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In-Vitro Regeneration of Interspecific F1 Hybrid (*Eucalyptus citriodora* and *Eucalyptus torelliana*) of Eucalyptus

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Abstract: Tissue culture technique was standardized for *in vitro* shoot multiplication, using nodal segments of 25-30 years old trees i.e., promising interspecific F1 hybrid of Eucalyptus (*Eucalyptus citriod*ora and *Eucalyptus torelliana*). Due to the phenolic exudation explants did not survive and eventually died without regenerating buds. The explants collected from July to September were the best for *in vitro* studies for micropropagation. The axillary buds were surface sterilized with 0.1% Mercuric chloride solution for 10-15 minutes, followed by 0.1% Bavistin treatment for 1 minute and subsequently washed 3-4 times with sterilized distilled water. These surface sterilized axillary buds were cultured on MS medium supplemented with cytokinin and auxin. MS medium supplemented with 1.5mg/l BAP + 0.1mg/l NAA proved to be the best hormonal combination for induction of axillary bud which resulted in the development of 1-3 axillary shoots. The proliferated shoots were cultured on MS medium with different concentration of BAP (0.1 – 3.0 mg/l) alone or in combination with NAA (0.1-1.5mg/l) and supplemented with sucrose at 3%. The aim of the paper was to obtain maximum rate of in vitro regeneration of FRI-15 by using micropropagation technique.

Keywords: *Eucalyptus* F_1 hybrids • Phenolic exudation • Axillary bud proliferation • In vitro shoot multiplication • MS medium

Introduction

Tissue culture, a major aspect of biotechnology, has a lot of potential for rapid mass multiplication and large-scale clonal propagation in plant. The Forest Research Institute, Dehradun has generated promising interspecific F_1 hybrids of Eucalyptus that have shown a hybrid vigour as compared to parents' plants species.There are thirteen mature F1 Eucalyptus hybrid pairings at the Forest Research Institute in Dehradun. Work on Eucalyptus hybridization was started in the 1970s at the Forest Research Institute in Dehradun (Venkatesh and Sharma, 1977c). Studies were started to create controlled and natural hybrids using half-sibling progeny generated from seeds gathered from stands of two inter crossable species growing close to one another based on cross-ability pattern. The hybrids are created mechanically or naturally through selection in the Dehradun campus of the Forest Research Institute. Arya et al., 2009worked on two importantinterspecificF1 hybrids, namely FRI-5(*Eucalyptus camaldulensis* Dehn *x E. tereticornis* Sm) and FRI-14(*Eucalyptus torelliana* F.V.Muell X *E. citriodora* Hook), for investigation from among these several interspecific hybrids that were created.

FRI-15 hybrid is one of the important hybrids of Eucalyptus (*Eucalyptus citriod*ora and *Eucalyptus torelliana*). FRI-15 hybrid combines commercially desirable traits like better quantity and quality of citrionella (used in perfumery

industries) and dense crown, better site control properties similar to those of *E. torellianaand* resistance to fungus was observed.The earlier published reports revealed that this hybrid had a very high degree of hybrid vigour, having three to five times superior growth than the parent species (Kapoor and Sharma, 1984; Dasand Mitra, 1990;Arya et al., 2009).Promising interspecific F_1 hybrids *(Eucalyptus citriod*ora and *Eucalyptus torelliana)* of Eucalyptus developed in India by Forest Research Institute, Dehradun has displayed a very high degree ofhybridvigour (Dasand Mitra, 1990;Arya et al., 2009; Brondani et al., 2012).

Material And Methods

Collection of Explants

Explants were collected from mature trees of 25-30 years old.These mother plants are growing at Forest Research Institute, Dehradun.Nodal segments were used as source material for micropropagation.

Sterilization and Inoculation of Explants

Nodal segments measuring 2.0 to 2.5 cm were cut and washed in with Cetrimide (ICI ltd. India) solution for 5 min and thereafter surface sterilized with 0.1% Mercuric chloride solution (10-15 min) followed by repeated washing with sterilized distilled water to remove traces of contamination. Other sterilant like $NaOCl_2$ (4%) and H_20_2 (20%) were also tested for sterilization of nodal segments. The surface sterilized axillary buds were cultured on semi-solid Murashige and Skoog's (MS) medium supplemented with cytokinin (BAP and Kinetin). The pH of the medium was adjusted to 5.8 prior to autoclaving the medium at 121° C for 15 min. Cultures were maintained at 25 ± 2 ° C with 16 hrsillumination with the photonflux density of2500 lux, from white fluorescent tubes. (Bisht et al., 2002; Joshi *et al*., 2003; Sowmya et al., 2019)

Statistical Analysis

All experiments were repeated thrice.Each treatment consists of 12 replicates.The data representing means of three experiments were analyzed with the help / use of statistical packages viz. ExcelVer 2.0 and GenStat Ver 8.0 for data of a completely randomized design. The data recorded for various parameters during the study were subjected to one and two way analysis of variance (ANOVA).The significance of the data was ascertained by F-test and the critical difference (C.D.) values at 5% computed,for comparing different means of various treatments.

Results And Discussion

Axillary budproliferation

Axillary bud break response in mature F1 hybrid of FRI-15 (*E. citriodora* X *E. torelliana*) was difficult to obtainas most of the explants did not respond and they became black and died. Nodal segments inoculated in hormone free medium do not yield bud break response and produced callus from the basal region of the explants. A maximum bud break response of 66.6% was obtained in MS medium supplemented with 1.5mg/l BAP + 1.0mg/l NAA (Table 1).This formulation was considered to be the optimal condition for axillary bud break in FRI-15 (*E. citriodora* X *E. torelliana*)shown in Fig 1.

Fig-1: In vitro shoot multiplication of F1 Hybrid (FRI-15)

Table-1: Effect of combination of BAP+NAA on axillary bud induction in MS medium using nodal segments. Data recorded after 5 weeks.

Hormonal concentration (mg/l) BAP+NAA	No. Of replicates inoculated	Response %
0.5+1.0	24	12.5 ± 0.12
0.5+1.5	24	8.3 ± 0.06
1.0+0.1	24	25.0 ± 0.06
1.0+0.5	24	33.3 ± 0.17
1.0+1.0	24	37.6 ± 0.06
1.0+1.5	24	58.0 ± 0.29
1.5+0.1	24	33.3 ± 0.12
1.5+0.5	24	25 ± 0.17
1.5+1.0	24	66.6 ± 0.06
1.5+1.5	24	37.6 ± 0.29
Significance		***
CD		**0.37**

*NS- Non-Significant *- Significance at 5% **- Significance at 1% ***-Significance at 0.1% ± Values represent the Standard deviation*

Table-2: Effect of months on axillary bud break and phenolic exudation using nodal segments in MS medium supplemented with BAP along with NAA. Data recorded after 5 weeks.

Months	Initial No. of replicates inoculated	Response%	Phenolics Exudation
January	24	12.50±0.29	+++
February	24	29.16±0.09	++
March	24	29.16±0.09	++
April	24	20.87±0.23	+++
May	24	8.30±0.12	+++
June	24	12.50±0.04	+++
July	24	33.30±0.09	++
August	24	54.16±0.09	+
September	24	62.30±0.09	+
October	24	33.30±0.02	++
November	24	8.30±0.06	+++
December	24	4.16±0.03	+++
Significance		***	
CD		**0.388**	

*NS – Non-Significant *- Significance at 5% **- Significance at 1% , ***-Significance at 0.1%, ± Values represent the Standard deviation, + low, ++ moderate and +++ high phenolic exudation*

Table -3: Effect of Cytokinin (BAP) on *in vitro* shoot multiplication. MS mediumused. Data recorded after 5 weeks.

Hormonal Concentration BAP (mg/l)	Average no. of shoots developed	Multiplication rate	Average no. of shoots length (cm)
0.1	10.6 ±0.92	2.12 ±0.17	1.15 0.08
0.5	42.91± 2.53	8.58 ±0.23	2.38 0.11
1.0	52.58 ±1.68	10.51± 0.31	3.21 0.06
1.5	41.6 ±71.82	8.33 ±0.23	2.90 0.06
2.0	33.58± 1.43	6.72 ±0.41	2.20 0.06
2.5	26.75± 1.74	5.35± 0.55	1.30 0.08
3.0	25.08 ±1.60	5.02± 0.55	0.8 0.09
Significance	***	***	***
CD	**4.84**	**0.40**	**0.22**

NS – Non - Significant **- Significance at 5% **- Significance at 1% , ***-Significance at 0.1%*

Effect of Phenolics on *in vitro* axillary bud proliferation

Leaching out of phenolics from the cultured explants (nodal segments) was the main concern which prevented establishment of *in vitro* cultures to a large extent.Nodal segments cultured on MS medium exudated phenolics (after 2 to 3 weeks of subculturing).The quantity of phenolics exudation by nodal segments and bud break response varied with the month starting from January to December.It was found that explants collected during February to April and July to September were the best for *in vitro* studies as they showed least phenolic exudation and better bud break response than other months (Kamal and Jadon,2015).In October to January and from May to June the phenolic exudation was high accompanied by poor bud break responses (Table 2).The maximum axillary bud response (54-65%) was obtained during the month of August–September, which drastically drops to 4% in month of December (Dasand Mitra, 1990;Kapoor and Chauhan 1992; Bishtet al., 2002; Joshi *et al*., 2003; Boulekbache et al., 2010).

***In vitro* shoot multiplication**

Effect of Phytohormones: The proliferated axillary *in vitro* shoots were excised from the mother explants and cultured on semi-solid MS medium supplemented with 0.1-3.0 mg/l BAP for further *in vitro* shoot multiplication.A high rate of shoot multiplication was obtained due to BAP in the medium, which stimulated the growth of multiple shoots during shoot multiplication cycle.These multiplied *in vitro* shoots were later dissected out into propagule (group of 6-7 shoots) and were subcultured on MS medium supplemented with 0.1-3.0mg/l BAP alone or along with combination with NAA for further *in vitro* shoot multiplication. The best shoot multiplication rate was obtained in MS medium supplemented with 1.0 mg/l BAP.On this optimal medium the shoot multiplication of 9-10folds in every 5 weeks subculture duration was obtained (Table-3). MS medium proved to be the best medium for the establishment of shoot cultures in Eucalyptus hybrids.In earlier reports on Eucalyptus F_1 hybrids MS medium has been successfully used for shoot initiation and establishment of Eucalyptus F_1 hybrids cultures (Gupta *et al*.,

1981, 1983; Kapoor and Chauhan 1992; Chang *et al.*, 1992; Bennett,1994; Bisht *et al*., 2000a and 2000b; Joshi *et al.*, 2003) and *Eucalyptus* F1 hybrids (Arya *et al*., 2009; Kamal et al., 2016; Trueman,2018).

Conclusion

Micropropagation technique was utilized to standardize *in vitro* shoot multiplication, using nodal segments of 25-30 years old trees of interspecific F1 hybrids of Eucalyptus. Nodal segments in the form of explants collected during February to April and July to September were the best for *in vitro* studies as they showed least phenolic exudation and better bud break response than in other months. The conclusion of this paper is to obtainmaximum rate ofin vitro Axillary bud proliferation and in vitro shoot multiplication of FRI-15 (*E. citriodora X E. torelliana*).

References

Arya, ID, Sharma, S and Arya, S (2009) Micropropagation of superior eucalyptus hybrids FRI-5 (Eucalyptus camaldulensis Dehn X E. tereticornis Sm) and FRI-4 (E. torelliana F V Muell X E. citriodora Hook): A commercial multiplication and field evaluation. Afr. J. of Biotechnol. 8:5718-5726.

Bisht, P, Joshi, I, Chauhan, JMS and Sharma, VK (2000 a) In vitro clonal propagation of mature Eucalyptus F_1 hybrid FRI-5 (E.camaldulensis Dehn. X E.tereticornis). Indian J. of Forestry. 23(1): 28-32.

Bisht, P, Joshi, I, Chauhan, JMS, Sharma, SK and Bagchi, SK (2000 b). Micropropagation of a 23 years old candidate Plus tree of Eucalyptus tereticornis SM. Indian J. of Forestry 23(2): 149-154.

Bisht, P, Sharma, VK and Uniyal, DP (2002) In vitro clonal propagation of mature Eucalyptus F_1 hybrids. Indian J. of Forestry. Vol. 25 (4): 481-485.

Boulekbache-Makhlouf L, Meudec E, Chibane M, et al. (2010) Analysis by high-performance liquid chromatography diode array detection mass spectrometry of phenolic compounds in fruit of Eucalyptus globulus cultivated in Algeria. J Agric Food Chem. 58(24): 12615-24.

Brondani, G. E., de Wit Ondas, H. W., Baccarin, F. J. B., Gonçalves, A. N., & de Almeida, M. (2012). Micropropagation of Eucalyptus benthamii to form a clonal micro-garden. In Vitro Cellular & Developmental Biology-Plant, 48(5), 478-487.

Chang, S.H., Donald, D.G.M., Jacobs, G. (1992). Micropropagation of Eucalyptus radiate ssp. Radiate using explants from mature and coppice material (1992). South African For. J. 162: 43-47.

Das and Mitra, GC (1990). Micropropagation of Eucalyptus tereticornis Sm. Plant Cell Tissue and Organ Culture, 22: 95-103.

Emara S, Shalaby AE. (2011) Seasonal variation of fixed and volatile oil percentage of four Eucalyptus spp. related to lamina anatomy. Afr J Plant Sci. 5(6): 353-9.

Gupta, PK, Mascarenhas, AF and Jagannathan. V. (1981). Tissue culture of forest trees-clonal propagation of mature trees of eucalyptus citriodora Hook. by tissue culture Pl. Sci. lett. 20: 195-201.

Joshi, I, Bisht, P, Sharma, VK and Uniyal, DP (2003) In vitro clonal propagation of mature Eucalyptus F_1 hybrid. Silvae Genat. 52: 3-4.

Kamal, Barkha & Jadon, Vikas Singh. (2015). Effect of season on in vitro shoot multiplication of Eucalyptus F1 hybrids (È. tereticornis X E. grandis) of Eucalyptus. Universities' Journal of Phytochemistry and Ayruvedic Heights. 19.

Kamal B, Arya I. D., Sharma V., & Jadon V. (2016). In Vitro Enhanced Multiplication And Molecular Validation Of Eucalyptus F1 Hybrids. Plant Cell Biotechnology And Molecular Biology, 17(3-4), 167-175

Kapoor, ML and Chauhan, JMS (1992). In vitro clonal propagation of mature F_1 hybrid (E. torelliana F.V. Muell X E.citriodora Hook). Silvae Genet. 41 (6): 305-307.

Sowmya K., Seenivasan R., Subramanian S., Chinnaraj S., Rajasree S., Kathirvel B., Thamaraiselvi K., (2019) Optimizing the sterilization methods for initiation of the five different clones of the Eucalyptus hybrid species. Biocatalysis and Agricultural Biotechnology, 22: 101-361, ISSN 1878-8181

Trueman,S.J., Hung,C., Wendling.,I 2018."Tissue Culture of Corymbia and Eucalyptus" Forests 9, no. 2: 84

Stackpole DJ, Vaillancourt RE, Alves A, Rodrigues J, Potts BM. (2011) Genetic variation in the chemical components of Eucalyptus globulus wood. G3 (Bethesda); 1(2): 151-9. Usman LA, Zubair MF, Adebayo SA, Oladosu IA. NO M, Akolade JO. Chemical composition of leaf and fruit essential oils of hoslundia opposita vahl grown in Nigeria. Am-Eurasian J Agric Environ Sci 2010; 8(1): 40-3.

Venkatesh, CS and Sharma, VK hybrid (1979) Comparision of a Eucalyptus tereticornis X E.grandis controlled hybrid with E. grandis X E. tereticornis putative natural hybrid. Silvae Genet. 28: 127-131.