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Plantlet Regeneration through Indirect Organogenesis of Flame Gold Tree (*Koelreuteria elegans* Laxm.)

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Abstract

Koelreuteria elegans, popularly known as "Flame Gold" is an ornamental tree. *In vitro* callus induction and regeneration from various explants (eaf segments and cotyledonary leaf) were studied on modified MS medium. The highest callus induction rate (80%) and multiplication was obtained in 2 mg/l 2,4-D from leaf segments. Calli transferred in 1.5 mg/l BAP resulted in efficient shoot regeneration (70%) and development (4.35 shoots). MS half strength medium supplemented with 0.2 mg/l NAA reported 80% rooting after 21 days of implantation. Mostly, the roots were long and healthy. Plants were successfully transferred in sterilized mixture of vermiculite: soil: sand (3:1:1) with 65% survival rate under field conditions. The *in vitro* regenerated plantlets were hardened and acclimatized successfully.

Keywords: Auxins, Cytokinins, *Koelreuteria elegans*, Ornamental tree.

Abbreviations: BAP- 6-Benzylaminopurine, Kn- Kinetin, NAA- α-Naphthalene Acetic Acid, 2,4-D- 2,4-Dichlorophenoxyacetic acid.

INTRODUCTION

Koelreuteria elegans Laxm. (Sapindaceae) popularly known as "Flame gold" is a fast growing medium-sized evergreen ornamental tree, capable of reaching up to 25 m in height having bipinnate compound leaves with relatively small leaflets and bright yellow flowers (Anonymous, 2003). The tree is native to Taiwan and is locally naturalised in the subtropical, tropical and warmer temperate regions of Australia, south-eastern USA, Hawaii and Guam (Meyer, 1976). The tree is often recommended for arboretum, parking lots, plantings along the highway, shade tree, residential street tree in urban areas (Gilman and Watson, 1993).

Biotechnology has emerged as a strong tool in mass multiplication and improvement of all plant species. Clonal multiplication is production of true of type plants in large number, in short period of time. It offers a method to increase valuable genotype rapidly and expedite release of large number of plantlets. Biotechnology involving modern tissue culture, cell biology and molecular biology offers an opportunity to develop new germplasm that are well adapted to changing demands (Yadav *et al.*, 2013). Plant tissue culture facilitates the accomplishment of a large number of uniform plants irrespective of season and serves as an alternative source of seed materials. I*n vitro* preservation of germplasm is also a safe method in protecting the species by reducing the risk of natural vagaries (Yadav and Singh, 2012).

Many ornamental plants like *Euphorbia pulcherrima* (Osternack *et al.*, 1999), *Ficus religiosa* (Nagaraju *et al.*, 1998), *Saintpaulia ionantha* (Mithila *et al.,* 2003) *Rosa hybrid* (Van der Salm *et al.*, 1996), (Atta-Alla and Van Staden, 1997) have been successfully propagated under *in vitro* conditions using various concentrations of different plant growth regulators.

Till now, there is no report of *in vitro* propagation of this species. The aim of this work was to achieve mass multiplication of *Koelreuteria elegans* under *in vitro* conditions.

MATERIALS AND METHODS

Plant Material

The seeds of this plant were collected from a mature tree growing in Herbal Garden of Botany Department, Kurukshetra University, Kurukshetra, India. Seeds were initially washed under running tap water with liquid detergent and sterilized with freshly prepared 0.1% (w/v) mercuric chloride solution for 6-7 minutes under aseptic conditions. After this, the seeds were rinsed 4-5 times thoroughly with sterilized double distilled water to remove any traces of mercuric chloride. Then, seed were inoculated on MS medium (Murashige and Skoog, 1962).

Medium preparation and Culture conditions

MS medium containing 30 g/l sucrose and solidified with 8 mg/l agar with and without growth regulators (Table 1 and 2) was prepared. The pH of media was adjusted to 5.8 with 1 N NaOH or 1 N HCl.

Table 1. Effect of 2,4-D on callus induction on the explants of *Koelreuteria elegans*.

Concentration of 2,4-D in MS media (mg/l)	Explant	Average number of days required for callus induction	% Response/ callus induction	Visual growth of callus	Color and texture of callus
0.5	Leaf	19.09^{de}	55	++	Light Yellow, Fragile
	Cotyledonary leaf	21.00^{e}	30	+	Light Yellow, Fragile
1.0	Leaf	16.23^{bc}	65	++	Light Yellow, Fragile
	Cotyledonary leaf	18.14^{cd}	35	+	Light Yellow, Fragile
1.5	Leaf	15.13^{ab}	75	+++	Light Yellow, Fragile
	Cotyledonary leaf	17.55^{cd}	45	+	Light Yellow, Fragile
2.0	Leaf	13.12^{a}	80	+++++	Light Yellow, Fragile
	Cotyledonary leaf	14.90^{ab}	55	++	Light Yellow, Fragile

Data based on 20 explants per treatment on fourth week of culture. (–) No Response, (+) Poor growth, (++) Moderate growth, (+++) Good growth.

Table 2. Effect of cytokinins on shoot regeneration from calli of *Koelreuteria elegans.*

Cytokinins (mg/l)	Callus source	Visual growth of callus	Calli forming shoots (%)	No. of shoots per culture	Shoot Length
BAP 0.5	LC	+	50	2.10^{cd}	1.58^{bcd}
BAP 1.0	LC	+	60	3.16^{abc}	2.11^{bc}
BAP 1.5	LC	++	70	4.35^{a}	3.17^{a}
BAP 2.0	LC	++	65	3.76^{ab}	2.56^{ab}
BAP 0.5	CC	+	55	1.72^{cd}	0.69^{d}
BAP 1.0	CC	+	55	2.09^{cd}	0.92^{d}
BAP 1.5	CC	++	60	2.16^{cd}	1.29^{cd}
BAP 2.0	CC	++	60	2.38^{bcd}	1.70^{bcd}
Kinetin 0.5	LC	+	20	1.00^{d}	0.85^{d}
Kinetin 1.0	LC	+	20	1.25^{d}	1.17^{cd}
Kinetin 1.5	LC	++	45	1.77^{cd}	1.54^{bcd}
Kinetin 2.0	LC	+++	60	2.50^{bcd}	2.25^{abc}
Kinetin 0.5	CC	+	10	1.00^{d}	0.60^{d}
Kinetin 1.0	CC	+	10	1.50^{d}	0.80^{d}
Kinetin 1.5	CC	++	20	1.50^{d}	1.22^{cd}
Kinetin 2.0	CC	+++	45	1.77^{cd}	1.45^{bcd}

*Data based on 20 explants per treatment on 28th day of culture. (–) No Response, (+) Poor growth, (++) Moderate growth, (+++) Good growth. LC – Callus from Leaf, CC – Callus from Cotyledons.

10 ml of medium was poured into each glass culture tubes and sterilized by autoclaving at 1.05 kg/cm^2; 121 °C for 18 min. Cultures were maintained at 25±2ºC temperature in 16/8 h light/dark photoperiod of 20 µmol m^{-2}s^{-1} photon flux intensity produced from cool white 40 watt tube lights.

Callus induction

Leaves and cotyledonary leaves from *in vitro* grown seedlings (25 days old) were excised and cut into small segments and inoculated on MS medium fortified with different concentrations of 2,4-D (0.5, 1.0, 1.5, 2.0 mg/l) for callus induction. Subculturing was done after every four weeks for maintenance and increasing the amount of calli. Black, brown or dead calli was discarded during sub culturing. Visual observations like per cent callus induction, growth of callus, colour and texture of callus was recorded.

Shoot and root induction

The fourth subcultured calli was transferred to MS medium supplemented with various concentration (0.5, 1.0, 1.5, 2.0 mg/l) of cytokinins (BAP and Kn) (Table 2) for shoot regeneration. Observations like per cent shoot regeneration and average number of days required for regeneration was recorded. The *in vitro* developed shoots (1-3 cm) were excised and implanted in culture tubes containing half strength MS medium (Murashige and Skoog, 1962) fortified without or with NAA (0.2, 0.5, 0.1 mg/l) under aseptic conditions for root initiation. After development of sufficient roots, the plantlets were gradually removed and transferred to polycups containing sterilized mixture of vermiculite: soil: sand (3:1:1) maintained under high humidity.

Table 3. Effect of different concentrations of NAA on root development.

MS half strength without growth regulators	60	28.05c	Thin
MS half strength +0.2 mg/l NAA	80	21.00a	Long, Healthy
MS half strength +0.5 mg/l NAA	40	22.95b	Short, Callus formation
MS half strength +1.0 mg/l NAA	-	--	Profuse Callus, formation

*Data based on 20 explants per treatment. (–) No sustainable rooting.

Data analysis

% response = (No. of explants with response/Total no. of explants cultured) × 100

% calli forming shoots = (No. of calli producing shoots/Total no. of calli cultured for shooting) × 100

% of culture forming roots = (No. of shoots producing roots/ Total no. of shoots inoculated for rooting) × 100

Statistical analysis

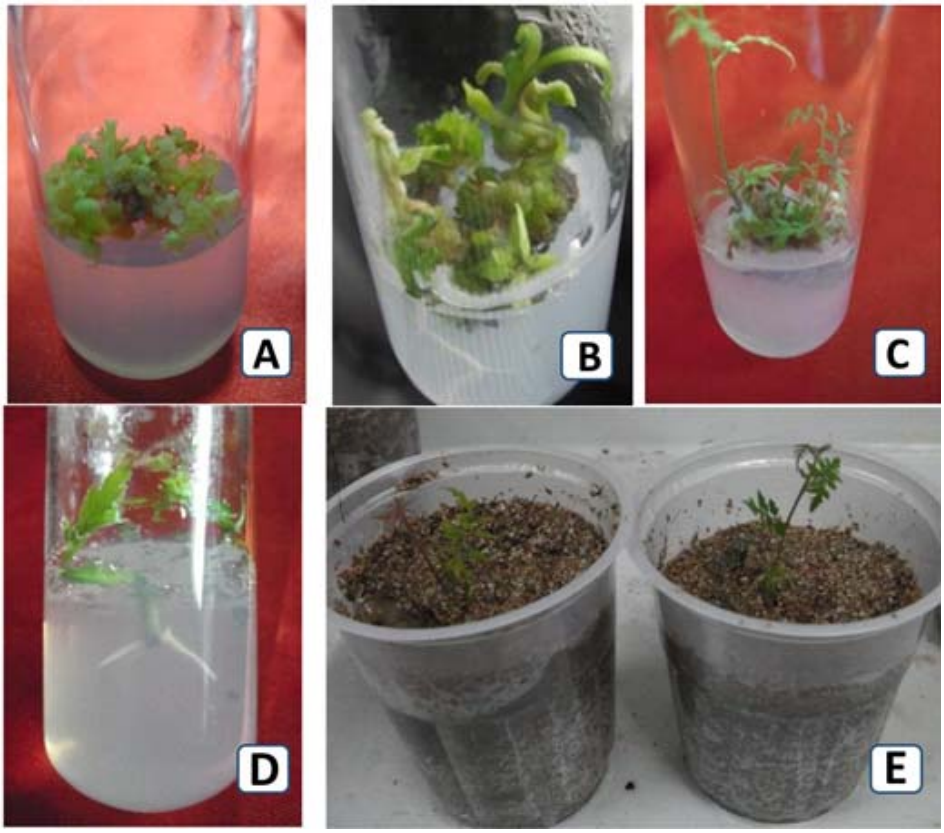
All the experiment were conducted with a minimum of 20 replicates per treatment. The data was analyzed statistically using (SPSS) one-way analysis of variance (ANOVA) and the differences contrasted using a Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Micropropagation offers a viable alternative for conventional methods because it can also be used as a complimentary strategy for conservation and utilization of genetic resources. Further, *in vitro* plant regeneration is an easy and economic way for obtaining a large number of consistently uniform and true- to- type plants within a short span of time (Yadav *et al.*, 2014).

All the explants (leaf segments and cotyledonary leaf) induced calli in MS media supplemented with different concentration of 2,4-D (0.5, 1.0, 1.5, 2.0 mg/l) (Table 1). Better growth was observed in media containing 2 mg/l 2,4-D. This concentration was efficient in term of less number of days required for callus induction and per cent response. Callus induction started at the cut ends

Fig. 1. Different stages of micropropagation of *Koelreuteria elegans* from callus formation to acclimatization.



(A) Undifferent mass of callus
(B) Shoots regenerated from callus in 1.5 mg/1 BAP
(C) Shoots
(D) Rooting of excised shoots in rooting medium supplemented with 0.2 mg/1 NAA
(E) Plants after one week of transfer in acclimatizing mixture Vermiclite: Soil: Sand (3:1:1)

of the explants, which later involved the whole surface. Leaf segments were proved to be the better explants for callus induction, multiplication and showed best percent response (80%) with less average number of days required for induction (13.12) as compared to cotyledonary leaf (Table 1, Fig.1 A). The supremacy of 2,4-D in callus induction has also been reported in *Momordica charantia* (Agrawal and Kamal, 2004); *Spilanthes acmella* (Yadav and Singh, 2010); *Aegle marmelos* (Yadav and Singh, 2011); and *Cissus quadrangularis* (Teware *et al.*, 2012).

Calli derived from leaf segments were better in terms of regeneration of shoots on media fortified with cytokinins (BAP and Kn). BAP was found to be more suitable than Kn for shoot regeneration. Highest per cent response (70%) of calli forming shoots, maximum number of shoots (4.35) with highest shoot length (3.17 cm) were observed in media supplemented 1.5 mg/l of BAP (Table 2, Fig.1 B and C) from leaf derived calli. Any deviation from this concentration resulted in the decreased response. In case of Kn, maximum per cent response was recorded in medium supplemented with 2 mg/l in both the explants derived calli. Shoot regeneration using BAP or Kn has been observed in *Momordica charantia* (Agarwal and Kamal, 2004); *Ipomoea batatas* (Getu and Feyissa, 2013); *Aconitum violaceum* (Rawat *et al.*, 2013).

The shoot regenerated from calli were excised aseptically and implanted on half strength MS media fortified with NAA (0.2 - 1.0 mg/l) (Fig. C; Table 3). Long, healthy roots were observed in 80% of shoots on half strength MS medium supplemented with 0.2 mg/l NAA after 21 days of implantation (Fig. D; Table 3). Further increase in concentration of NAA (0.5, 1.0 mg/l) decreased the per cent root formation. The use of NAA in enhancing the root formation has also been observed in *Eucalyptus grandis* (Sita and Rani, 1985); *Celastrus paniculatus* (Lal *et al.,* 2010); Kinnow (Sharma *et al.,* 2012) and *Hippeastrum johnsonii*, (Zakizadeh *et al.*, 2013).

After successful production of sufficient roots, the plantlets were gently taken out from rooting medium and washed carefully with a soft brush in sterilized water to remove the adhering agar-agar with plant tissue. The plants were transferred in the sterilized mixture of vermiculite: soil: sand (3:1:1) in polythene cups (Fig. E). Each of the transferred plants was covered with a polythene bag to maintain high humidity and check morality due to dehydration. Each plant was irrigated with ¼ strength of MS salt solution on every second day. After two weeks, the covering of polythene bags was removed for 2-3 hours daily to acclimatize the plants to the natural environment. After about 4-5 weeks of transfer, the plantlets were transferred in field conditions of natural photoperiod and temperature. Sixty five percent of the plants survived after acclimatization. Successful acclimatization and field transfer of the *in vitro* regenerated plantlets have also been reported in *Hildegardia populifolia* (Lavanya *et al.*, 2012); *Salvadora persica* (Kumari and Singh, 2012) and *Gloriosa superba* (Yadav *et al.*, 2013).

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Litrature Cited

Agarwal M., and Kamal, R. 2004. *In vitro* colonal propagation of *Momordica charantia* L. Indian Journal of Biotechnology, 3: 426-430.

Atta-Alla, H. and Van Staden, J. 1997. Micropropagation and establishment of *Yucca aloifolia*. Plant Cell Tissue Organ Culture;48(3):209-212.

Feng, D.L., Zhang, J., Liu, X., Peng, W.X. and Wu, T.Y. 2009. *In vitro* calture of immature embryo from *Koelreuteria bipinnata* var 'integrifolia'. Foresty Studies in China, 11(3): 179-184.

Getu, T., and Feyissa, T. 2013. *In vitro* regeneration of sweet potato (*Ipomoea batatas* (L.) Lam.) Convolvulaceae, from leaf and petiole explants. Ethiopian Journal of Biological Sciences, 11: 147-162.

Gilman, E.F., and Watson, D. G. 1993. *Koelreuteria elegans* Flame Gold. U.S. Forest Service Fact Sheet ST-337. Available http://hort.ifas.ufl.edu/database/documents/pdf/tree_fact_sheets/koeelea.pdf (viewed 8-28-2014).

Kumari, S., and Singh, N. 2012. *In vitro* plantlet regeneration from cotyledonary node explants of *Salvadora persica* L. a medicinally important desert tree. Journal of Agricultural Technology, 8: 1839-1854.

Lal, D., and Singh, N., Yadav K. 2010. *In vitro* studies on *Celastrus paniculatus.* Journal of Tropical Medicinal Plants, 11: 169-174.

Lavanya, A.R., Muthukrishnan, S., Kumaresan, V., Franklin Benjamin, J.H., and Rao, M.V. 2012. *In vitro* micropropagation of *Hildegardia populifolia* (Roxb.) Schott & Endl., an endangered tree species from Eastern Ghats of Tamil Nadu. Indian Journal of Agricultural Technology, 8: 1727-1744.

Mithila, J., Hall, J., Victor, J.M.R. and Saxena, P.K. 2003. Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl.). Plant Cell Reports;21(5):408-414.

Meyer, F. G. 1976. A revision of the genus *Koelreuteria* (Sapindaceae). Journal of the Arnold Arboretum, 57: 129–166.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum, 15: 473-495.

Nagaraju, S., Reddy, S.K. and Farook, S.A. 1998. Propagation of *Ficus reliosa* L. from maxillary buds and shoot tips. Adv Plant Science;11(2):287-290.

Osternack, N., Saare-Surminski, K., Preil, W. and Lieberei, R. 1999. Induction of somatic embryos, adventitious shoots and roots in hypocotylstissue of *Euphorbia pulcherrima* Willd. Ex Klotzsch: comparative studies on embryogenic and organogenic competence. Journal of Applied Botany; 73:197-201.

Rawat, J. M., Rawat, B., Chandra, A., and Nautiyal, S. 2013. Influence of plant growth regulators on indirect shoot organogenesis and secondary metabolite production in *Aconitum violaceum* Jacq. African Journal of Biotechnology, 12: 6287-6293.

Sharma, T., Khan, M. K., Misra, P., and Shukla, P. K. 2012. Micropropagation of kinnow through nodal explants. The Biosean, 7: 295-297.

Sita, G. L., and Rani, B. S. 1985. *In vitro* propagation of *Eucalyptus grandis* L. by tissue culture. Plant Cell Reports, 4: 63-65.

Teware, K., Singh, P., and Mehta, R. 2012. *In vitro* callus induction from stem explants of *Cissus quadrangularis* L. (Hadjod). International Journal of Ayurvedic and Herbal Medicine 2: 135-138.

Van der Salm, T.P.M., Van der Toorn, C.J.G., Hanisch-ten Cate, C.H. and Dons, H.J.M. 1996. Somatic embryogenesis and shoot regeneration from excised adventitious roots of the root stock *Rosa hybrida* cv. 'Money Way'. Plant Cell Reports;15:522-526.

Yadav, K., and Singh, N. 2010. Micropropagation of *Spilanthes acmella* Murr. an important medicinal plant. Nature and Science, 8: 5-11.

Yadav, K., and Singh, N. 2011. *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., Aggarwal, A. and Singh, N. 2013. Evaluation of genetic fidelity among micropropagated plants of *Gloriosa superba* L. using DNA-based markers—a potential medicinal plant. Fitoterapia, 89:265-270.

Zakizadeh, S., Kaviani, B., and Onsinejad, R. 2013. *In vitro* rooting of amaryllis (*Hippeastrum johnsonii*), a bulbous plant, via NAA and 2-iP. Annals of Biological Research, 4: 69-71.