International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; SP6: 914-917

Pradip Kumar Sarkar
ICAR Research Complex for Eastern Region, FSRCHPR, Ranchi, Jharkhand, India

Animesh Sinha
Institute of Forest Productivity, Lalgutwa, Ranchi, Jharkhand, India

BP Bhatt
ICAR Research Complex for Eastern Region, Patna, Bihar, India

Rajneesh Kumar
Institute of Forest Productivity, Lalgutwa, Ranchi, Jharkhand, India

Atanu Sarkar
Institute of Forest Productivity, Lalgutwa, Ranchi, Jharkhand, India

Sushit Banerjee
Institute of Forest Productivity, Lalgutwa, Ranchi, Jharkhand, India

Jyotirmoy Ghosh
IINRG, Namkum, Ranchi, Jharkhand, India

Ruby S Kujur
Institute of Forest Productivity, Lalgutwa, Ranchi, Jharkhand, India

Nitin Kulkarni
Institute of Forest Productivity, Lalgutwa, Ranchi, Jharkhand, India

Corresponding Author:
Pradip Kumar Sarkar
ICAR Research Complex for Eastern Region, FSRCHPR, Ranchi, Jharkhand, India

(Special Issue -6)
3rd National Conference
On
PROMOTING & REINVIGORATING AGRI-HORTI, TECHNOLOGICAL INNOVATIONS [PRAGATI-2019]
(14-15 December, 2019)

Effect of pretreatment of explants and *in vitro* culture media in controlling infection and browning in Kusum [*Schleichera oleosa* (Lour.) Oken]

Pradip Kumar Sarkar, Animesh Sinha, BP Bhatt, Rajneesh Kumar, Atanu Sarkar, Sushit Banerjee, Jyotirmoy Ghosh, Ruby S Kujur and Nitin Kulkarni

Abstract
The Kusum tree [*Schleichera oleosa* (Lour.) Oken] is being exploited in India mainly for lac production but also being known for its multiple uses like ethno-medicinal value, edible-fruits, seed oil as medicinal and biodiesel uses. But, very little attention has been given in the direction of tree improvement of this species. Moreover, *in vitro* propagation of woody species poses problems due to high contamination rate and exudation of phenolics. Hence, to overcome such problems, an experiment was conducted to study the effect of pretreatment of explants and *in vitro* culture media in controlling infection and browning in Kusum. Thus, for disinfection of explants of Kusum, nine different treatments as surface sterilants were considered. While, for controlling browning, the ten treatments consisting of different additives including antioxidants were prepared for the study. The results revealed that, the nodal explants treated with $HgCl_2$ (0.1%) for 7 minutes and NaOCl (7%) for 5 minutes were found to be the best treatments in controlling infection (*i.e.*, 83.33% and 70.91% respectively) in the culture media with respect to maximum survival of explants on Day 30 (*i.e.*, 78.37% and 73.06%, respectively). On the other hand, Ascorbic acid (ranges from 100 mg L^{-1} to 200 mg L^{-1}) added in MS (Murashige and Skoog) media had played a significant role and had effectively controlled the browning when compared with control. The best season for *in vitro* culture of Kusum was found to be the summer season mainly during the months from February to May end.

Keywords: Kusum tree, *in vitro* culture, browning, infection, shoot initiation

Introduction
The Kusum tree (*Schleichera oleosa* (Lour.) Oken; Syn. *S. trijuga* Willd, *Pistacia oleosa* Lour., *Cussambium oleosum* O. Kuntze), belongs to Sapindaceae family, is being exploited in India mainly for lac production. All plant parts are useful and extracted for fruit, fodder, fuelwood, timber, medicine, *etc.* [1]. Bark and fruits are of medicinally valuable [2, 3]. Seed oil also posses medicinal value [4] as well as act as an alternative source of biodiesel [5]. Based on usefulness of this species, one can find an ample scope to develop suitable cultivars through different plant breeding approaches for plant multiplication and its conservation. Forest areas are getting shrink day-by-day and the individuals of many species including Kusum tree is getting outnumbered. Hence, mass multiplication of the species is the need of the hour to maintain its diversity and conservation. Conventional propagation through seeds may get resulted into genetic heterogeneity and very long juvenile phase due to slow growth of Seedlings [6]. Availability of Kusum seed for planting is not always sufficient, proper seed

storage to ensure the availability and maintenance of the seed quality need to be known [7]. Hence, in the contrary, vegetative propagation through tissue culture can play an important role in solving problems related to improvement of forest and fruit trees [8]. The *in vitro* techniques are being increasingly applied to supplement the conventional methods of vegetative propagation of forest trees. Moreover, *in vitro* propagation of woody species poses problems of high contamination rates and exudation of phenolics [9]. With this background, an experiment was conducted at Tissue culture laboratory of Institute of Forest productivity, Lalgutwa, Ranchi during 2017-19, to study the effect of pretreatment of explants and *in vitro* culture media in controlling infection and browning in Kusum.

Methodology

The experiment was conducted at Tissue culture laboratory of Institute of Forest Productivity, Lalgutwa, Ranchi, Jharkhand, India, during 2017-19. The explants of single nodal segments of 2-3 cm length (procedure extracted from Sinha and Akhtar) [10] were collected from 10 - 15 years old trees of Kusum based on the selection criteria as described by Sinha [11]. Once the explants were collected, surface sterilization (initially washing the explants under running tap water for half an hour, followed by washing with Cetrimide (0.1%) along with Tween 20 (2 drops) for 10 min, washing with 10% Savlon for 10 min and then dipping them in 0.1% Bavistin solution for one hour and washing them with distilled water) was done. The explants then treated with Ascorbic Acid (0.2%) and kept it for one hour for chilled treatment followed by treating them again with different surface sterilants *viz.*, $HgCl_2$ (0.1%) and NaOCl (7%) for varying time period as considered, just before inoculating them into culture media.

Effect of pretreatments of explants in controlling infection under *in vitro* conditions

A total of nine different surface sterilants as treatments with three replications each, having 50 explants per replication (Table 1), were considered for controlling infections in culture media.

Table 1: Pretreatments for controlling infections in culture media

Treatments (TRT)	Particulars
T1	Control
T2	Explants washed with 0.1% $HgCl_2$ for 3 minutes
T3	Explants washed with 0.1% $HgCl_2$ for 5 minutes
T4	Explants washed with 0.1% $HgCl_2$ for 7 minutes
T5	Explants washed with 0.1% $HgCl_2$ for 10 minutes
T6	Explants washed with NaOCl (7%) for 3 minutes
T7	Explants washed with NaOCl (7%) for 5 minutes
T8	Explants washed with NaOCl (7%) for 7 minutes
T9	Explants washed with NaOCl (7%) for 10 minutes

Effect of different *in vitro* culture media in controlling browning in Kusum

For controlling of browning, different combinations of Murashige and Skoog (MS) media and varying concentrations of additives (like activated charcoal, ascorbic acid, BAP, PVP, Citric Acid, Silver Nitrate, *etc*.) were prepared (Table 2). A total of ten different treatments with three replications, having 50 explants per replication were considered for the study.

Table 2: Different treatments for controlling browning in culture media

TRT	Particulars
T1	Murashige and Skoog media with agar (MS) as Control
T2	MS media without Agar (*i.e.*, Liquid medium)
T3	MS + BAP (3 mg L^{-1})
T4	MS + Activated Charcoal (2 mg L^{-1})
T5	MS + Ascorbic acid (100 mg L^{-1})
T6	MS + Ascorbic acid (200 mg L^{-1})
T7	MS + Ascorbic acid (200 mg L^{-1}) + BAP (3 mg L^{-1})
T8	MS + Ascorbic acid (100 mg L^{-1}) + BAP (3 mg L^{-1}) + PVP (50 mg L^{-1})
T9	MS + Ascorbic acid (100 mg L^{-1}) + BAP (3 mg L^{-1}) + PVP (50 mg L^{-1}) + Citric Acid (50 mg L^{-1})
T10	MS + Ascorbic acid (100 mg L^{-1}) + BAP (3 mg L^{-1}) + PVP (50 mg L^{-1}) + Citric Acid (50 mg L^{-1}) + Silver Nitrate (3 mg L^{-1})

Observations and data analysis

Various observations related to treatment's effect on control of infection *viz.*, contamination (%), explants free from contamination (%) and survival on Day 30 (%) were recorded and for the other experiment, data on control of browning (%) was recorded. All data were subjected to analysis of variance (ANOVA) to quantify the differences between applied treatments by using SYSTAT software [12]. Treatment means were compared from the estimated values of critical difference (CD) at 5 per cent level of significance for the error degree of freedom.

Results and discussions

Effect of pretreatments of explants in controlling infection under *in vitro* conditions

Nodal explants treated with $HgCl_2$ (0.1%) for 7 minutes (T4) and NaOCl (7%) for 5 minutes (T7) were found to be the best treatments in controlling infection (*i.e.*, 83.33% and 70.91% respectively) in the culture media having maximum survival of explants on Day 30 (*i.e.*, 78.37% and 73.06%, respectively). Similar results were also reported by Kumar *et al.* [13] against micropropagation of plum (*Prunus domestica* L.). Bisht *et al.* [14] and Razdan [15] stated that the type of sterilant to be used, its concentration and time depends on the nature of explants and species. The maximum contamination of *in vitro* culture of Kusum (Fig. 1 & 3) was recorded during rainy season (*i.e.*, June to september), followed by winter season (October - January) and the minimum was recorded during summer (*i.e.*, the months from February to May). The best season for *in vitro* culture of Kusum was found to be the summer season (February to May end), but sometime the visible performance was even recorded upto the period till the onset of monsoon. The highest contamination during rainy season might be attributed to existence of high humidity during rainy season [10].

Table 3: Effect of pretreatments on control of infection in culture media

Treatment	Contamination (%)	Explants free from contamination (%)	Survival on Day 30 (%)
T1	$100.00^{a}\pm0.00$	$0.00^{f}\pm0.00$	$0.00^{g}\pm0.00$
T2	$95.60^{a}\pm6.78$	$4.40^{e}\pm0.99$	$0.00^{g}\pm0.00$
T3	$90.80^{b}\pm5.42$	$9.20^{d}\pm2.13$	$57.28^{c}\pm5.08$
T4	$16.67^{e}\pm2.87$	$83.33^{b}\pm10.01$	$78.37^{a}\pm6.17$
T5	$8.77^{f}\pm1.46$	$91.23^{a}\pm6.85$	$14.10^{f}\pm2.44$
T6	$90.14^{b}\pm9.45$	$9.86^{d}\pm2.56$	$0^{g}\pm0.00$
T7	$29.09^{c}\pm5.33$	$70.91^{c}\pm5.11$	$73.06^{b}\pm5.59$
T8	$21.57^{d}\pm3.91$	$78.43^{bc}\pm6.06$	$27.58^{d}\pm3.68$
T9	$19.43^{de}\pm2.80$	$80.57^{b}\pm5.97$	$21.24^{e}\pm3.33$

Data represent the mean ± standard deviation. Means within a column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

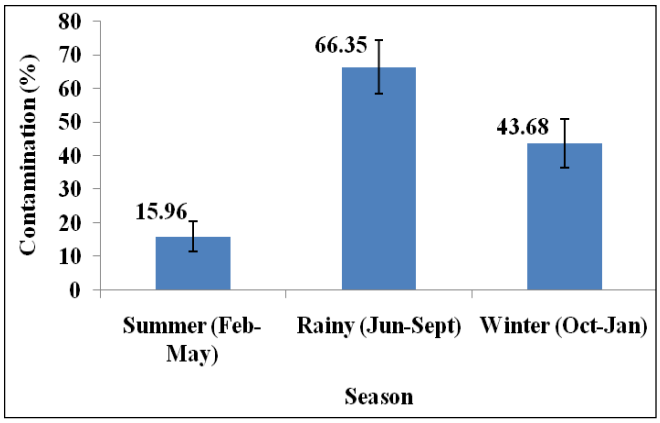

Fig 1: Contamination of *in vitro* culture of Kusum during different seasons

Effect of different *in vitro* culture media in controlling browning in Kusum

In all the cases, Ascorbic acid (ranges from 100 mg L^{-1} to 200 mg L^{-1}) added in MS media had played a significant role and had effectively controlled the browning when compared with control (Fig. 2 & 3). There was a significant control of browning of *in vitro* culture of Kusum and was recorded maximum in case of treatment T9 (*i.e.*, MS + Ascorbic acid (100 mg L^{-1}) + BAP (3 mg L^{-1}) + PVP (50 mg L^{-1}) + Citric Acid (50 mg L^{-1}), followed by T8, T7, T6, T10, T5, and so on. But there was no any control of browning was observed in case of T1 (*i.e.*, Murashige and Skoog media with agar). The control of browning could be due to complete control of exudation of phenolics and the result was at par with findings reported by Kumar *et al.* [13].

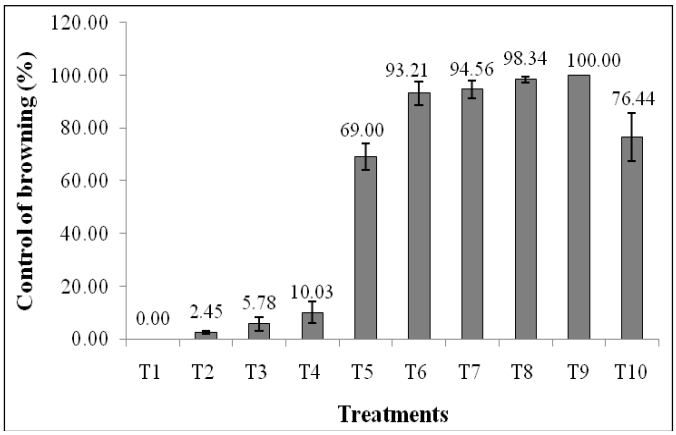

Fig 2: Control of browning in culture media

Fig 3: *In vitro* culture of Kusum; a. Infection; b. Browning; c. Control of browning and infection

Conclusion

Tissue culture can play an important role in solving problems related to improvement of forest and fruit trees. The *in vitro* techniques are being increasingly applied to supplement the conventional methods of vegetative propagation of forest trees. Exudation of phenolics and high rate of contaminations are the major problem of *in vitro* culture of Kusum. Hence, different surface sterilants and antioxidants were tried to control infections and browning of *in vitro* culture of Kusum, respectively. It can be concluded that, the nodal explants treated with $HgCl_2$ (0.1%) for 7 minutes can be the best treatments for controlling infection and for controlling browning, antioxidants like Ascorbic acid (ranges from 100 mg L^{-1} to 200 mg L^{-1}) added in MS (Murashige and Skoog) media can play a significant role. The best season for *in vitro* culture of Kusum was found to be the summer season mainly during the months from February to May end.

References

1. Jaiswal AK, Singh JP. *Schleichera oleosa* and Lac Cultivation. Indian Institute of Natural Resins and Gums. Tech bulletin, 2010; No.06, pp. 1-38.
2. Guleria H, Vaidya M. Anatomical studies of *Schleichera oleosa* (Lour.) Oken. World Journal of Pharmaceutical Research. 2015; 4(12):1178-1188.
3. Khan MJ, Saraf S, Saraf S. Anti-inflammatory and associated analgesic activities of HPLC standardized alcoholic extract of known ayurvedic plant *Schleichera oleosa*. J Ethnopharmacol. 2017; 197:257-65.
4. Thind TS, Singh R, Kaur R, Rampal G, Arora S. In vitro antiradical properties and total phenolic contents in methanol extract/fractions from bark of *Schleichera oleosa* (Lour.) Oken. Medicinal Chemistry Research. 2011; 20:254-260.
5. Kumar N, Pali HS. Kusum oil as a potential fuel for CI engines. 2012. DOI: 10.13140/2.1.3313.6966 (accessed on. 15.03.2018).
6. Radhamani J, Singh AK, Sharma A. Exploring conventional seed storage in some tropical species, in seed conservation: turning science into practice, edited by RD Smith, JB Dickie, SH Linington, HW Pritchard and RJ Probert, Royal Botanic Gardens, Kew, 2003, pp. 777-784.
7. Siahaan FA. Effect of Storage Conditions and Storage Periods on Seed Germination of Kesambi (*Schleichera*

oleosa (Lour.) Merr). Journal Perbenihan Tanaman Hutan, 2017; 5(1):1-11.
doi: //doi.org/10.20886/bptpth.2017.5.1.1-11.
8. Arya HC, Shekhawat NS. Clonal multiplication tree species in the Thar Desert through tissue culture. For. Ecol. Manag. 1986; 16:201-208.
9. Dalal MA, Sharma BB, Rao MS. Studies on stock plant treatment and initiation culture mode in control of oxidative browning in *in vitro* cultures of grapevine. Sci. Hort., 1992; 51: 35-41.
10. Sinha A, Akhtar S. Nodal bud culture in *Schleichera oleosa*: aseptic culture establishment, explant survival and influence of plant growth regulators. Indian J Genet. 2008; 68(2):219-221.
11. Sinha A. Selection of plus trees of *Schleichera oleosa* (Lour.) Oken – A multipurpose tree. In: Multipurpose Trees in the Tropics: Management and Improvement Strategies (eds. Tewari, V.P. and Srivastava, R.L.). AFRI, Jodhpur, 2003, pp. 719-721.
12. Wilkinson L, Coward M. SYSTAT: Statistics-II. (Version 12) Systat software Inc, Sanjose, CA-95110, 2007.
13. Kumar A, Vandana, Kumar A, Nautiyal MC. Micropropagation of plum (*Prunus domestica* L.). In: Trends in Plant Tissue Culture and Biotechnology. (eds. Pareek, L.K. and Swarnkar, P.L.). Agrobios (India), Jodhpur, 2002, pp. 241-247.
14. Bisht P, Joshi I, Chauhan JMS, Bagchi SK. Micropropagation of a 23 year old candidate plus tree of *Eucalyptus tereticornis* SM. Indian J For. 2000; 23(2):149-154.
15. Razdan MK. Inroduction to Plant Tissue Culture (2nd Edn.), Science Publishers, Enfield, USA, 2003, 375.