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Induction of callus and multiple shoots from nodal cultures of Pueraria tuberosa Roxb. ex.willd.

KEYWORDS: Multiple shoots, callus culture, nodal culture, clonal propagation.

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ABSTRACT *Pueraria tuberosa is a wild and medicinal plant belongs to one of the largest family i.e Papilionaceae. It is growing sandy areas of the forest of southern states of India. Tuberous roots of plant are used for cardiovascular benefits and ailments of human health. Very few reports are on callus induction and no report on regeneration and somatic embryogenesis. We are attempting for clonal propagation and regeneration of plantlets form various explants, particularly from nodal cultures of endangered medicinal plant and achieved large scale callus induction and multiple shoot induction on MS medium fortified with BAP (4.0 mg/l) + IBA (0.5 mg/l) with 3.5 % sucrose in a short span of time.*

Introduction:
Pueraria tuberosa is a perennial woody climber belongs to the family papilionaceae. It is commonly known as Indian kudzu. It is geographically distributed in India, Nepal and Pakistan. *Pueraria* species are popular Chinese herbal medicine with antioxidantive and antithrombotic effects. It has been reported that Puerarin plays effective role in the terminated of hypertension, diabetes, arteriosclerosis and metabolic syndrome (Teng *et al*. 2009, Luo *et al*. 2010, Zhu *et al*. 2010). The flavonoids are used in the treatment of cardiovascular diseases (Mizushige *et al*.2007), osteoporosis and post menopausal symptoms (Dai *et al*.2008). Leaves, shoots, flowers, seeds and roots in tempura, pressed salads and pickles. Kudzu fibers are used to stuff cushions, beds and chairs. *In vitro* culture facilitates a potential alternative to the mass harvesting of plants for the purpose of obtaining crude drug extracts. Wide spread harvesting of *Pueraria* for its tubers restricts its reproduction and regeneration these by made it a threatened species by these great efficacies urge *in vitro* studies to develop the technologies for their large scale production.

Material and methods:
Explants like nodes and inter nodes were collected from *Pueraria tuberosa* from our research filed for tissue culture studies. These explants were thoroughly washed under running tap water for 10minutes and surface sterilized with 0.1% $Hgcl_2$ for 7-8minutes, rinsed 3-4times with sterilized distilled water. The sterilized nodes and inter nodes were cut into small species and inoculated on MS medium supplemented with BAP + 2, 4-D and BAP + IBA Combinations with 3.0% (Callus) and 3.5% (Multiple shoots) sucrose and 0.9% agar-agar, pH was adjusted to pH 5.8 and autoclaved at 121°c for 20minutes. These cultures were incubated at 25 ± 2°c under 16 hours day photoperiod. The cultures were responded after 10 days of culture and results were recorded with different intervals of time.

Results and Discussion
The nodes and internodes were inoculated on MS medium supplemented with different combinations of BAP + 2,4-D with 3.0% sucrose. Only nodal portions responded positively for the induction of callus and initiated callus from the cut ends. The response and quality of the callus was good on MS + 2.5 mg/l BAP +0.5 mg/l 2,4-D, the callus turned to black in color after four weeks (Table-1, Fig. **A**). By increasing the concentration of BAP from 2.5 mg/l to 4.0 mg/l along with 1.0 mg/l 2,4-D, green compact callus was induced after three weeks of sub culture, significant growth and percent of response was maximum. Higher the concentration of BAP did not show significant result (Table-1 Fig. **B**). Increased concentration of 2, 4-D in the medium was inhibitory to callus formation and the formed callus was white friable which is not suitable for regeneration experiments.

When the nodes were inoculated on MS medium with different concentrations of BAP + IBA with 3.5%, The explants induced little amount of callus and 2-4 shoots on MS+1.0-2.0mg/l BAP + 0.5mg/l IBA (Table-2 Fig. **C**) after four weeks of inoculation. The subcultures were maintained at an interval of four weeks. The frequency of multiple shoots was increased by increasing the concentration of BAP from 2.0mg/l to 4.0mg/l by keeping the concentration of IBA constant (0.5 mg/l) (Table-2 Fig. **D**). The percent response and frequency of multiple shoots was maximum on MS medium with 4.0 mg/l BAP + 0.5 mg/l IBA (Table-2 Fig. **E**). This is the first report of multiple shoot induction in *Pueraria tuberosa*. The higher concentration of IBA (1.0 mg/l) inhibited shoot induction but promoted callus. The shoots were remain fresh for longer time and the percent response was also higher in our investigation.

Callus induction from nodal explants was good on MS + BAP + 2, 4-D combination. The leeching of phenolics compounds could be inhibited by increase the concentration of BAP from 1.0 mg/l to 2.5 mg/l and 4.0 mg/l, similar results were observed by Udomsuk *et al*, (2008) by using BAP and NAA combinations. The initial callus was subcultured on the same medium for further growth and effective callus formation and also proved as the nodal explant was suitable for callus induction. Till to data no standardized protocol is developed for the induction of high amount of callus (Kim *et al*, 2005, Lualon *et al*, 2008), but we succeeded in the induction of Green friable and compact callus on MS medium fortified with BAP + 2,4-D (Table-1).

Nodal explants responded positively in *Phaseolus vulgaris* on MS medium fortified with 1.5 mg/l BAP and 0.5 mg/l NAA

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(Mohamed *et al*. 1992). Although specific studies on tissue culture on *Pueraria species* are not available, some basic investigations are undertaken in our studies. Nodal explants of *Canavalis virosa* fortified with BAP, GA3 and KN yielded multiple shoots, while IBA induced roots (Kathiravan and Ignacimuthi, 1999).

The successful reports on regeneration in legumes were achieved using various cytokinins BAP (Avenido and Hattori, 2000; Saini and Jaiwal 2002), TDZ (Keneda *et al*. 1997; Das *et al*. 1998; Yoshida 2002). Therefore we studied with the effects of BAP and IBA on *in vitro* plant production. The present study attempted micropropagation of different Nodal and internodal explants of *Pueraria tuberosa* using different methods, media with growth factors and culture conditions *in vitro* and *in vivo*. Thiem (2003) has developed a micropropagation system for *Pueraria lobata*, while Thanonkeo and Panichajakul (2006) have reported on successful micropropagation *of Pueraria condollei, Pueraria minifrica*. Moreover Kimtzios *et al* (2004) have reported on production of puerarin from hairy root cultures.

Such previous results supported the induction of callus just for the compound increment where as our target is to achieve the high amount of green compact callus formation. There is a variation in callus texture with respect to the various concentration of BAP in combination with 2,4-D.

Table 1: Induction of callus from nodal explant of *Pueraria tuberosa* Roxb on MS medium supplemented with BAP + 2,4-D mg/l.

S.NO	MS medium with BAP + 2,4-D (mg/l)	Percentage of showing response	Morphogenic response
1	0.5 + 0.5	10	Light black callus
2	1.0 + 0.5	20	White callus
3	1.5 + 0.5	20	White friable callus
4	2.0 + 0.5	40	Brown friable callus
5	2.5 + 0.5	30	Light green callus
6	3.0 + 0.5	40	White friable callus
7	3.5 + 0.5	50	White friable callus
8	4.0 + 0.5	30	Brown compact callus
9	0.5 + 1.0	20	White compact callus
10	1.0 + 1.0	30	Brownish white friable callus
11	1.5 + 1.0	20	Brown compact callus
12	2.0 + 1.0	30	Light green friable callus
13	2.5 + 1.0	50	Light greenish brown friable callus
14	3.0 + 1.0	40	Green friable callus
15	3.5 + 1.0	50	Green compact callus
16	4.0 + 1.0	60	Green compact callus

*Data was collected after 3 weeks of culture.

Table 2: Induction of multiple shoots from nodal explant of *Pueraria tuberosa* Roxb on MS medium Supplemented with BAP + IBA mg/l.

S. NO	MS medium with BAP + IBA (mg/l)	Percentage of response	No. of shoots / explant (Mean ± SE)	Length of shoot (cm) (Mean ± SE)
1	0.5 + 0.5	10	2.6 ± 0.45	2.54 ± 0.12
2	1.0 + 0.5	20	3.2 ± 0.35	2.62 ± 0.14
3	1.5 + 0.5	30	3.1 ± 0.41	2.57 ± 0.13
4	2.0 + 0.5	40	4.1 ± 0.28	2.21 ± 0.15
5	2.5 + 0.5	50	3.2 ± 0.51	2.74 ± 0.12
6	3.0 + 0.5	50	6.1 ± 0.52	2.21 ± 0.11
7	3.5 + 0.5	60	7.1 ± 0.54	1.89 ± 0.09
8	4.0 + 0.5	70	8.2 ± 0.44	1.15 ± 0.11
9	4.5 + 0.5	50	5.8 ± 0.46	2.39 ± 0.14
10	5.0 + 0.5	60	6.4 ± 0.59	2.21 ± 0.12

*Data was collected after 5 weeks of culture.

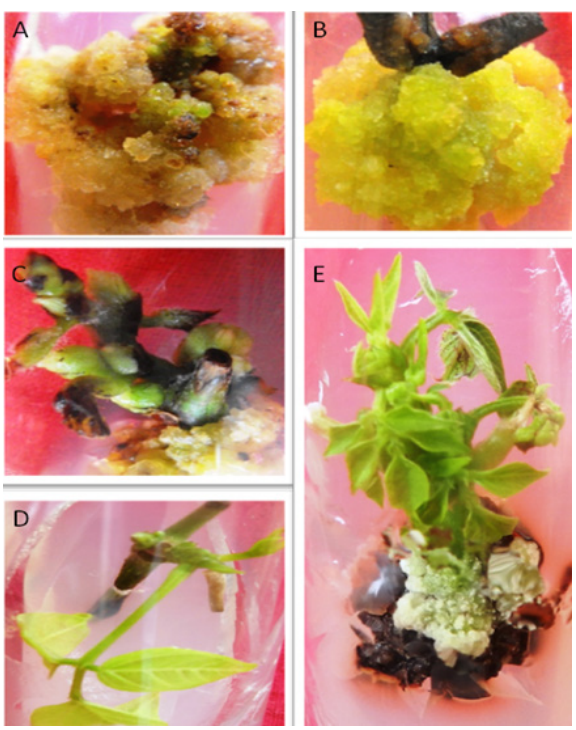

Figure: Induction of callus and multiple shoots from nodal cultures of *Pueraria tuberosa*

A. Induction of white friable callus on MS + 1.0 mg/l BAP + 0.5 mg/l 2,4-D.
B. Induction of green campact callus on MS +4.0 mg/l BAP+ 1.0 mg/l 2,4- D.
C. Initiation of multiple shoots on MS + 2.0 mg/l BAP + 0.5 mg/l IBA.
D. A shoot with trifoliate compound leaf on MS + 2.5 mg/l BAP + 0.5 mg/l IBA.
E. High frequency of multiple shoots on MS + 4.0 mg/l BAP + 0.5 mg/l IBA.

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