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Effect of Phytohormones on Leaf Explants of *Bacopa Monnieri* L. Penn: An Endangered Medicinal Plant

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ABSTRACT

The present investigation is focussed on producing an efficient protocol for rapid and large scale in vitro propagation of a valuable medicinal herb, Bacopa monnieri. The effect of phytohormones such as 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA), indole acetic acid (IAA), indole-3- butryric acid (IBA), thidiazuron (TDZ), kinetin (KN) and 6-benzyl amino purine (BAP) at concentrations of 0.5, 1.0, 2.0, 5.0 and 10.0 µM was studied by inoculation of leaf explants of B. monnieri after surface sterilization on Murashige and Skoog (MS) media with (20g/l) sucrose. The shoots obtained were transferred to the rooting media. For hardening the plantlets were transferred to plastic cups containing mixture of soil and vermicompost (1:1). KN was found to be superior for induction of adventitious shoots with 85±0.5 shoots/explants in semi-solid and 200±0.5 shoots/explants in liquid medium. Whereas, 10.0 µM NAA showed maximum percentage (95%) of callus induction. 10.0 µM IBA resulted in maximum mean length (5.00±0.5) of the roots and maximum number (17.0±0.5) of root. The results thus indicate the use of leaf explants with the influence of phytohormones as a reproducible, dependable, simple - two steps procedure for clonal propagation for conservation of B. monnieri with high-rate of adventitious shoot formation.

Keywords: Bacopa monnieri, in vitro plant regeneration, Kinetin, NAA, leaf explants.

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INTRODUCTION

Bacopa monnieri belongs to the family Scrophulariaceae and are found to possess many medicinal properties. It is commonly known as water hyssop or brahmi. It is a perennial, creeping herb whose habitat includes wetlands and muddy shores [1,2]. National Medicinal Plant Board (NMPB), Government of India and Technology, Information, Forecasting and Assessment Council have listed B. monnieri as highly endangered medicinal herb [3]. In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory and neuro pharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress [4, 5]. Due to the progressively increasing demand, more than 90% of plant species used by industry are collected from the wild source of which 70% involves unorganized harvesting, leading to extinction of the plant [6]. Thus, there is an urgent need for using the alternative methods such as micropropagation for the management of traditional medicinal resources. It has superiority over conventional method of propagation because of high multiplication rate within short period and limited space. The plants produced by this method are independent of climatic changes or soil conditions [7, 8].

There are reports on multiple shoot regeneration from different explants of B. monnieri such as leaf, intermodal, nodal segments and apical buds of B. monnieri [6, 9-14]. However, considerable variation has been reported regarding the capacity of regeneration. It is observed that, apart from the explant source, plant growth regulators or phyto-hormones play a decisive role in regeneration and in vitro multiplication [15]. Kothati et al. [16] emphazised that the synergistic obligation of both cytokinin and auxins for initiation of cell division and growth in plant tissue culture. The current study establishes the high regenerative capacity of leaf explants for induction of adventitious shoots. The study also focuses on the effect of different concentrations of various phytohormones on direct and indirect organogenesis.

MATERIALS AND METHODS

Source of Plant materials

The plant material (leaves) of B. monnieri was collected from Muthathi, Karnataka, India. Plant sample was authenticated by Dr. S. B. Sullia, Botanist and Microbiologist, Jain University and the voucher specimen have been deposited in Jain University herbarium (voucher no.3425).

Explants Sterilization

The plant material was washed under running tap water for 10 minutes and they were rinsed with 70% alcohol for 1 minute. The final step of sterilization was carried out in a horizontal laminar air flow chamber by rinsing the plant material twice in sterile distilled water, followed by 0.01% mercuric chloride solution for 3 minutes.

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Micro Propagation

The explants were trimmed and inoculated on MS (1962) medium containing 20g/l sucrose, 0.8% (w/v) agar (Hi-Media, Mumbai) and supplemented with different concentrations (0.5, 1.0, 2.0, 5.0 and 10.0 µ M) of BAP, KN, NAA, 2, 4-D and TDZ for induction of multiple shoots.

Rooting and Hardening

For rooting, the in vitro raised shoot lets were sub-cultured on MS media containing 20g/l sucrose and supplemented with different concentrations (0.5, 1.0, 2.0, 5.0 and 10.0 µM) of IAA and IBA. The pH of media was adjusted to 5.8 ± 0.1 prior to autoclaving and the cultures were incubated at 24 ± 2°C under 16-h photoperiod. For acclimatization, plantlets with well developed roots were removed from culture tubes, washed to remove the remnants of agar and planted separately in plastic cups filled with mixture of vermicompost and soil (1:1). The hardened plants were then transferred to pots.

RESULTS AND DISCUSSION

In the present study, the leaf explants inoculated on medium supplemented with above mentioned phytohormones, showed two responses i.e, in direct organogenesis, shoots were regenerated on explant surface directly without callus formation (via adventitious shoot bud proliferation); and in indirect organogenesis, plantlets were originated via callus formation (indirect organogenesis). Adventitious shoots were induced from cut ends of leaf explants within 2 weeks (Figure 1a) and the shoot length increased in the 3rd week (Figure 1b). After period of 4 weeks, adventitious shoots developed on entire leaf surface and each explant was transformed into a dense mass of regenerating shoots which made it impossible to count the number of shoot buds (Figure 1c). After 4 weeks of culture, explants were sub cultured on fresh MS medium supplemented with cytokinin as BAP, KN and TDZ (0.5, 1.0, 2.0, 5.0 and 10.0 µM), upon which shoots grew further (Figure1d). There was an increase in the extent of shoot regeneration with an increase in the concentration of cytokinins (Figure 1d). Among the three cytokinins tested, KN was found to be best for induction of adventitious shoots from leaf explants (Table1). An optimum of 85 multiple shoots were developed on semisolid medium supplemented with 10.0 µM KN at the end of 8 weeks of culture. In the absence of cytokinins, 10-15 shoots were developed from the leaf explants. The fresh weight and dry weight were also influenced by type and concentration of growth regulators supplemented to the medium (Table 1). After culturing leaf explants on semisolid medium for 4 weeks, explants were transferred to MS liquid medium with 10.0 µM KN and were incubated for another 4 weeks. In liquid medium, leaf explants showed proliferation of adventitious shoot buds and further growth of the shoots. An increase in the shoot length and biomass was observed. An optimum of 200 shoots/explant proliferated on each leaf explant at the end of 8th week in MS medium. Media supplemented with NAA and 2, 4-D at all concentrations. The callus observed in NAA was friable, pale yellow in colour at 5.0 and 10.0 µM concentration callus and the callus produced by 2, 4-D was compact. Swelling of explants was observed within 12-14 days of inoculation. The

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influence of both the auxin was observed after two weeks, the callus induction rate was recorded. Rapid callus growth (95%) response was observed in the MS medium with 10.0 µM NAA followed by 5.0 µM NAA with a response rate of 92%. Minimum callus formation (20%) was noted in the MS medium containing 0.5 µM 2, 4-D (Figure 2). Among the various concentrations of IAA and IBA, IBA proved to be superior. 10.0 µM concentration of IBA was optimum for induction of root. The optimum root length of 5.00 ± 0.5cm and optimum number of roots were 17.0±0.5 (Figure 3). After transplantation, there was 50% survival rate was observed (Figure 1e, 1f, 1g).

Table 1: Comparison between the different concentrations of cytokinins for induction of multiple shoots in semi solid media (mean ± SE)

Name of hormone	Concentration of hormone (µM)	fresh weight (g)	Dry weight (g)	No of shoots	Length of shoot (cm)
Control	---	1.7±0.02	0.15±0.005	13±0.5	7±0.5
BAP	0.5	2±0.05	0.15±0.005	19±0.5	6±0.5
	1	2.1±0.06	0.17±0.005	45±0.5	7±0.5
	2	2.2±0.02	0.17±0.01	53±0.5	10±0.5
	5	2.5±0.05	0.19±0.005	55±0.5	12±0.5
	10	2.8±0.05	0.2±0.005	70±0.5	15±0.5
KN	0.5	1.5±0.02	0.09±0.005	22±0.5	7±0.5
	1	2.32±0.01	0.15±0.005	45±0.5	10±0.5
	2	2.5±0.02	0.19±0.005	55±0.5	12±0.5
	5	2.5±0.02	0.19±0.005	60±0.5	12±0.5
	10	2.95±0.02	0.2±0.005	85±0.5	15±0.5
TDZ	0.5	1.25±0.12	0.12±0.01	-	-
	1	1.75±0.05	0.15±0.01	-	-
	2	1.45±0.02	0.11±0.01	-	-
	5	1.50±0.02	0.14±0.005	-	-
	10	2.00±0.45	0.19±0.005	-	-

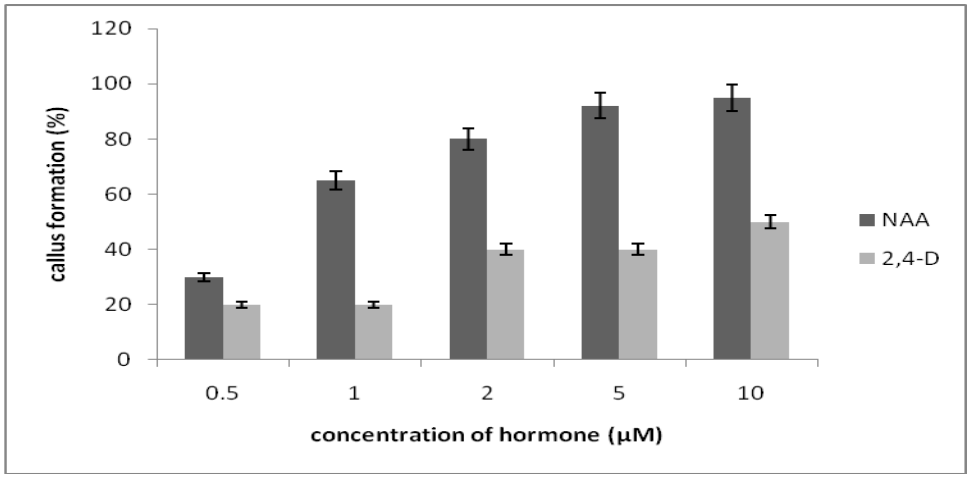

Figure 2: Effect of various concentration of 2,4-D and NAA on callus formation of *B. monnieri*

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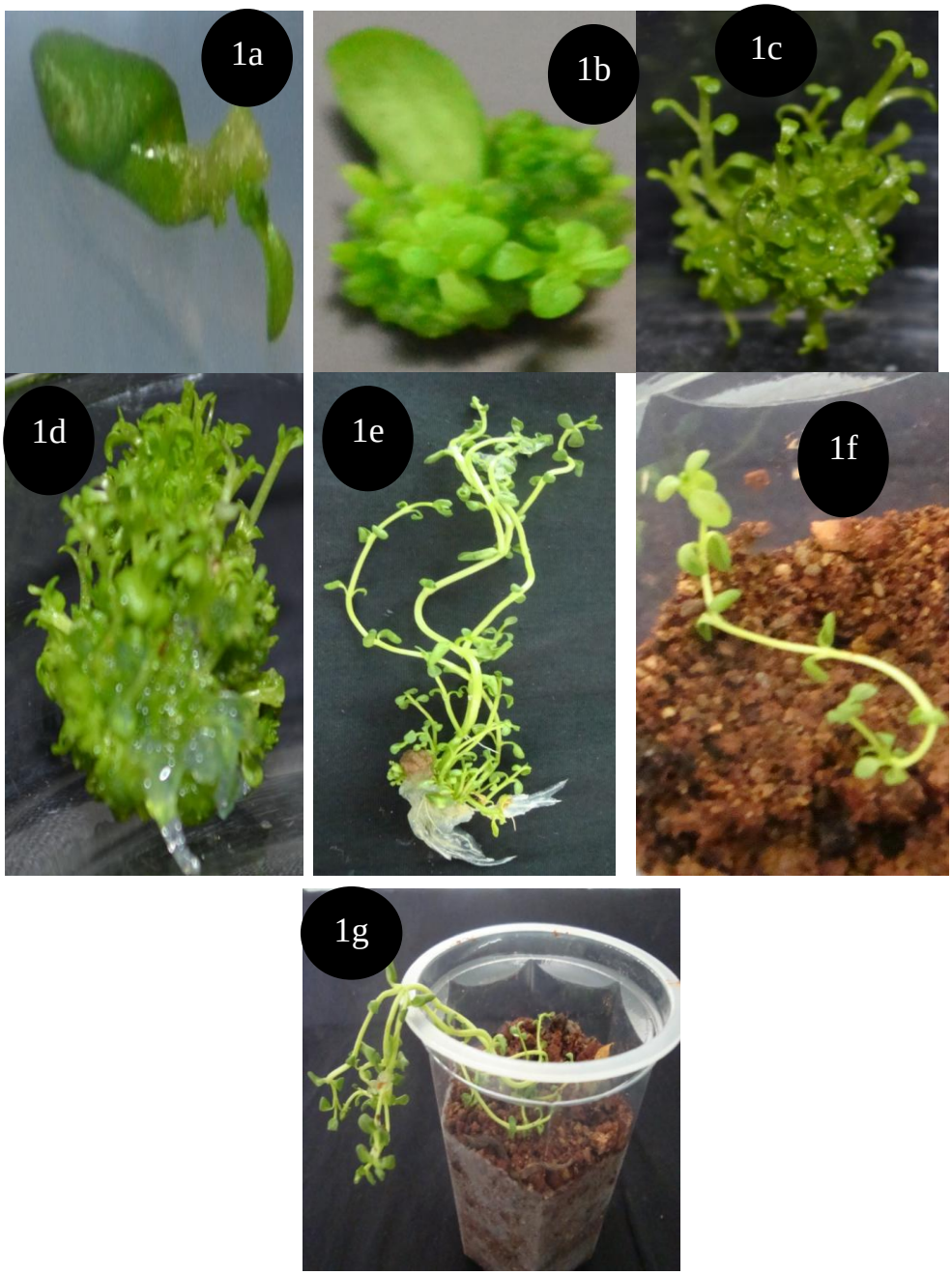

Figure 1: Induction of multiple shoots, 1a. 2nd week, 1b. 3rd week 1c. 4th week, 1d. 8th week, 1e. *in vitro* raised plantlets of *B.monnieri*, 1f. Hardened plant of *B.monnieri* in plastic cups, 1g. Hardened plant of *B.monnieri* in plastic cups

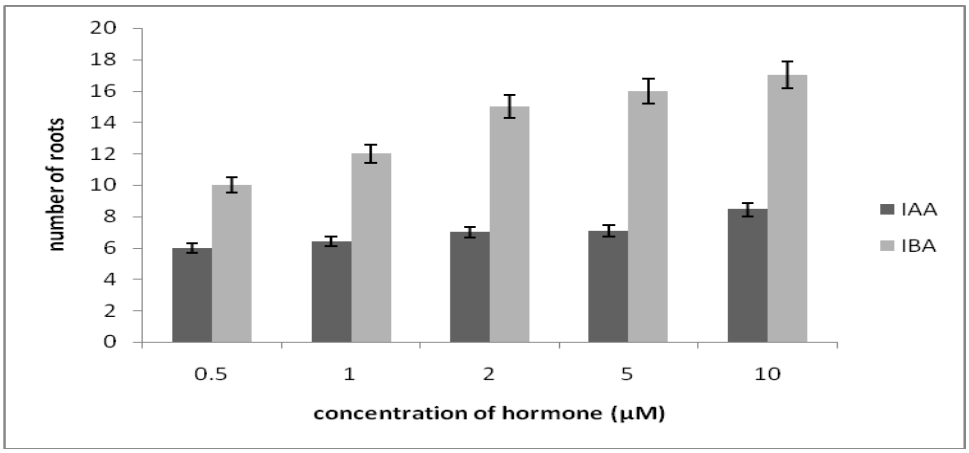

Figure 3: Effect of various concentrations of IAA and IBA on induction of root

Plant growth regulators are the most important factors for successful plant regeneration. In the present study, the cytokinins produced adventitious shoots by direct organogenesis whereas the auxin resulted in indirect organogenesis by induction of callus. According to the literature, leaf explants were most efficient for multiple shoot induction when compared to nodal or internodal explants, also the reports suggest that leaves are most widely used explants for studying adventitious shoot formation [11, 18, 19].MS media supplemented with 10.0 µM KN were most effective for the induction of multiple shoots when compared to BAP [1, 11, 20]. 10.0 µM KN was found to be superior as it induced highest number of multiple shoots from leaf explants and it is also responsible for fresh weight and dry weight of regenerated shoots [1], whereas, induction of callus followed by shoots were observed in media supplemented with TDZ in the second weeks [1]. Further, the shoot buds developed on the above medium were small and remain stunted and they required repeated sub culturing to produce sturdy growth. These observations are in accordance with the earlier results of Tiwari et al. [12]. In the current study higher number of adventitious shoots (85±0.5) was observed when compared to Naik et al. [13] where optimum number of shoots (79.00 ± 2.30) with fresh weight 2.41 g; dry weight 0.136 g was obtained by using leaf explants of B. monnieri cultured for 8 weeks on MS medium supplemented with 2 mg/l KN at pH 5.8. When MS suspension medium supplemented with 2 mg/l KN with 2% sucrose at pH 4.5 showed 150.50 ± 2.84 shoots/explant with fresh weight 6.31 ± 0.12 g; dry weight 0.250 ±5.00 g was also observed by Naik et al. [13]. Joshi et al., [6] observed the largest numbers of adventitious shoot buds 6.70 ± 0.34 were in MS media supplemented with 6 µM BAP. 18 multiple shoots were observed with maximum 16.33 shoots on addition of 80 mg/l adenine sulphate with combinational treatment of IAA (0.2 mg/l) and BAP (1.5 mg/l) by Tiwari et al. [12].The regenerated shoots were rooted on MS medium with NAA 0.5 mg/1 and IBA 1 mg/1.In the present study, the proliferation of multiple shoots and elongation growth of shoots was found to be higher in liquid medium as compared to the solid medium [19,26,27]. This might be due to better uptake of nutrients as large surface areas of explants were in contact with the liquid increasing the growth and multiplication [10]. Furthermore, the liquid medium helps in maintaining O_2:CO_2 balance [29]. The indirect organogenesis of present study was not in accordance with Tiwari et al. [9] who has reported greater callus formation observed for nodal explants of Bacopa cultured on MS

medium containing 0.5 mg/l 2,4-D. The best callus induction (71±2.2%) was found in MS medium supplemented with 2.0 mg l -1 BAP + 0.5 mg l -1 NAA from leaf explant by Rout et al. [30] .In the present study the induction of root was in not accordance with Chaplot et al. [19] where MS medium with 0.5–1.0 mg/l NAA ensuing excellent response for root induction but, MS medium supplemented with 0.5 mg/l of IAA as a root inducing hormone showed good response for the implanted explants. 100% in vitro rooting was obtained when shoot clusters were culture on MS medium supplemented with 0.15 mg/l IBA **(**24.9 ± 4.19) was observed by Sharma et al.[31]. Rooting was achieved in microshoots on ½ strength basal liquid medium supplemented with sucrose (1%) and indole-3-butyric acid (2 µM) by Rout et al. [30]. Finally the well developed plantlets were transferred to polycups containing the mixture of soil, sand and compost (in the ratio of 1:1:1) for hardening.

CONCLUSION

Medicinal plants are under severe threat of overexploitation and depletion of biodiversity. So, there is an urgent need of their ex situ conservation. Thus by the use of tissue culture technique such as micropropagation of medicinal plants, genetic material could be conserved and also the plants could further be used in producing active compounds in the laboratories. By the various research focusing on the advances in the tissue culture technique, nature's natural drugs could be preserved and also enable the sustainable use of the medicinal plants. Thus, the present work has deciphered methods of improving in vitro propagation by developing a two- step protocol highlighting efficient reproducible and reliable techniques for mass multiplication of a medicinally and economically important herb B. monnieri.

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REFERENCES

[1] Praveen N, Naik PM, Manohar SH, Nayeem A and Murthy HN. Acta Physiol Plant 2009; 31:723–728.
[2] Karthikeyan A, Madanraj A, Pandian KS and Ramesh M. Genetic Res Crop Evol 2011; 58:769-782.
[3] Russo A and Borrelli F. Phytomed 2005; 12:305–317.
[4] Rahman L, Verma CP, Singh DV, Gupta MM and Banerjee S. Biotechnol Lett 2002; 24:1427-1429.
[5] Das A, Shanker G, Nath C, Pal R, Singh S and Singh KH. Pharmacol Biochem Behavior 2002; 73:893-900.
[6] Joshi A, Pathak RA, Sharma MA and Singh S. Environm Exp Biol 2010; 8:81- 84.
[7] Yadav K, Singh N and Verma N. J Agr Sci Technol 2012; 8:305-318.
[8] Parale A, Barmukh R and Nikam T. Physiol Mol Biol Plants 2010; 16:167-175.
[9] Tiwari V, Singh BD and Tiwari KN. Plant Cell Rep 1998; 17:538-543.

[10] Tiwari V, Tiwari KN and Singh BD. Phytomorphol 2000; 50:337–342.
[11] Tiwari V, Tiwari KN and Singh BD. Plant Cell, Tissue and Organ Culture 2001; 66:9-16.
[12] Tiwari V, Tiwari KN and Singh BD. Plant Cell Rep 2006; 25:629-635.
[13] Naik P M, Manohar SH, Praveen N and Murthy HN. Plant Cell, Tissue and Organ Culture 2010; 100:235–239.
[14] Naik PM, Manohar SH and Murthy HN. Acta Physiol Plant 2010; 11-30.
[15] Gantait S, Mandal N and Das PK. American J Plant Physiol 2009; 5:325-37.
[16] Kothari SL, Joshi A, Kachhwaha S and Ochoa-Alejo N. Biotechnol Adv 2010; 28:35-48.
[17] Murashige T and Skoog F. Physiolog Plantarum 1962; 472-497.
[18] Hedayat M, Abdi GH and Khosh-Khui M. American-Eurasian J Agr Environm Sci 2009; 6:81-87.
[19] Chaplot BB, Dave MA and Jasrai TY. Plant Tissue Culture and Biotechnology 2005; 15:167-175.
[20] Shrivastava N and Rajani M. Plant Cell Rep 1999; 18:919-923.
[21] Peeters AJM, Gerards W, Barendse GWM and Wullems. Plant Physiol 1991; 97:402-8.
[22] Jain SM and Ochatt SJ. Protocols for in vitro propagation of ornamental plants, Springer protocols. Humana Press 2010; pp. 15-20.
[23] Mamidala P and Nanna RS. Plant Omics J 2009; 2:98-102.
[24] Hoque ME. Plant Omics J 2010; 3:7-11.
[25] Kumar A, Sood A, Palni UT, Gupta AK and Palni LMS. J Horti Sci Biotechnol 2001; 76:30-34.
[26] Joy PP, Thomas SMJ and Baby SP. Nestle Res News 2008. 92-103.
[27] Biondi S and Thorpe TA. Requirements for tissue culture facility. In: plant tissue culture: methods and application in agriculture, TA Thorpe academic press (Eds), Newyork, USA, 1981; pp. 1-20.
[28] Patil PK, Rath SP, Sharma M, Sood A and Ahuja PS. Biotechnol Adv 2005; 94- 114.
[29] Hashemabadi D and Kaviani B. Australian J Crop Sci 2010**;** 4:216-222.
[30] Rout GR, Samantaray S and Das P. Biotechnol Adv 2000**;** 18: 91–120.
[31] Sharma S, Kamal B, Rathi N, Chauhan S, Jadon V, Vats N, Gehlot and Arya S. African J Biotechnol 2010; 9:8318-8322.