Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 1, 2015

Original Article

ENHANCED PRODUCTION OF PSORALEN THROUGH ELICITORS TREATMENT IN ADVENTITIOUS ROOT CULTURE OF *PSORALEA CORYLIFOLIA* L.

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Received: 15 Sep 2014 Revised and Accepted: 13 Oct 2014

ABSTRACT

Objective: The present study aimed to determine the effect of two elicitors namely Methyl Jasmonate (MeJ) and Salicylic acid (SA) on adventitious root production of *Psoralea corylifolia* L. as the elicitors and at different concentrations.

Methods: Adventitious roots of *P. corylifolia* L. were treated with different concentration of elicitors such as MeJ (10, 20, 30 and 40 µM/l) and SA (50, 100, 150 and 200 µM/l) to enhance the psoralen contents. After the treatment, psoralen content was analyzed through the analytical HPLC experiments (Methanol: Water (50:50) at 0.8 ml/min^{-1} flow rate and the injection volume as 20 µl).

Results: MeJ and SA treatments at the concentrations of 30 µM/l and 150 µM/l respectively were found to increase the net wet weight of adventitious root production to 8 fold at 8 hours of elicitor treatment periods. Further, the quantity of psoralen was determined from the elicitors (MeJ and SA) treated roots and mother plant through the analytical HPLC experiments in order to estimate the psoralen content and it was found as 3.73 mg/ml, 0.015 mg/ml and 0.56 mg/ml respectively.

Conclusion: In the present study we achieved enhanced production of psoralen through abiotic elicitors (MeJ and SA) treatment and concluded that the MeJ at 30 µM/l concentration shows significant enhancement of psoralen production.

Keywords: *Psoralea corylifolia* L. Psoralen, Isopsoralen, Elicitors, MeJ, SA, Adventitious roots, HPLC.

INTRODUCTION

Medicinal plants are the natural sources for different forms of alkaloids and chemical substances which are being used to cure a variety of diseases among human beings worldwide. *Psoralea corylifolia* Linn. (Fabaceae) is an important medicinal plant used in folk, siddha and ayurvedic system of medicine. It is an endangered and rare herbaceous medicinal plant distributed in the tropical region of the world [1]. From time to time, the fruits, seeds and roots of *P. corylifolia* have been examined and a large number of pharmacologically important compounds have been reported [2]. This plant species is also characterized by the presence of essential oil, terpenoids and resins. In addition, the most important compounds are such as alkaloids, flavonoids, glucosides, essential and fatty oils, resins, gums, mucilage, tannins and etc. are also have largely used in pharmacology. These active principles might present in the storage organs of plants *viz* roots, seeds, leaves and etc [3].

This plant contains major bioactive compounds such as psoralen, isopsoralen, angelicin and daidzein [4]. Among them psoralen is one of the important pharmacological active compounds and it is used to treat various skin diseases such as psoriasis, mycosis, fungoides and eczema [5]; [6]. Psoralen and isopsoralen are being investigated against several diseases including AIDS [7]. It is also used in indigenous medicine as the laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions [8]. These compounds are specially recommended for the treatment of leucoderma, leprosy, psoriasis and inflammatory diseases of the skin and prescribed for both oral administration and external application in the form of a paste or ointment [9]; [10].

Elicitor treatment is one of the effective strategies for improving secondary metabolites production in *in vitro* plant cell culture [11]. Exogenous application of Methyl Jasmonate (MeJ) and Salicylic acid (SA) are involved in the signal transduction pathways that induce particular enzymes to catalyze biochemical reactions to produce defense compounds with lower molecular weight in plants like polyphenols, alkaloids, quinines, terpenoids, and polypeptides [12]. The accumulation of secondary metabolites in plants is part of their defense response, which triggered and activated by elicitors and acts as signal compounds of plant defense responses. There are voluminous literature showing that the positive influence of MeJ and SA on an enhancing secondary metabolites production in cell culture [13]. In our previous study on rapid seed germination and highest survival rate of seeds of *P. corylifolia* was achieved by heat treatment at 70 °C [14]. The effect of abiotic and abiotic elicitors at various concentrations on total isoflavonoid accumulation was studied in the hairy root culture of *Pueraria candollei* [15]. SA has been identified as a stress signaling molecule involved in plant defense responses [16] and enhance the production of phytoalexin in suspension culture [17]. Sivanandhan *et al.,* [18] reported that the different concentrations of MeJ and SA were increased the secondary metabolites level in adventitious root formation of *Withania sominifera* L. MeJ is involved in signal transduction pathway which induces the enzyme to catalyze biochemical reaction [19] and SA is also involved in stress signaling pathway, plant resistance to pathogens and other environmental stress factor [20].

Field-grown plants are more prone to attack by pests which consequently affect the quality and quantity of psoralen production. Hence it is desirable to depend on *in vitro* cell culture for avoiding any contamination during plant growth and yield of psoralen. So the present study was aimed to enhance the production of psoralen content through elicitors treatment in *in vitro* root culture of *P. corylifolia*.

MATERIALS AND METHODS

Seed material

P. Corylifolia seeds were kindly provided by Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV) Jabalpur, Madhya Pradesh, India.

Aseptic seed germination

Healthy seeds were washed thoroughly once in tap water for 10 minutes, followed by soaking in soap solution (2% Teepol - commercial soap solution) for 5 minutes and then the seeds were kept under running tap water for 30 minutes. After washing, the seeds were disinfected with 70% ethanol for 45 seconds and rinsed with double distilled water for 3 times, followed by exposure in

0.1% (w/v) aqueous mercuric chloride for 5 minutes. After decanting the mercuric chloride solution, the seeds were rinsed 5 times in sterile distilled water and then disinfected seeds were inoculated in test tubes containing moistened cotton for seed germination. Initially the cultures were maintained in dark condition for 48 h at 25±2°C and then under 16 h photoperiod conditions with the light intensity of 3000 lux. All the surface sterilization and inoculation works were performed under aseptic condition. Before inoculation, the test tubes were autoclaved at 121°C for 15 minutes with 1.06 Kg cm^{-2} pressures (15 lb). After germination, healthy and vigorously growing seedlings were selected and used as the source of explants.

Root culture

The trimmed leaf explants from 20 days old *in vitro* seedlings were inoculated in MS medium. The leaf explants were inoculated in rooting medium containing 3% sucrose, NAA (0.5-2.5 mg/l), IAA (0.5-2.5 mg/l) and IBA (0.5-2.5 mg/l). The cultures were maintained in dark condition for 48 h at 25±2°C and then under 16 h photoperiod conditions with the light intensity of 3000 lux. After the production of roots from explants, they were transferred into liquid root culture medium containing 3% sucrose, NAA (0.5-2.5 mg/l), IAA (0.5-2.5 mg/l) and IBA (0.5-2.5 mg/l).

Optimization of elicitors concentrations

Different concentrations of MeJ *viz.*,10, 20, 30 and 40 µM/l and SA *viz.*, 50, 100, 150 and 200 µM/l were used along with optimum auxin concentration. The culture was maintained in an orbital shaker at 120 rpm/min. The 28th day of liquid root culture was exposed for 8 h contact time with the elicitors for the production of psoralen. After 8 hours of elicitor treatment, the root samples were collected, extracted with methanol and stored at - 20°C. All the experiments were conducted in triplicate.

HPLC analysis of elicitor treated root samples

After the elicitation treatment, the root samples were taken from the liquid root culture medium, dried and ground to fine powder. The powder was extracted with 10 ml methanol using sonication for 30 min. The extracted material was then subjected to dry at 50°C for 1 week and then the sample was again dissolved in 5 ml methanol and centrifuged at 8000 rpm for 25 min. The supernatant was then filtered through 0.22 µM membrane filter and it was subjected to HPLC analysis (Waters, C18 silicon column, reverse phase, Australia). The analytical HPLC experiments were performed in Methanol: Water (50:50) at 0.8 ml/min^{-1} flow rate and the injection volume was set as 20 µl (Fig. 1 - 4) [26-27].

Estimation of Psoralen from Elicitors Treated Root Samples:

Estimation of psoralen content in the treated root samples was compared with the mother plant and calculated by the following formula.

$$\text{Sample concentration} = \left\{\frac{\text{Sample area}}{\text{Mean STD Area}}\right\} \times \left\{\frac{\text{STD weight}}{\text{STD Dilution}}\right\} \times \left\{\frac{\text{Sample Dilution}}{\text{Sample Weight}}\right\}$$

RESULTS AND DISCUSSION

The present study was carried out to identify the role of MeJ and SA for enhanced production of psoralen in adventitious root culture of *P. corylifolia*. The leaf explants were inoculated on MS medium supplemented with auxins at different concentrations. The medium containing 3% sucrose with NAA 0.5mg/l, IAA 1.0mg/l and IBA 1.5mg/l were found as the optimum concentration for the high number of root induction. The production of psoralen was influenced by the age of the culture and elicitation period, and by the different concentrations of two elicitors (MeJ and SA). The 28th day liquid culture, treated with the MeJ for 8 h contact time showed better results than SA in terms of visible changes in root morphology such as color and texture of the root and increased weight.

Table 1 and 2 indicates that the best result was observed in 30 µM/l of MeJ and 150 µM/l of SA respectively. The best concentration alone was repeated for further evaluation in order to find the optimal time periods. In which, the best result was observed in 30 µM/l of MeJ treated roots after 8 hours of elicitation treatment and obtained 2.76 fold increase in weight (Plate 1).

Table 1: Effect of different concentrations of Methyl Jasmonate for adventitious root production in MS liquid medium

Concentration (µM/l)	Inoculated Root Weight (g)	Harvested Root Weight (g)*	Fold Increase
10	3	5.033 ± 0.15	1.67
20	3	5.96 ± 0.15	1.98
30	**3**	**8.3 ± 0.2**	**2.76**
40	3	5.5 ± 0.2	1.83

*values are represented as Mean ± SD

Table 2: Effect of different concentrations of Salicylic Acid for adventitious root production in MS liquid medium

Concentration (µM/l)	Inoculated Root Weight (g)	Harvested Root Weight (g)*	Fold Increase
50	3	3.99 ± 0.15	1.32
100	3	4.3 ± 0.2	1.43
150	**3**	**6.033 ± 0.15**	**2.01**
200	3	5.5 ± 0.3	1.83

*values are represented as Mean ± SD.

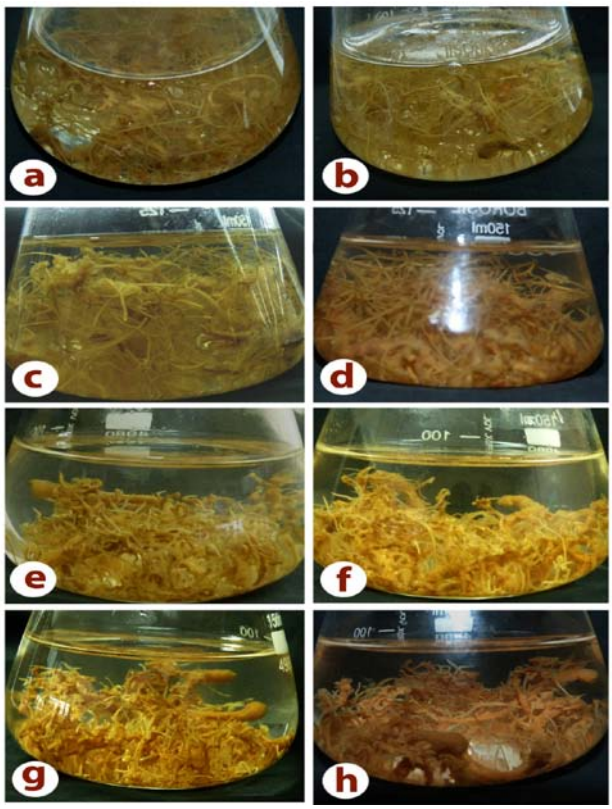

Plate: 1. Effect of different concentrations of elicitors treatment (28th day old culture and 8 h contact time) on *in vitro* adventitious root growth of *Psoralea Corylifolia L.*

a to d - MeJ treated adventitious root (a, 10 µM/l; b, 20 µM/l; c, 30 µM/l; d, 40 µM/l)

e to h - SA treated adventitious root (e, 50 µM/l; f, 100 µM/l; g, 150 µM/l; h, 200 µM/l)

Addition of MeJ and SA showed enhanced root growth which subsequently increased the psoralen content in adventitious root culture of *P. corylifolia*. Bulging of root was observed after 8 hours of contact time with MeJ at 30 µM/l and SA at 150 µM/l concentrations showed profuse root growth.

Amit Shinde *et al*., [21-23] reported that the addition of SA at 1 mM concentration stimulated high accumulation of isoflavones in hairy root culture of *P. corylifolia* after 2 days of elicitation, but further increasing the concentration of SA beyond the optimal concentration and incubation period it found that the reduction in root growth and psoralen accumulations. Elicitor treatment has been proved to enhance the production of secondary metabolites in adventitious root culture of some important medicinal plants [11]. MeJ and SA were used as elicitors for higher production of with anolides in adventitious root culture of *Withania somnifera* [24]. Similarly, MeJ was showed to induce inulin accumulation at 150 µM/l in combination with *Aspergillus niger* extract in *Helianthus tuberosus* [25].

HPLC analysis of elicitor treated root sample

Psoralen standard chromatogram (RT 20.628)

HPLC analysis of methanolic extract of MeJ treated root samples showed a single peak at the retention time of 21.622 and estimated psoralen concentration is found as 3.73 mg/ml. SA treated root sample peak at the retention time of 21.651 and estimated as the concentration of psoralen is as 0.015 mg/ml. Comparatively, we obtained mother plant root sample peak at the retention time of 21.312 and estimated the concentration of psoralen is as 0.56 mg/ml. This HPLC analysis showed that MeJ treated root samples indicated the good results when compared with SA (Fig 1 - 4).

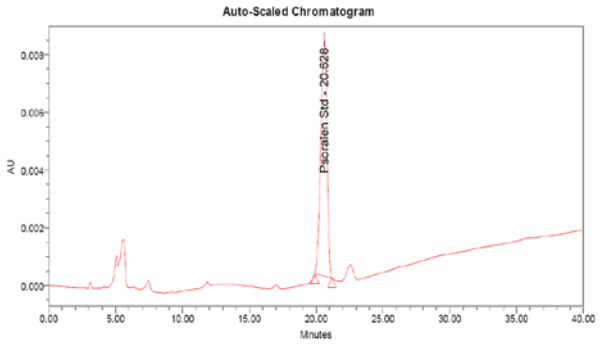

Fig. 1: Psoralen Standard Chromatogram

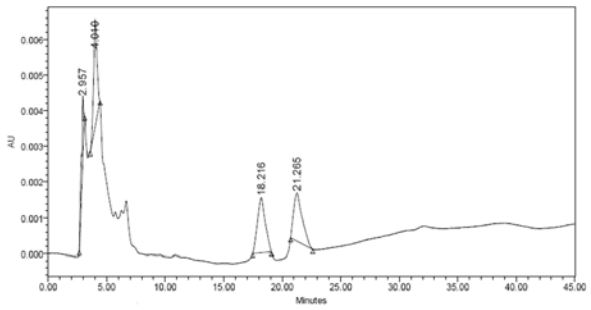

Fig. 2: Root Sample Chromatogram from the MeJ Treated Plant 30 µM/l

Baskaran and Jayabalan [26] performed HPLC analysis of psoralen from both *in vitro* and *ex vitro* grown *P. corylifolia* plants and reported that the psoralen content was high *in vitro* grown plants when compared to *ex vitro* grown plants. The analytical HPLC experiments was performed in Methanol: Water (50:50) at 0.8 ml/min^{-1} flow rate and the injection volume was set as 20 µl. HPLC analysis of psoralen was performed for *in vitro ex vitro* grown roots and seeds part of *P. corylifolia* plants [27].

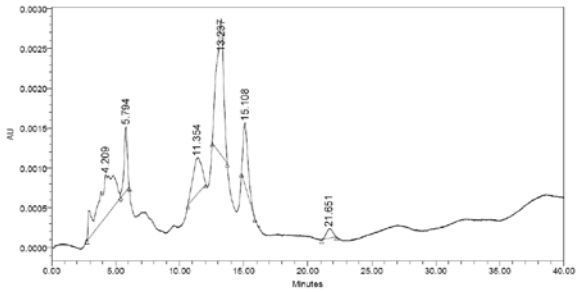

Fig. 3: Root Sample Chromatogram from the SA Treated Plant 150 µM/l

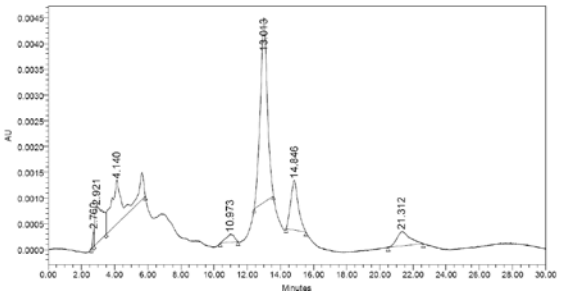

Fig. 4: Root sample Chromatogram from Mother Plant control

In the above said factors, the elicitors treatment is important to improve the secondary metabolites production in medicinal plants. In earlier reports stated the psoralen is very important in pharmaceutical and medicinal industries to prevent and cure some of the skin diseases. So the psoralen production is commercially very important for pharmaceutical industries. In this present study, two types of elicitors were used for improving the psoralen production in *P. corylifolia*. In which, MeJ treated roots shows best result in psoralen production. MeJ 30 µl/l treated roots produced 3.73 mg/ml of psoralen concentration when compared with the SA and mother plant.

CONCLUSION

Psoralen is the major compound present in root parts of *P. corylifolia*. In the present study we achieved enhanced production of psoralen through abiotic elicitors (MeJ and SA) treatment. Leaf explants from 20 days old *in vitro* raised seedlings were cultured on MS medium containing 3% sucrose, 8% agar along with NAA 0.5 mg/l, IAA 1.0 mg/l and IBA 1.5 mg/l for root induction. These roots were transferred to suspension culture medium containing the above said plant growth regulators. After 28th day mass production of roots, MeJ (30 µM/l) and SA (150 µM/l) were added to determine the increase in root weight and accumulation of psoralen. After 8 hours of elicitation period, root samples were extracted and subjected to HPLC analysis and psoralen content was determined as 3.73 mg/ml in MeJ treated root sample, 0.015 mg/ml in SA treated root sample and 0.56 mg/ml in control plant. From the present study, it is concluded that MeJ at 30 µM/l concentration showed the best result for enhanced production of psoralen.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENT

This work was supported by the University Grants Commission, New Delhi, India (Letter no. 41-393/2012(SR) Dt.16.07.2012) in form of project fellow, and grateful to the Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV) Jabalpur, Madhya Pradesh for valuable assistance for seed Material. The authors are grateful to Prof. N. Jayabalan, Coordinator, UGC - SAP and Mr. S. Muthukrishnan, Technical Assistant, UGC- SAP, Department of Plant Science Bharathidasan University, Tiruchirappalli for their help with HPLC Analysis.

REFERENCES

1. Jain SK. Ethno botany and research in medicinal plants in India. Ethno botany and the search for New. Drugs 1994;185:153-68.
2. Bajwa BS, Pyare LK, Seshadri TR. A new chromenochalcone bavachromene from the seeds of *Psoralea corylifolea.* Cur Sci 1972;41(22):814-15.
3. Khan IA, Khanum A. Role of Biotechnology in medicinal and aromatic plants-Retrospect and prospect. Ukaaz publications, Hyderabad, India; 1998. p. 1.
4. Baskaran P, Jayabalan N. Rapid micropropagation of *Psoralea corylifolia L*. using nodal explants cultured in organic additive-supplemented medium. J Horticulture Sci Biotechnol 2007;82:908-13.
5. Frank S, Caffieri S, Raffaelli A, Vedaldi D, Dall Acqua F. Characterization of psoralen-oleic acid cycloadducts and their possible involvement in membrane photo damage. J Photochem Photobiol B 1998;44:39-44.
6. Yones SS, Palmer RA, Kuno K, Hawk JLM. Audit of the use of Psoralen phochemotherapy (PUVA) and narrowband UVB phototherapy in the treatment of psoriasis. J Dermatol Treat 2005;16:108-12.
7. Bhattacharjee SK. In: Hand book of medicinal plants. (Eds.) pointer publishers, Jaipur; 1998. p. 287-8.
8. Rastogi, Mehrotra. In: Compendium of Indian medicinal plants; 1990;1:332-3.
9. Anonymous. In: The Wealth of India. A dictionary of Indian raw materials and Industrial products. 2, CSIR: New Delhi, India; 1988. p. 116-8.
10. Orient longman. In: Indian medicinal plants (Eds.) Orient Longman Ltd. Madras 1996;4:374.
11. Ganeshan Sivanandhan, Ganapathi. Citossan enhances withanoides production in adventitious root culture of *Withania somnifera L.* Dunal Insdustrial Crops Prod 2011;37:124-9.
12. Saenz-Carbonell L, Loyola-Vargas VM. *Datura stramonium* hairy roots tropane alkaloid content as a response to changes in Gamborg's B5 medium. Applied Biochem Biotechnol 1997;61:321-37.
13. Dicosmo F, Misawa M. Eliciting secondary metabolism in plant cell cultures. Trends Biotechnol 1985;3(12):318-32.
14. Siva G, Jayabalan N. Enhanced seed germination of *Psoralea Corylifolia* L. by heat treatment. World J Agric Res 2014;2-4-2:151-4.
15. Latiporn Udomsuk, Kanokwan Jarukamjorn, Hiroyuki Tanaka, Waraporn Putalun. Improved isoflavonoid production in *Pueraria candollei* hairy root cultures using elicitation. Biotechnol Lett 2011;33:369-74.
16. Draper J. Salicyliate × superoxide synthesis and cell suicide un plant defense. Trends Plant Sci 1997;2:162-5.
17. Mehmetoglu U, Cuurtis WR. Effects of abiotic inducers on sesquiterpene synthesis in hairy root and cell suspension cultures of Hyoscyamus. Appl Biochem Biotechnol 1997;67:71–77.
18. Ganeshan Sivanandhan, Ganapathi. Optimization of elicitation conditions with Methyl jasmonate and salicylic acid to improve the productivity of Withnolides in the adventitious root culture of *Withania somnifera L*. Dunal Appl Biochem Biotechnol 2012a;168:681-6.
19. Yu Kw Gao, Hahn EJ, Peak KY. Jasmonic acid improving ginsenoside accumulation in adventitious root culture of panax ginseng CA Meyer. Biochem Engg J 2002;11:211-5.
20. Rao MV, Lee H, Creelman RA, Mullet JE, Davis KR. Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. Plant Cell 2000;12:1633-46.
21. Amit N Shinde, Nutan Malpathak, Devanand P Fulzele. Optimized production of isoflavones in cell cultures of *psoralea corylifolia l.* using elicitation and precursor feeding. Biotechnol Bioprocess Eng 2009;14(5):612-8.
22. Amit N Shinde, Nutan Malpathak, Devanand P Fulzele. Studied enhancement strategies for phytoestrogens production in shake flasks by suspension culture of *Psoralea corylifolia*. Bioresour Technol 2009b;100:1833-9.
23. Amit N, Shinde a, Nutan Malpathak a, Devanand P, Fulzele. Determination of isoflavone content and antioxidant activity in *Psoralea corylifolia L.* callus cultures. Food Chem 2010;118:128-32.
24. Ganeshan Sivanandhan, Ganapathi. Effect of culture conditions of cytokinins, MeJ and SA on the bio mass production in multiple shoot culture of *Withania sominifera* L. Dunal Acta Physiol Plant 2012.
25. HS Taha, AM Abd E1-Kawy, M Abd-Kareem Fathalla. A new approach for achievement of inulin accumulation in suspension cultures of Jerusalem artichoke (*Helianthus tuberosus*) using biotic elicitors. J Genet Eng Biotechnol 2012;10:33-8.
26. Baskaran P, Jayabalan N. Effect of growth regulators on rapid micropropagation and psoralen production in *Psoralea corylifolia L*. Acta Physiol Plant 2008;30:345-51.
27. Baskaran P, Jayabalan N, Van Staden J. Production of psoralen by *in vitro* regeneration plants from callus cultures of *Psoralea corylifolia L*. Plant Growth Regul 2011;65:47-54.