INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE & TECHNOLOGY
© VISHWASHANTI MULTIPURPOSE SOCIETY (Global Peace Multipurpose Society) R. No. MH-659/13(N)
www.vmsindia.org

IN-VITRO REGENERATION OF PSORALEA CORYLIFOLIA

Moghe Sandhya, Mangesh Narad, Deepti Laud, Gauri Ade, Ishani Bansod, and Ravindra Moghe

Abstract

Psoralea corylifolia L. is an important medicinal plant. It is commonly called as Babachi and is an excellent source of psoralene and other major drug compound which are used for the treatment of various deadly diseases such as genital cancer, leucoderma, antidotes effect against poison etc. As the plant is of medicinal value and natural population is drastically reduced, it was investigated for in-vitro regeneration. For regeneration studies of this plant, seeds of good quality was obtained from local nursery and used for in-vitro germination to get various explants. Four different explants such as. Shootip, Cotynodes, Hypocotyl and Leaf explants were selected and they were screened for In-vitro response using Murashige and Skoog modified medium containing different combinations of BAP + KIN + NAA and TDZ. All these growth regulator combinations showed favorable response towards callusing and multiple shoots induction. Result revealed that Leaf explants showed cent percent response against all combinations, while hypocotyl explants sections were less responsive to majority of growth regulators but more favorable to TDZ combinations. The shoot tip and cotynode explants were responded to multiple shoot induction in BAP and NAA combination. The efforts were made to establish regenerated plants after hardening in soil..

Key words: Babachi, psoralea, hypocotyls, shoot tip, cotynode, MS, regeneration, somatic embryogenesis

Introduction

Medicinal plants are of global importance and have been one of the most important sources of medicine. Beginning of human's civilization, it is estimated that around 70,000 plants species from lichens to flowering trees, have been used for medicinal purposes (Purohit and Vyas, 2004). About 70% of the 3,000 plants from tropical forests were identified by the National Cancer Institute (NCI) as source of cancer fighting chemicals. (Tayler Miller, 1996).

At present *Psoralea corylifolia Linn*, is an endangered plant belonging to the family Fabaceae. The plant is well recognized in Chinese and Indian folkloric medicine as a laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions. The seeds have been recommended in the treatment of leucoderma, leprosy, psoriasis and inflammatory diseases of the skin. The active components of the plants are resin, alkaloid, fixed oil, and an essential oil. It is also used in perfumery industry due to its specific aroma.

The exploitation of this plant from their natural habitat has resulted in a marked decline in the population. It has been observed that plants of *P. corylifolia* are propogated by seed germination. A conventional method of propagation of *P. corylifolia* through seeds is not adequate, as seeds have hard seed coat and it poses problem in germinations. In-vitro propagation using different plant part and growth regulators would help to identify most potential and regenerable explants. In the present paper we report a simple and reproducible protocol of regeneration. Keeping in view all fact, an experiment was designed to study the effect of different growth regulator combination on regeneration efficiency of different explants.

Material and Method

Babachi is found in Maharashtra region, mainly as weed in fields having capacity to treat diseases and is tolerant to drought resistance to water lodging. Seeds of this plants was collected from medicinal plant nursery, Nagpur

Seeds of Babachi have hard coat and therefore easily not germinated in half MS medium. We soaked the seeds in water for 5-6 hrss and then were sown in earthen pots containing soil and sand. The pots were kept moist with water in college garden under natural condition. The seed germination was observed in all the pots. About 15-20 germinated pots were selected for experimentation. The explants used for present for tissue culture study was shoot tip, cotynode, hypocotyl, leaf section, and roots sections.

Explant were washed properly under running tap water to remove dust and dirt. They were rinsed in liquid detergent for 10 min then washed 2-3 times by double distilled water to remove traces of detergent. Then Explants were treated with 2% Bavistin (antifungal agent) with continuous shaking for 15 minutes. After this, all the further procedure were carried out under Laminar Air Flow. Bavistin was then decanted and explants were washed with autoclaved doubled distilled water to remove traces of Bavistin. Explants were then treated with 0.1% sodium hypochlorite for 5-7 min with gentle shaking followed by sterilization with 0.1% $HgCl_2$ for 10-15 sec and washing with ddw 3- 4 times to remove traces of $HgCl_2$. All the explants were collected in sterile Petri plate overled with tissue paper. The explants were cut by sharp

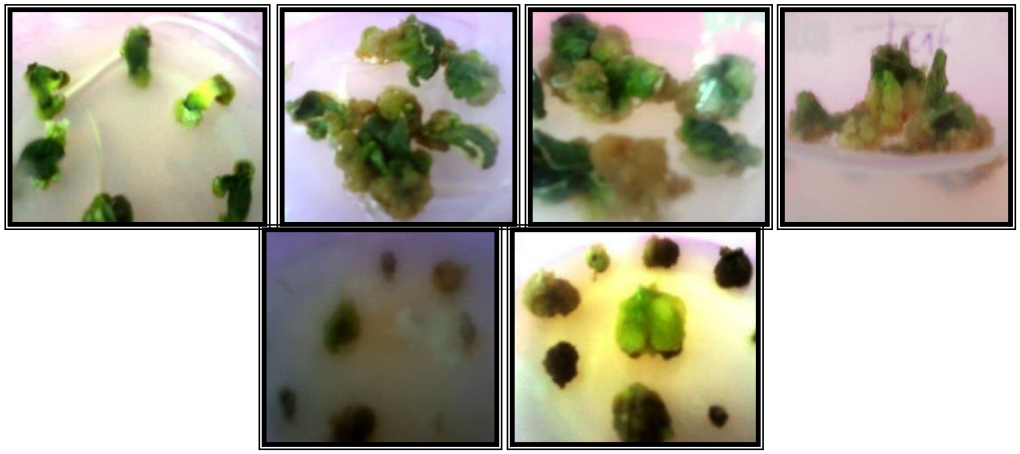
blade into different types such as shoot tip, leaf, cotynode, root section, and these were inoculated in to full MS medium in different combination for further development in MS basal salt with 30gm Glucose , 100mg Inositol , 10mg Thiamin , 7gm/L agar along with the combination of various growth regulators like TDZ,BAP,NAA, and KINETIN. After isolation and inoculation culture flask and bottles were incubated in culture room at 27± 2 °c at 16:8 hrs. photoperiod.

Result

IN- VIVO SEED GERMINATION RESPONSE OF BABACHI

For in–vivo seed germination, seeds were collected from medicinal plant nursery, Nagpur. The healthy selected seeds were germinated in 20 different pots. After 5-6 days, germination of these seeds was observed. Various age group of seedlings were selected for isolation of explants. The explants as given above were inoculated in different growth regulator combinations to study their response towards callusing and multiple shoots.

	Shoot Tip				**Hypocotyl**			**Cotynode**			**Leaf Section**		
	Media Combination (Mg/L)	Explant inoculated	No. of explants responded to regeneration	Regeneration %	Explant inoculated	No. of explants responded to regeneration	Regeneration %	Explant inoculated	No. of explants responded to regeneration	Regeneration %	Explant inoculated	No. of explants responded to regeneration	Regeneration %
1	TDZ (2)	25	21	84	20	20	100	15	15	100	25	25	100
2	TDZ(4)	25	20	80	20	16	80	15	14	93.3	25	22	
3	TDZ (6)	25	25	100	20	11	55	15	8	53.3	25	25	100
4	TDZ (8)	25	23	92	20	20	100	15	15	100	25	25	100
5	BAP+ KIN (1:1)	25	22	88	20	20	100	15	15	100	25	25	100
6	BAP+ KIN (2:1)	25	21	84	20	18	90	15	13	86.6	25	25	100
7	BAP+ KIN (2.5:1)	25	25	100	20	0	0	15	13	86.6	25	23	
8	BAP+ KIN (2:2)	25	25	100	20	0	0	15	8	53.3	25	24	
9	BAP+ KIN (3:3)	25	15	60	20	9	45	15	13	86.6	25	25	100
10	BAP+ NAA (1:1)	25	22	88	20	11	55	15	0	0	25	23	
12	BAP+ NAA (3:1)	25	19	76	20	6	30	15	12	80	25	21	
13	BAP+ NAA (4:1)	25	25	100	20	4	20	15	9	60	25	25	100



In-vitro* regeneration of *Psoralea corylifolia from hypocotyl and leaf section

In-vitro* regeneration of *Psoralea corylifolia from shoot tip and cotynode explant

The table showed response of different explants towards callusing and shoot formation. All the explants were cultured in MS medium supplemented with different combinations of TDZ, BAP + Kin and BAP + NAA combinations. It has been observed that shoot tip explants showed 100 % response to regeneration in 6 mg/L TDZ. This was followed by 8 mg/L and 2 mg/L in which 92 and 84 percent response was observed. In case of hypocotyls 2 mg/L TDZ reported cent percent regeneration, followed by 80 % in 4 mg/L. In cotynode explants however 8 and 2 mg/L TDZ combination recorded 100 % response to regeneration, followed by 93 % in4 mg/L. Leaf explants appeared to be most responsive in all the combination of TDZ.

In BAP and Kinetin combinations, shoot tips have recorded 100 % response to regeneration in 2.5:1 and 2:2 mg/L, followed by 88 % in 1 mg/L each. In hypocotyls explants however 2:1 mg/L BAP:Kin combinations have recorded 100 and 90 % response to regeneration.Cotynode explants however were responsive in 1:1, 2:1 and 2.5:1 BAP and Kin combination respectively. Response of leaf explants was comparatively good in 1:1, 2:1 and 3:3 mg/L BAP and Kin combination.

In BAP and NAA combination, response of shoot tip explants to regeneration was 100 % in 4:1 mg/L combinations, this was followed by 1:1 mg/L and 3:1 mg/L BAP and NAA combinations where percentage response to regeneration was 88 and 76 % respectively. In case of hypocotyl explants however response to regeneration was very poor in which 1:1 mg/L BAP and NAA has only recorded 55% regeneration. In rest of the combination regeneration response was below 50 %. In coty node also maximum regeneration of 80 % was in 3:1 mg/L followed by 60 % in 4:1 mg/L respectively. Leaf explants amongst all were very poor except 4 and 1 mg BAP and Kin combination where regeneration response was cent percent. In all other growth regulators there was negligible response.

Discussion

Babachi is an herbal crop. In India, Babachi is not known widely to the people. It is used in Ayurvedic, Unani, and Tibb medicine. It contained most essential chemical compounds used for treating deadly diseases. In the present study efforts were made to regenerate this plant under tissue culture with the objective to develop a protocol of regeneration.

Seeds of the plant under study were collected and sown in pots. Different explants from healthy seedlings were used for culture of explants. Shootip explant and cotynode were used for induction of multiple shoot and hypocotyl and leaf explant were used for callus induction (*Schiavone and cook 1987; Michalezuk etrrl.,* 1992) In the present study MS medium i.e. Murashige & Skoog basal salt supplemented with 30 gm/L glucose, 100 mg/L Inositol, 10mg/L thiamine and different combination of phytohormones like BAP (Benzylaminopurine), Kinetin, TDZ (Thiadiazurine), NAA (Naphthalene acetic acid) were used.

Jayakumar and Jayabalan (2001) have reported BAP as the most effective cytokinins for shoot bud regeneration. *Saxena st. al.* (1957) reported plant regeneration via organogenesis from callus derived from mature leaves stems, petioles and roots of young seeding in BAP & NAA In IAA, IBA & 2, 4- D also (Tanaka et al 2000) Induced high frequency shoot regeneration and enhanced isoflavones production in *Psoralea corylifolia in* TDZ (Thidiazuron) and N6-benzilaminz purine.

Thidiazuron (N-phenyl-N'-1, 2.3-thiadiazol-5-ylurea; TDZ), a substituted urea, with cytokinins- like activity, stimulated shoot proliferation in chickpea (Cicer arierinum L.) TDZ induced high frequency of shoot formation as compared to BAP and also minor salts of MS medium played an important role in increasing the number of shoots. Roots could be induced in these shoots in MS medium supplemented with 0.5pM IBA (*Rajendar et al.*, 2002). TDZ facilitates multiple shoot proliferation of many plant species (*Huetteman and Preece*, 1993; Lu, 1993; Murthy et al., 1998). It has been found to be less susceptible to plants degrading enzymes than endogenous cytokinin is active at lower concentrations than the amino purine cytokinins *(Mok et al.*, 1987) Moreover, plant regeneration can be stimulated through exposure to TDZ for a relatively short time (*Visser,* 1992). TDZ has exhibited a strong cytokinin activity in several culture systems (Thomas and *Katterman, 1986; Mok et al.*, 1987). TDZ has been reported to induce multiple shoot formation in various dicots. It has been shown to promote shoot regeneration at a much lower concentration than other cytokinins, and shoot regenerated with comparable or greater efficiency than with other cytokinins (Lu, 1993).

Jayakumar and Jayabalan (2001) have reported BAP as the most effective cytokinins for shoot bud regeneration in *Psoralea corylifolia*. The maximum percentage of shoot bud formation (82.2%) was obtained on MS medium fortified with the combination of BAP (3.0 mg/L) and & 4 mg/L.

Saxena et al. (1998) reported an average of 3-5 fold multiplication in Psoralea corylifolia when axillary shoots were allowed to continue in primary cultures for 8 weeks on MS medium supplemented with 2.5 mg/L, BA+ 1.0 mg/L NAA + 5.0 mg/L adenine sulphate.

In the present investigation, multiple shoot were induced by using shootip and cotynode isolated from 10-12 days old seedlings obtained from in-vivo germinated seedlings. . Multiple shoot induction was favourable when MS medium supplemented with BAP + KIN + NAA and TDZ were used. Multiple shoots from shootip were observed in BAP + NAA + KIN and TDZ combinations with good proliferation observed in 6 mg/L TDZ, BAP + KIN (2:2)mg/L and BAP + NAA (4:1)mg/L and low percent proliferation in BAP + KIN (3:3)mg/L. Also, multiple shoots from cotynode were observed cent percent proliferation response, in 2mg/L TDZ, 8mg/L TDZ , BAP + KIN (1:1)mg/L and low percent proliferation response of 33.30 in BAP + NAA (2:1)mg/L. Proliferation response for cotynode in BAP + NAA + KIN media combinations were below 50 percent in major combinations.

Study reveals that 10-12 days old seedlings have shown proliferation response to callus induction from hypocotyl shows cent percent response in 2mg/L TDZ, 8mg/L TDZ and BAP + KIN (1:1)mg/L and BAP + KIN (4:1)mg/L showed 14.28 percente response which is low amongest all. Proliferation response of hypocotyl was not good in BAP + KIN + NAA medium than TDZ combination. Leaf showed tremendous proliferation response for callusing in all combinations. Complete leaf gives more proper response of callusing than isolated leaf. Somatic embryogenesis occurred in leaf callus in two different combinations out of 13 different

combinations used. Cytological study revealed the stages of somatic embryos.

References

1. WHO, IUCN and WWF, Guidance on the conservation of medicinal plant (IUCN Gland Switzerland), 1993.

2. Sahrawat AK, Chand S. Continuous somatic embryogenesis and plant regeneration from hypocotyle segment of *Psoralea corylifolia* L. an endangered and medicinally important plant.Curr.sci. 2001; 81:10 -25.

3. Sharma PV. Hand book of Ayurveda (dravyagun) 2005;2 :175 -178.

4. Hyo JA, Seo JM, Choi Y, Park RK, Jeong S, Lee JY, Kim HM, Um JY, Hong SH., Induction of nitric oxide and tumor necrosis factor - ∝ by *Psoralea corylifolia* ,Indian J. Med. Res. 2008;128:752-758.

5. Liu R, Aifeng LI, Sun A, Kong L. Preparative isolation and purification from *Psoralea corylifolia* by high speed counter current chromatography. of chro.2004;1057:225 -228.

6. Oudhiya P. Traditional medicinal knowledge about herb Bemchi (*Psoralea corylifolia*) in Chhatisgarh, India. *Pharmacy*. 2001; 61(7): 312-332.

7. Chand S, Sahrawat AK. Somatic embryogenesis and plant regeneration from root segment of *Psoralea corylifolia* L. An endangered medicinal plant, In vitro. Cell. Dev. Biol. 2002; 33-38.

8. Jayakumar M, Jayabalan N., *In vitro* plant regeneration from cotyledonary node of *Psoralea corylifolia* L. Plant Tissue cult.2002; 12(2): 125-129.

9. Rajput SJ, Vijaya Z, Pallavi R., Studies on extraction ,isolation and estimation of Psoralea corylifolia, Pharmaco.Magazine, 2008;4 :13 Jan-Mar.

10. Shinde AN, Malpathak N, Fulzele DP., Induced high frequency shoot regeneration and enhanced isoflavones productin in *Psoralia corylifolia*. Rec.Nat.Prod. 2009; 3:38-45.

11. Jeyakumar M, Jayabalan N., An efficient method for regeneration of plantletsfrom nodal explants of *Psoralea corylifolia* L. Plant. Cell Biotech. Mol. Biol.2000; 1(1&2): 37

❖❖❖❖