Factors influencing *in vitro* plant regeneration of Liquorice (*Glycyrrhiza glabra* L.)

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Abstract
An efficient and reproducible *in vitro* protocol for large-scale multiplication of Liquorice (*Glycyrrhiza glabra* L.) has been described. Multiple shoots formation was significantly influenced by growth regulators, photoperiod, explant position, season of explant collection and culture passage. Nodal explants were collected at monthly intervals to initiate *in vitro* cultures. The highest bud-break (86.6%) with the longest shoot length (8.0 cm) and maximum number of shoots (3.0) was obtained when middle order nodes (3rd to 5th node from apex) collected between May to August were inoculated on MS medium supplemented with 6-Benzylaminopurine (2.0 mg/l) + α-naphthalene acetic acid (0.5 mg/l) under photoperiod of 16/8 h (light/dark cycle). The induction of multiple shoots was also affected by photoperiod and subculture cycle. Multiple shoots formation increased from the first (2.2) to the fourth subculture (6.6). The *in vitro* regenerated shoots were induced on half strength MS medium enriched with 1.0 mg/l IAA resulting early rooting and maximum root growth. Plantlets were hardened and successfully established in the soil. Concentration of chlorophyll, total sugars, reducing sugars and proteins were estimated in leaf tissues from both *in vivo* and *in vitro* raised plants. Chlorophyll content was higher in *in vivo* plants, whereas other three components were higher in micropropagated plants. The present optimized micropropagation protocol offers the possibility of germplasm conservation and mass cultivation of this important medicinal plant.
Keywords**:** Biochemical analysis; Explanting season; Multiple shoots; Nodal segments; Photoperiod; Subculture time; Liquorice

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INTRODUCTION

Glycyrrhiza glabra L. (Leguminoseae) commonly known as Mulaithi or Liquorice is a small perennial shrub native to the Mediterranean region and central and southwest Asia. The roots and rhizomes of this shrub contain principal active component glycyrrhizin, used commercially as a non-nutritional sweetening and flavoring agent in some candies and pharmaceuticals (Wang *et al*., 2000). Diverse medicinal properties of *G. glabra* for example antidiabetic, anti-inflammatory, antiulcer, antiallergy, anticarcinogenesis, laxative, and antipyretic have attracted entrepreneurs to set eyes on this plant (Wang and Nixon, 2001; Brown, 1995).

This plant is cultivated in Russia, UK, USA, Italy, France, Germany, Spain, China and Northern India (Punjab and Sub-Himalayan tracts). Large scale commercial cultivation is seen in Spain, Sicily and England (Chopra and Chopra, 1958). Liquorice occupies the land for a period of 5 or sometimes 4 years. A yield of two tons of roots per acre for bailing, plus 3-4 centum weight of trimmings or offal is considered satisfactory (Vispute and Khopade, 2011).

The conventional method for propagation of *G. glabra* is via seed. However, poor germination potential restricts its multiplication (Sawaengsak *et al*., 2011). Micropropagation is an effective means for rapid multiplication of species in which conventional

methods have limitations (Sudha and Seemi, 1994). The success of *in vitro* regeneration depends on a series of stages, each with a specific set of requirements. Some of these include physiological and developmental state of the explant, growth regulators, photoperiod and sequential subculture cycles (Yadav and Singh, 2011b; Shou *et al*., 2008).

Although, there are many reports on the *in vitro* propagation of *G. glabra* (Sawaengsak *et al*., 2011; Fu *et al*., 2010; Arya *et al*., 2009; Shams-Ardakani *et al*., 2007; Mousa *et al*., 2006), however, only a few are available on the effect of growth regulators and culture environment on shoot multiplication of this important medicinal plant. So, the present study was undertaken with an objective to establish an efficient protocol on micropropagation by manipulating growth regulators, culture conditions and other external factors influencing *in vitro* multiplication of *G. glabra*.

MATERIAL AND METHODS

Healthy nodal explants (1.0-1.5 cm) were excised from the plants growing in Herbal Garden of Botany Department, Kurukshetra University, Haryana, India. The collections were made in three seasons: January to April, May to August and September to December. Nodal explants were divided into three groups based on their position along the length of the branch, from the apex to the base: distal order (containing 1st and 2nd nodal position), middle order (containing 3rd-6th nodal position) and basal order (containing 7th-10th nodal position). The explants were washed with liquid detergent under running tap water to remove dust particles. The explants were then treated with 0.1% (w/v) mercuric chloride for 3-5 min under aseptic conditions. These explants were then thoroughly washed 4-5 times with sterilized double distilled water to remove the traces of mercuric chloride. The nodal segments after trimming the ends were finally inoculated on MS (Murashige and Skoog, 1962) medium containing 30 g/l sucrose and 8 g/l agar supplemented with various concentrations of 6-Benzyl amino purine (BAP) alone and in combination with α-naphthalene acetic acid (0.5 mg/l). The pH of the medium was adjusted to 5.8 with 1 N NaOH or 1 N HCl prior to autoclaving at 121°C for 20 min.

The cultures were incubated at a temperature of 25±2ºC and a 16-h photoperiod (intensity of 4000 lux). Subsequently, the cultures were maintained by regular subculture at three week intervals on fresh MS medium supplemented with BAP (2.0 mg/l). Effect of different durations of photoperiod, 3 light/dark cycles i.e.,12/10, 16/8 and 20/4 h on the growth and number of *in vitro* produced shoots was also tested. Each experiment consisted of 5 replicates with one explant per culture tube and was repeated thrice. Visual observations like percentage of bud break, number and length of shoots regenerated per explants were recorded after 3 weeks of culture. To establish root induction, the *in vitro* raised shoots (2.5-3.0 cm) from shoot multiplication cultures were excised and transferred to cultural tubes containing half strength MS medium fortified with various concentrations of IAA (0.5-2.0 mg/l). When adequate rooted shoots were obtained, the plantlets were thoroughly washed to remove the adhering agar particles and transferred to pots containing sterilized soil and sand mixture (3:1) for 2 weeks for hardening. The pots were kept in the greenhouse for acclimatization.

Four different biochemical parameters viz. chlorophyll, total sugars, reducing sugars and proteins were estimated from the leaf tissue of *in vivo* and *in vitro* grown plants. The analyses were conducted at Plant Physiology Laboratory, Department of Botany, Kurukshetra University, India. Protein was estimated by the method of Bradford (1976) using coomassie brilliant blue G-250 dye. The total soluble sugar was measured following the method of Hart and Fisher (1971). Amounts of reducing and non reducing sugars were calculated against a standard curve of glucose. The content of chlorophyll was measured according to Arnon (1949). Three replicates were used for each biochemical analysis.

Data were analyzed for significance using ANOVA and the differences contrasted using a Duncan's multiple range test at $P \leq 0.05$. All statistical analyses were performed using the SPSS (version 11.5).

RESULTS

No shoot formation occurred on MS medium without cytokinins. Moreover, the concentration of BAP strongly influenced the number and length of shoots developed per explant (Table 1). In general, with an increase in concentration of BAP, an increase in the number of shoot formation was noted. An experiment was also conducted with BAP (1.0 and 2.0 mg/l) and NAA (0.5 mg/l) in order to determine the optimum concentration of cytokinin and auxin needed for shoot

formation. Of the various concentrations tried, BAP (2.0 mg/l) + NAA (0.5 mg/l) was found to be most effective as this concentration favored early sprouting with maximum length of shoot and number of shoots. Combinations of BAP and NAA gave better response than BAP alone. Addition of NAA (0.5 mg/l) also decreased the number of days required for bud break.

Here the multiple shoots were formed directly with considerable callusing at the basal cut ends of nodal segments.

The highest frequency of bud break (86.6%) with maximum number of shoots per explant (3) was observed when the explants were collected between May to August, due to the presence of active meristematic cells in the fresh sprouts (Table 2). Explants collected between September to December exhibited the lowest bud break (33.3%).

Nodal segments obtained from middle zone showed the highest percentage of bud break with three shoots and performed better. The distal order nodal segments responded bud break with solitary, thin and weak shoot from single axils (Fig. 1 A-B) (Table 3). Basal zone nodal explants had serious contamination problems under the disinfection conditions used (Fig. 1 C). Surface sterilization for longer duration resulted in necrosis of explants.

The *in vitro* produced shoots was also affected by photoperiod (Table 4). Higher multiplication was achieved at 16 h photoperiod. The rate of multiplication was low when photoperiod either increased or decreased.

The axillary shoots (1 cm or longer) regenerated from mother explants were separated and transferred to fresh MS medium supplemented with BAP (2.0 mg/l) after 3 weeks for further shoot regeneration and multiplication. The rate of shoot multiplication

Table 1. Effect of various concentrations of BAP alone and in combination with NAA on shoot initiation from mature nodal explants of *G. glabra* after 30 days of culture.

Auxins/ cytokinins (mg/l)	Plant growth regulators (mg/l)	Bud break (%)	Number of shoots	Shoot Length (cm)	Extent of callusing
MS+BAP	0.5	40	1.16 ± 0.40d	1.68 ± 0.34^{d}	–
	1.0	60	1.44 ± 0.52^{c}	2.8 ± 0.41^{cd}	+
	2.0	73.3	2.09 ± 0.53^{bc}	3.63 ± 0.74^{c}	++
MS+BAP+ NAA	1.0 + 0.5	80	2.16 ± 0.57^{b}	5.75 ± 1.30^{b}	+
	2.0 + 0.5	86.6	3.0 ± 0.81^{a}	8.0 ± 1.87^{a}	++
($P \leq 0.05$) ANOVA ($F_{4,10}$)			0.7269 10.226*	1.60155 18.870*	

(-) No Response, The number of '+' sign donates extent of callusing,Values represent mean ± standard error, n = 15. Mean value followed by different alphabet/s within a column do not differ significantly over one other at P≤0.05 lead by Duncan's Multiple Range Test. *Significant at p≤0.05

Table 2. Influence of explanting period on culture establishment of *G. glabra* on MS medium containing 2.0 mg/l BAP + 0.5 mg/l NAA after 30 days of culture.

Months of collection	Bud break (%)	Number of shoots
January to April	60.0	2.0 ± 0.70^{b}
May to August	86.6	3.0 ± 0.81^{a}
September to December	33.3	1.5 ± 0.57^{b}
($P \leq 0.05$) ANOVA ($F_{2,6}$)		0.81785 13.549*

Values represent mean ± standard error, n = 15. Mean value followed by different alphabet/s within a column do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test. *Significant at p≤0.05

Table 3. Effect of relative position of nodes along the stem length of *G. glabra* on MS medium containing 2.0 mg/l BAP + 0.5 mg/l NAA after 30 days.

Relative position of nodes (from apex) along the stem length	Bud break (%)	Number of shoots
1st -2nd	60.0	1.66 ± 0.70^{b}
3rd -6th	86.6	3.0 ± 0.81^{a}
7th -10th	33.3	1.60 ± 0.54^{b}
($P \leq 0.05$) ANOVA ($F_{2,6}$)		0.7823 15.637*

Values represent mean ± standard error, n = 15. Mean value followed by different alphabet/s within a column do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test. *Significant at p≤0.05

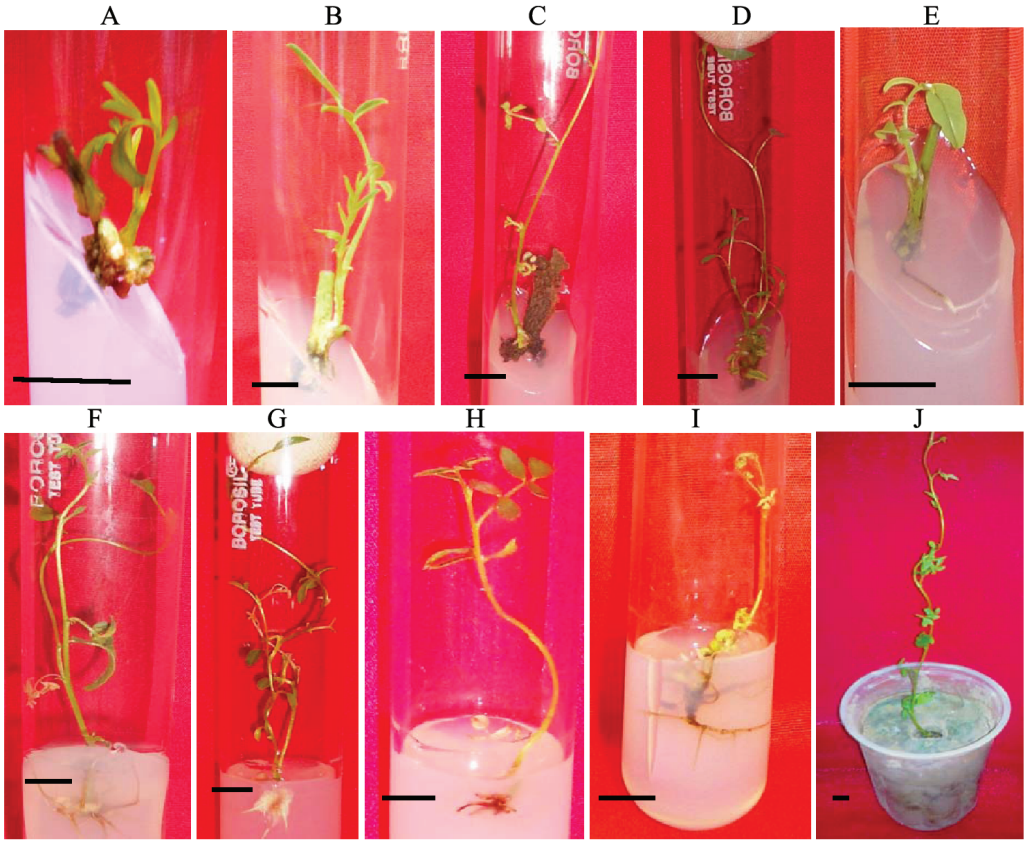

Figure 1. *In vitro* propagation of *G. glabra*: A-C: Initiation of multiple shootlets derived from different nodal position (1st, 4th and 7th respectively) on MS + BAP (2.0 mg/l) + NAA (0.5 mg/l); D: Clump of proliferating shoots obtained in 3rd subculture on MS + BAP (2.0 mg/l); E: root proliferation along with *in vitro* developed shoots from the mother explants collected between August to September on MS + BAP (2.0 mg/1) F: *In vitro* derived plantlet with shootlets and rootlets; G-I: Root formation in shoots harvested from 1st, 2nd and 3rd subculture passage (respectively) of micro shoots cultured on ½ MS + IAA (1.0 mg/l) after three weeks of culture; J: Hardened plants in polycups. *Bar= 1.0 cm and it represents the length of the plant.

Table 4. Effect of different photoperiods on *in vitro* growth of plantlets on MS medium containing 2.0 mg/l BAP + 0.5 mg/l NAA after 30 days.

Photoperiod (light/dark)	Bud break (%)	Number of shoots (Mean±SE)
12/12	60.0	1.55 ± 0.52^{c}
16/8	86.6	3.0 ± 0.81^{a}
20/4	80.0	2.16 ± 0.57^{b}
($P \leq 0.05$)		0.7995
ANOVA ($F_{2,6}$)		8.849*

Values represent mean ± standard error, n = 15. Mean value followed by different alphabet/s within a column do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test. *Significant at p≤0.05.

Table 5. Effect of subculture passages on shoot multiplication of *G. glabra* on MS medium containing 2.0 mg/l BAP (after 4 weeks).

Passages	Bud break (%)	Number of shoots (Mean±SE)
First	86.6	2.23 ± 0.43^{d}
Second	93.3	3.0 ± 0.78^{c}
Third	100	4.06 ± 1.22^{b}
Fourth	100	6.66 ± 1.98^{a}
($P \leq 0.05$)		1.00445
ANOVA ($F_{3,8}$)		35.925*

Values represent mean ± standard error, n = 15. Mean value followed by different alphabet/s within a column do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test. *Significant at p≤0.05.

depended on the number of subculture. The average number of shoots per explant increased with each successive subculture cycles (Fig. 1 D) (Table 5).

G. glabra required a longer time to initiate rooting. Excised shoots taken from first subculture failed to develop roots on both full and half strength MS medium without growth regulators. Amongst different concentrations of IAA (0.5 to 2.0 mg/l) used, the best

Table 6. Root formation on different concentrations of IAA in *G. glabra* after 30 days.

Media composition (mg/l)	Time required for root induction (days)	Rooting (%)	Extent of callusing
MS full strength without growth regulators	–	–	–
MS half strength without growth regulators	–	–	–
MS half strength + 0.5 mg/l IAA	22.08 ± 1.08^{b}	80	+
MS half strength + 1.0 mg/l IAA	16.73 ± 1.09^{a}	100	++
MS half strength + 2.0 mg/l IAA	21.27 ± 1.34^{b}	73.3	+++
LSD ($P \leq 0.05$)	5.73725		
ANOVA ($F_{4,10}$)	0.265*		

(-) No Response, The number of '+' sign donates extent of callusing, ++= swelling and callusing at base of shoot followed by rooting. ++= swelling and extensive callusing. Values represent mean ± standard error, n = 15. Mean value followed by different alphabet/s within a column do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test. *Significant at p≤0.05.

response was obtained with 1.0 mg/l IAA considering all the parameters for rooting, like, number of days required, length of roots and the extent of swelling followed by callusing at the shoot base (Table 6; Fig. 1 F). Though rooting was greater than 70 % with callusing at the shoot base in all the cases, however, at 2.0 mg/l IAA, delayed rooting with few small thick roots and restricted growth developed from a large amount of intervening callusing. However, the rooting response varied with the subculture of shoot multiplication (Fig. 1 G-I). The number of days required for root initiation gradually decreased (14.2 to 7.5) from the 1st passage to the 4th passage of shoot multiplication (Fig. 2). None or slight swelling at the shoot base followed by long roots (about 2.0 cm) was observed from the maximum shoots (>70%) harvested from the third subculture of shoot multiplication.

Irrespective of the rooting treatments, excessive root proliferation along with *in vitro* developed shoots was also observed from middle order (3rd-6th position) mother nodal explants collected between August to September with BAP (2.0 mg/1) as well (Fig. 1 E).

The micropropagated plantlets grew well after acclimatization in potted soil and sand mixture (3:1) under glasshouse conditions (Fig. 1 J). Three different biochemical parameters viz. total sugars, reducing sugars and proteins were found to be higher in content in the leaves of *in vitro* regenerated plants as compared to *in vivo* plants (Table 7). The leaf chlorophyll content recorded in the *in vivo*-grown plants was typically higher than the *in vitro* regenerated plants. This shows that the rate of photosynthesis increased with maturity, the tissue cultured plants requiring further establishment under natural environmental conditions.

Table 7. Comparison of various biochemical contents (mg/100 mg f.wt.) between *in vivo* and *in vitro* regenerated leaves of *G. glabra.*

Biochemical Parameters	Sample 1	Sample 2
Chlorophyll a	0.93 ± 0.13^{b}	1.30 ± 0.30^{a}
Chlorophyll b	0.28 ± 0.01^{b}	0.34 ± 0.02^{a}
Total Sugars	0.16 ± 0.00^{a}	0.09 ± 0.00^{b}
Reducing Sugars	0.05 ± 0.00^{a}	0.03 ± 0.00^{b}
Proteins	0.09 ± 0.01^{a}	0.07 ± 0.00^{b}
($P \leq 0.05$)	0.1086	0.0404
ANOVA ($F_{4,10}$)	108.588*	1322.88*

Sample 1: Leaves sample collected from *in vitro* raised plants. Sample 2: Leaves sample collected from *in vivo* plants. Values represent mean ± standard error, n = 3. Mean value followed by different alphabet/s within a column do not differ significantly over one other at p≤0.05 lead by Duncan's Multiple Range Test. *Significant at p≤0.05.

DISCUSSION

Shoot multiplication is a function of cytokinin activity. BAP was crucial for stimulating explant growth and development (Barless and Skene, 1980). The stimulatory effect of BAP on multiple shoot formation has been reported earlier in other medicinal plants like *Aegle marmelos* (Yadav and Singh, 2011a) and *Spilanthes acmella* (Yadav and Singh, 2011b). As in

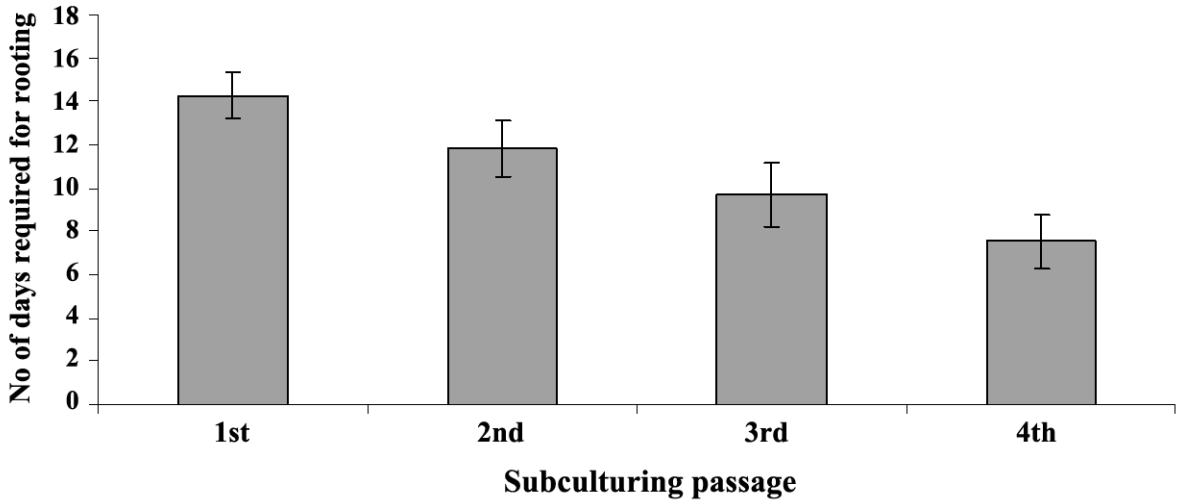

Figure 2. Effect of subculture passage on number of days required for rooting from multiplied shoots.

the present study, Lal *et al*. (2010) also noted the synergistic effect of BAP in combination with an auxin for efficient shoot regeneration.

Formation of multiple shoots along with considerable callusing at the basal cut ends of nodal segments was also reported in *Azadirachta indica* (Arora *et al*., 2010). It may be due to the action of accumulated auxin at the basal cut ends, which stimulates cell proliferation, especially in the presence of cytokinins (Marks and Simpson, 1994).

There is increasing evidence that seasonal differences influence the regulation of cell cycle and therefore affect the morphogenetic processes (Anderson *et al*., 2001). Similar results regarding the dominance of seasonal effect on the establishment of aseptic cultures were obtained by Rani and Rana (2010).

The response of distal order nodal segments with solitary, thin and weak shoot may be due to the inhibitory influence of unknown factors or others released from the shoot tip and the difference between the physiological states of the various nodes along the length of the branch (Gangaprasad *et al*., 2005). The use of nodes in preference of shoot tips is reported in other species such as *Azadirachta indica* (Arora *et al*., 2010) and *Aegle marmelos* (Yadav and Singh, 2011a).

The effects of photoperiod on growth and multiplication of *in vitro* produced shoots were also reported by Senapati and Rout (2008). Enhanced shoot multiplication in subsequent culture was also reported earlier by Martin (2002).

IAA has also been reported suitable for rooting in several medicinal plants, such as *Momordica cymbalaria* (Nikam *et al*., 2009), *Celastrus paniculatus* (Lal *et al*., 2010) and *Aegle marmelos* (Yadav and Singh, 2011a).The success of repeated sub-culturing in promoting efficient rooting has also been reported by Sha Valli Khan *et al*. (1999) and Prakash *et al*. (2006). Repeated sub-culturing may change the physiological state and gradually rejuvenate the shoots promoting better rooting (Ecnomou and Read, 1986). Similar to our results, root initiation along with multiple shoot formation from mature nodal explants was reported in *Spilanthes acmella* (Yadav and Singh, 2012).

The increase in the content of various biochemical parameters in the leaves of *in vitro* regenerated plants may be due to the effect of different phytohormones in *in vitro* raised plants (Mohapatra *et al*., 2008). Yadav and Singh (2011a) also observed significant difference in the chlorophyll content in the leaves of *in vitro* regenerated and natural plants.

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References

Anderson JV, Chao WS, Horvath DP (2001). A current review on the regulation of dormancy in vegetative buds. *Weed Sci*. 49: 581-589.

Arnon DI (1949). Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*. *24*: 1-15.

Arora K, Sharma M, Srivastava J, Ranade SA, Sharma AK (2010). Rapid *in vitro* cloning of a 40-year-old tree of *Azadirachta indica* A. Juss. (Neem) employing nodal stem segments. *Agroforest Syst*. 78: 53-63.

Arya S, Rathi N, Arya ID (2009). Micropropagation protocol for *Glycyrrhiza glabra* L. *Phytomorphol*. 59: 71-76.

Barless M, Skene KGM (1980). Studies of the fragmental apex of grapevine I. The regeneration capacity of leaf primordial fragments *in vitro*. *J Expt Bot*. 31: 483-488.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Annal Biochem*. 72: 248-254.

Brown D (1995). *The herb national society of America encyclopedia of herbs and their uses*. Dorling Kindersley Publishing Inc. New York. PP.135

Chopra RN, Chopra IC (1958). Indigenous drugs of India. 2nd edition. Kolkata: Academic Publishers; PP.183-187.

Ecnomou AS, Read PE (1986). Microcutting production from sequential reculturing of hardy deciduous azalea shoot tips. *Hort Sci*. 21: 137-139.

Fu C, Lei C, Gan L, Li M, Yang Y, Yu L (2010). Optimization of embryogenic-callus induction and embryogenesis of *Glycyrrhiza glabra*. *Afr J Biotechnol*. 9: 5823-5829.

Gangaprasad A, Decruse SW, Seeni S, Nair GM (2005). Micropropagation of ecorestoration of *Decalepis arayalpathra* (Joseph and Chandra) Venter–An endemic and endangered ethnomedicinal plant of Western Ghats. *Ind J Biotechnol*. 4: 265-270.

Hart FL, Fisher HJ (1971). Modern food analysis. Springer, New York.

Lal D, Singh N, Yadav K (2010). *In vitro* studies on *Celastrus paniculatus*. *J Trop Med Plant*. 11: 169-174.

Marks TR, Simpson SE (1994). Factors affecting shoot development in apically dominant *Acer* cultivars *in vitro*. *J Hort Sci*. 69: 543-551.

Mohapatra H, Barik DP, Rath SP (2008). *In vitro* regeneration of medicinal plant *Centella asiatica. Biologia Plantarum*. 52: 339-342.

Mousa N, Siaguru P, Wiryowidagdo S, Wagih ME (2006). Rapid clonal propagation of licorice *(Glycyrrhiza glabra)* by *in vitro* shoot culture. *Sugar Tech*. 8: 292-298.

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

Nikam TD, Ghane SG, Nehul JN, Barmukh RB (2009). Induction of morphogenic callus and multiple shoot regeneration in *Momordica cymbalaria* Fenzl. *Ind J Biotechnol*. 8: 442-447.

Prakash E, Sha Valli Khan PS, Sreenivasa Rao TJV, Meru ES (2006). Micropropagation of red sanders (*Pterocarpus santalinus* L.) using mature nodal explants. *J Forestry Res*. 11: 329-335.

Rani S, Rana JS (2010). *In vitro* Propagation of *Tylophora indica*-influence of explanting season, growth regulator synergy, culture passage and planting substrate. *J Am Sci*. 6: 385-392.

Sawaengsak W, Saisavoey T, Chuntaratin P, Karnchanatat A (2011). Micropropagation of the medicinal herb *Glycyrrhiza glabra* L., through shoot tip explant culture and glycyrrhizin detection. *Int Res J Plant Sci*. 2: 129-136.

Senapati SK, Rout GR (2008). Study of culture conditions for improved micropropagation of hybrid rose. *Hort Sci*. 35: 27-34.

Sha Valli Khan PS, Prakash E, Rao KR (1997). *In vitro* micropropagation of an endemic fruit tree of *Syzygium alternifolium* (Wight) Walp. *Plant Cell Rep*. 16: 325-328.

Shams-Ardakani M, Mohagheghzadeh A, Ghannadi A, Barati A (2007). Formation of glycyrrhizin by *in vitro* cultures of *Glycyrrhiza glabra*. *Chem Nat Comp*. 43: 353-354.

Sudha CG, Seeni S (1994). *In vitro* multiplication and field establishment of *Adhatoda beddomei* C.B. Clarke, a rare medicinal plant. *Plant Cell Rep*. 13: 203-207.

Vispute S, Khopade A (2011). *Glycyrrhiza glabra* Linn. - "Klitaka": a review. *Int J Pharma Bio Sci*. 2: 42-50.

Wang ZY, Nixon DW (2001). Licorice and cancer. *Nutr Cancer*. 39: 1-11.

Wang ZY, Athar M, Bickers DR (2000). Licorice in foods and herbal drugs: Chemistry, pharmacology, toxicology and uses. In Herbs, Botanicals & Teas, Mezza G, Oomah BD (eds). Technomic Publishing Co. Inc: Lancaster, PP. 321-353.

Yadav K, Singh N (2012). Rapid plant regeneration from nodal explants of *Spilanthes acmella* (L.) Murr. –an endangered medicinal plant. *Analele Universităţii din Oradea – Fascicula Biologie*. 19: 35-39.

Yadav K, Singh N (2011a). *In vitro* propagation and biochemical analysis of field established wood apple (*Aegle marmelos* L.) *Analele*. *Universităţii din Oradea – Fascicula Biologie*. 18: 23-28.

Yadav K, Singh N (2011b). *In vitro* flowering of shoots regenerated from cultured nodal explants of *Spilanthes acmella* Murr. - An ornamental cum medicinal herb. *Analele Universităţii din Oradea-Fascicula Biologie*. 18: 60-64.