinetin (KIN), α-Naphthalene Acetic Acid (NAA), Indole-3 Acetic Acid (IAA) and Indole 3-butyric acid (IBA) along with 3% sucrose were incorporated in the media and gelled with 0.6% Phytagel (P8169-Phytagel, Sigma-Aldrich, St. Louis. Mo. USA). Shoot induction was carried out in MS medium containing 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 & 3.0 mg/l BAP. In another experiment above described variants of BAP were used in combination with 0.5 mg/l KIN and 0.5 mg/l NAA. Rooting was carried out in MS medium containing 0.0, 1.0, 2.0 & 3.0 mg/l each of IAA and IBA. The pH of the media was adjusted to 5.8 before autoclaving at 121^{o}C for 15 minutes under pressure of 15 psi. The sterilised seeds were cultured on MS (Murashige and Skoog, 1962) media for germination under laminar flow (Technico Scientific, Lahore, Pakistan) to avoid contamination in glass bottles with plastic caps. Thereafter, the seeds were incubated at 25^{o}C under 16 hours of light. Intensity of light ranged from 2000 to 3000 lux of energy obtained by using 40 watts, normal cool white florescent tubes (Philips-TL40W54). In vitro grown one week old seedlings under aseptic conditions were used for the collection of nodal segments for establishing *in vitro* cultures. The nodal segments were cultured in vertical position. These were cultured on variants of BAP, Kin and NAA described above for shoot multiplication. Experiment was laid out and analysed according to completely randomized design (CRD) with 5 replicates per treatment. Established shoot cultures were sub-cultured after 4-6 months depending upon the growth and condition of media in the glass jars. The means were compared using Duncan's Multiple Range Test (DMR).

RESULTS

***i. Effect of various concentrations of BAP - NAA in MS medium on shoot regeneration*:** The results showed that various concentrations of BAP-NAA in MS medium had significant effect on shoot regeneration frequency and number of shoots per explant in *C. scindicus*. Means for shoot regeneration frequency (%) and number of shoots per explant ranged 2.0 to 66% and 0.20 to 4.70, respectively (Table 1). Maximum number of 4.70 shoots per explant with regeneration frequency% 47 was recorded on MS medium containing 2.0 mg/l BAP and 0.5 mg/l NAA. Negligible and significantly reduced number of 0.20 shoots per explant was recorded in the absence of plant growth regulators.

***ii. Effect of various concentrations of BAP - KIN - NAA in MS medium on shoot regeneration*:** The results showed that various concentrations of BAP-KIN-NAA in MS medium had significant effect on shoot regeneration frequency% and number of shoots per explant. The shoot regeneration frequency% and number of shoots per explant ranged 1.0 to 56 and 0.60 to 8.20, respectively (Table 2). Maximum number of 8.20 shoots per explant with regeneration frequency% 56 was recorded on MS medium containing 2.5 mg/l BAP, 0.5 mg/l KIN 0.5 mg/l NAA (Fig.1). A sharp decline in the mean number of shoots per explant was recorded in the absence of plant growth regulators.

***iii. Effect of various concentrations of IAA or IBA in MS medium on number of roots*:** Various concentrations of IAA or IBA in MS medium had significant effect on rooting frequency and number of roots per explant. The results showed that the maximum rooting frequency of 67% was recorded on MS medium containing 2.0 mg/l IAA. Contrarily IBA was not so promising and resulted in sharp reduction in rooting frequency on any concentration of IBA. Similarly, maximum number of 1.50 roots per explant was recorded on MS medium containing 2.0 mg/l IAA. In general, increase in the concentration of IAA resulted in corresponding increase in the number of roots per explant,

Table 1. Effect of BAP-NAA in MS medium on frequency and number of shoots per explant from nodal cuttings of seedlings of *Convolvulus scindicus*

BAP concentrations (mg/l)	NAA concentrations (mg/l)	Frequency of shoot regeneration (%)	Mean number of shoots per explant
0	0	2.0d	0.20d
0.5	0.5	25.0c	3.30c
1.0	0.5	39.0ab	4.20abc
1.5	0.5	66.0a	4.60a
2.0	0.5	47.0a	4.70a
2.5	0.5	43.0ab	4.30ab
3.0	0.5	35.0b	3.50bc

Values within column followed by small letters are significantly different in accordance with Duncan's Multiple Range Test

Table 2. Effect of various concentrations of BAP - KIN - NAA in MS medium on frequency (%) and number of shoots per explant of *C. scindicus*

BAP (mg/l)	Kin (mg/l)	NAA (mg/l)	Frequency (%) of shoot regeneration	Mean number of shoots per explant
0.0	0.0	0.0	1.0d	0.60c
0.5	0.5	0.5	17.0cd	1.70d
1.0	0.5	0.5	32.0bc	3.32c
1.5	0.5	0.5	33.2bc	3.60bc
2.0	0.5	0.5	46.0ab	4.60b
2.5	0.5	0.5	56.0a	8.20b
3.0	0.5	0.5	32.0bc	3.26c

Values within column followed by small letters are significantly different in accordance with Duncan's Multiple Range Test

Table 3. Effect of various concentrations of IAA and IBA in MS medium on *in vitro* rooting of *C. scindicus*

Conc. mg/L	Frequency (%) of root regeneration		Number of roots per explants	
	IAA	IBA	IAA	IBA
0	0.0b	0.0a	0.00b	0.00b
1.0	0.00b	0.0a	0.20b	0.00b
2.0	67.0a	3.0a	1.50a	0.40a
3.0	0.00b	0.00a	0.00b	0.00b

Values within column followed by small letters are significantly different in accordance with Duncan's Multiple Range Test

with maximum roots at 2 mg/l of IAA. IBA resulted in the decrease of roots per explant; even no roots were recorded on MS medium containing 1.0 mg/l IBA. Similarly, no roots were recorded on auxin free MS medium (control).

Figure 2. Multiple shoot regeneration of *C. scindicus* on MS medium containing 2.5 mg/l BAP, 0.5 mg/l Kin and 0.5 mg/l NAA from nodal explant.

Figure 3. Rooting of *C. scindicus* on MS medium containing 2.0 mg/l IAA.

DISCUSSION

In vitro conservation offers a viable tool for the maintenance of the wild populations through the preservation and reintroduction of endangered germplasm in its natural habitat (Holobiuc *et al*., 2009).

Kalia *et al.* (2004) reported that regeneration of targeted species in artificially stimulated/controlled environment (*in vitro)* is a quite complex interaction of genotype and exogenous growth regulators augmented in the nutrient medium, they mentioned that cytokinins and auxin resulted in enhanced shoot regeneration in regeneration in *Dalbergia sissoo*. Shushu (2001) found satisfactory results in shoot multiplication, when nodal segments of *Harpagophytum procumbens*, were cultured on MS supplemented with 0.1 - 0.5 mg/l BAP and 1.0 mg/l NAA.

Their results are in agreement with our findings, where higher number of 8.20 shoots per explant was achieved, when nodal segments of *in vitro* grown seedlings of *C. scindicus* were cultured on medium augmented with 2.5 mg/l BAP - 0.5 mg/l Kin - 0.5 mg/l NAA. Findings of Abdulaziz and Bahrany (2002) support the current studies that recorded 9 shoots per explant of *Citrus aurantifolia* in medium containing 2.0 mg/l BAP + 1.0 mg/l Kin + 1.0 mg/l NAA. Balaraju *et al*. (2008) also reported that variable number of shoots per explant using 2.0 mg/l BAP in combination with

0.1 mg/l Kin or 1.5 mg/l BAP in combination with 0.1 mg/l NAA. During this study, MS medium containing 2.0 mg/l BAP, 0.5 mg/l NAA without Kin failed to produce more than 4.70 shoots per nodal segments in *C. scindicus*.

Rathore *et al.* (2004) stated that nature and concentration of plant growth regulators required for *in vitro* root formation was highly specific and varies from species to species. According to Husain *et al.* (2008) IBA, shows strong rooting effect in a wide range of plants especially woody species. Dewan *et al*. (1992) and Krishnan and Seeni (1994) observed rooting on IAA supplemented MS medium in *Acacia nilotica* and *Woodfordia fruticosa,* respectively.

In our studies, the maximum number (1.50) of roots per explant was recorded on MS medium using 2.0 mg/l IAA with rooting percentage of 67%. This is in agreement with Kaur *et al*. (1998) who obtained rooting frequency of 60-80% at ¼ concentrations of macro, micro salts and vitamins of MS medium containing 3.0 mg/l IAA. No rooting was recorded on 0, 1.0 and 3.0 mg/l IBA, while weak and fragile root initials were recorded at 2.0 mg/l IBA.

It is concluded that MS medium containing BAP - Kin - NAA was appropriate shoot regeneration medium for *Convolvulus scindicus*, with best rooting in the presence of IAA. This protocol could be rightly used for conservation and re introduction of the plant in nature to avoid its extirpation.

REFERENCES

Abbas, H. and M. Qaiser. 2011. *Convolvulus scindicus*: conservation assessment and strategies to avoid extirpation. Pak. J. Bot. 43 (3):1685-1690.

Abdulaziz, M. and A.I. Bahrany. 2002. Effect of phytohormones on *in vitro* shoot multiplication and rooting of lime *Citrus aurantifolia* (Christm.) Swing. Sci. Hort. 95:285-295.

Austin, D.F. and S. Ghazanfar. 1979. Convolvulaceae. p.126: 1-64. In: E. Nasir and S.I. Ali (ed.). Flora of West Pakistan. Pakistan Agricultural Research Council, Islamabad, Pakistan.

Balaraju, K., P. Agastian, J.P. Preetamraj, S. Arokiyaraj and S. Ignacimuthu. 2008. Micropropagation of *Vitex agnus-Castus*, (Verbenaceae)-A Valuable medicinal plant. *In Vitro* Cell. Dev. Biol.: Plant 44:436-441.

Beck, S.L. and F.A. Dunlop. 2001. Micropropagation of the *Acacia* species: a review. *In Vitro* Cell. Dev. Biol.: Plant 37:531-8.

Dewan, A., K. Nanda and S.C. Gupta. 1992. *In vitro* micro-propagation of *Acacia nilotica* subsp. indica Brenan via cotyledonary nodes. Plant Cell Rep. 12:18-21.

Duncan, D.B. 1955. Multiple range and multiple F test. Biometrics 11:1-42.

Engelmann, F. 1997. *In vitro conservation methods.* p.119-162. In: B.V. Ford-Lloyd, H.J. Newburry and J.A. Callow, (ed.). Biotechnology and plant genetic resources: Conservation and use. CABI, Wallingford, UK.

Holobiuc, I., R. Blându and V. Cristea. 2009. Research concerning *in vitro* conservation of the rare plant species *Dianthus nardiformis* Janka, Biotechnol. Biotech. Eq. 23(2):221-224.

Husain, M.K., M. Anis and A. Shahzad. 2008. *In vitro* propagation of a multipurpose leguminous tree (*Pterocarpus marsupium* Roxb.) using nodal explants. Acta Physiol. Plant. 30:353-359.

Kalia, S., R.K. Kalia and S.K. Sharma. 2004. Evaluation of clonal variability in shoot coppicing ability and *in vitro* responses of *Dalbergia sissoo* Roxb. Silvae Genet. 53(5-6):12-220.

Kaur, K., B. Verma and U. Kant. 1998. Plants obtained from the Kahir tree (*Acacia catechu* Willd) using mature nodal segments. Plant Cell Rep. 17:427-429.

Khan, T.I., *A.K.* Dular and D.M. Solomon. 2003. Biodiversity conservation in the Thar desert with emphasis on endemic and medicinal plants. Environmentalist 23:137-144.

Krishnan, P.N. and S. Seeni. 1994. Rapid micropropagation of *Woodfordia fructicosa* (L.) Kurz. (Lythraceae), a rare medicinal plant. Plant Cell Rep. 14:55-58.

Krogstrup. P., J.I. Find, D.J. Gurskov and M.M.H. Kristensen. 2005. Micropropagation of Socotran fig, *Dorstenia gigas* Scrweinf. Ex. Balf. F.–a threatened species, endemic to the island of Socotra, Yemen. *In Vitro* Cell. Dev. Biol.: Plant 41:81-86.

Malda, G., H. Suzán and R. Backhaus. 1999. *In vitro* culture as a potential method for the conservation of endangered plants possessing crassulacean acid metabolism. Sci. Hort. 81:71-87.

Martin, K.P. and A.K. Pradeep. 2003. Simple strategy for the in vitro conservation of *Ipsea malabarica* an endemic and endangered orchid of the Western Ghat of Keral, India. Plant Cell Tiss. Org. 74:197-200.

Maunder, M., S. Higgens and A. Culham. 1998. Neither common nor garden; the garden as refuge for threatened plant species. Curtis's Bot. Mag. 15:124-132.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant. 15:473-497.

Oudhia, P. 2003. Traditional medicinal knowledge about flowers of indigenous herbs used to treat common diseases by natives and traditional healers of Chhattisgarh, India. (Available online with updates at http: //www.botanical.com/site/column_poudhia/48_ gandai_ region.html).

Pence, V.C. 2005. *In vitro* collecting (IVC). I. The effect of media and collection method on contamination in temperature and tropical collections. *In Vitro* Cell. Dev. Biol.: Plant 41:324-332.

Rajasekharan, P.E., S.R. Ambika and S. Ganeshan. 2009. *In vitro* conservation of *Tylophora indica*: A threatened medicinal plant. The IUP J. Genet Evol. 11:26-35.

Rajwana, I.A., I.A. Khan, A.U. Malik, B.A. Saleem, A.S. Khan, K. Ziaf, R. Anwar and M. Amin. 2011. Morphological and biochemical markers for varietal characterization and quality assessment of potential indigenous mango (*Mangifera indica*) germplasm. Int. J. Agric. Biol. 13:151–158.

Ramsay, M.M., A.D. Jackson and R.A. Porley. 2000. A pilot study for exsitu conservation of UK bryophytes. p.52-57. In: EuroGard 2000–II. European Botanic Gardens Congress. Canary Islands, Las Palmas de Gran Canaria, Spain.

Rathore, J.S., V. Rathore, N.S. Shekhawat, R.P. Singh, G. Liler, M. Phulwaria and H.R. Dagla. 2004. Plant Biotechnology and Molecular Markers. p. 195-205. Springer, Netherlands.

Roy, B.B. and S. Pandey. 1971. Expansion or contraction of the Great Indian Desert. Proc. Ind. National Sci. Acad. 36(6):331-334.

Sarasan, V., R. Cripps, M.M. Ramsay, C. Atherton, M. McMichen, G. Prendergast and J.K. Rowntree. 2006. Conservation *in vitro* of threatened plants - progress in the past decade. *In Vitro* Cell. Dev. Biol.: Plant 42:206-214.

Shushu, D.D. 2001. *In vitro* regeneration of the Kalahari devilès claw, *Harpagophytum procumbens* DC. South Afric. J. Bot. 67(2):378-380.