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RESEARCH ARTICLE :

Identification of best culture media from medicinal plant *Celastrus paniculatus* (Malkangni)

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SUMMARY : The present study was conducted with the objectives to find out the best culture media for callusing and to study shoot and root regeneration capacities of callus in the medicinal plant *Celastrus paniculatus* commonly known as Malkangni. Seven media were tried under the present study. Of them, five were of Murashigeand Skoog's (MS) medium with various concentrations and combinations of growth hormones and the other two media were Gamborg B5 and White's media. Different combinations and concentrations of 2,4- Di chloro phenoxy acetic acid (2,4- D), Kinetin (Kn), benzyl amino purine (BAP), Naphthalene acetic acid (NAA) were used. With regard to callusing percentage and callus growth medium M_3 was the best, which contained MS+ 2mg/l BAP +2mg/l Kinetin +1mg/l IAA and NAA, followed by Medium M_4 (MS + 2mg/l Kinetin + 1mg/l NAA) found the best callusing and shoot regeneration in *Celastrus paniculatus.* For root regeneration containing $^1/_2$ MS salts + 2mg/l BAP + 2mg/l Kinetin + 1mg/l IAA and NAA was found to be the best. Darkness was found to be favourable for root regeneration.

KEY WORDS :
Celastrus paniculatus, MS media, Callus culture, Nodal segment

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BACKGROUND AND OBJECTIVES

Celastrus paniculatus belongs to family Celastraceae commonly known as Malkangni, Jyotishmati, Bitter Sweet, is an important Indian medicinal, deciduous, forest climber seen growing mostly in the hilly regions of northern India at an altitude of 1250 meters. The plant has a distribution range in the sub-Himalayan region from Jhelum eastward upto 1875 meters throughout hilly parts of Bombay, South of Gujarat, Central India, Madras, Ceylon, Burma, Malay Peninsula and Archipelago. According to the Bhanumathy *et al.* (2010) it is a traditional Ayurvedic medicinal plant used for centuries as a memory enhancing, antiinflammatory, analgesic, sedative and antiepileptic agent. The bark is abortifacient, depurative and a brain tonic. The leaves are emmenagogue and the leaf sap is a good antidote for opium poisoning. The seeds are acrid, bitter, thermogenic, emollient, stimulant, intellect promoting, digestive, laxative, emetic, expectorant, appetizer, aphrodisiac, cardiotonic, antiinflammatory, diuretic, emmenagogue, diaphoretic, febrifuge and

tonic, abdominal disorders, leprosy, pruritus, skin diseases, paralysis, cephalalgia, arthralgia, asthma, leucoderma, cardiac debility, inflammation, nephropathy, amenorrhoea, dysmenorrhoea. The seed oil is bitter, thermogenic and intellect promoting and is useful in abdominal disorders, beri-beri and sores. The conventional method of propagation of *Celastrus paniculatus* is through seeds. Poor seed viability and germination restricts their use in multiplication. In addition, indiscriminate overexploitation from natural sources to meet the growing demand by pharmaceutical industry coupled with low seed germinability, lack of vegetative propagation methods and insufficient attempts for replenishment of wild stock of this medicinally important plant species have contributed to its threatened status. Realizing the threat of extinction there is a need to develop quick propagation protocols and conservation strategies. Regeneration in *Celastrus paniculatus* utilizing nodal and apical shoots tip as an explant has been reported recently by Sharada *et al.* (2003). Enhancement in the regeneration frequency would be an added advantage in improving the genetic transformation protocols. Thus a mass multiplication protocol is to be developed for its better future supply.

RESOURCES AND METHODS

Nodal segment of *Celastrus paniculatus* were collected from the nursery of Indore campus and present experiments were carried out at Tissue Culture Laboratory, College of Agriculture, Indore a constituent campus of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.) during year 2013-14.

To sterilize the explants, they were washed in tap water for 30 minutes to remove any remaining dirt or dead plant material. The explants were then cut with sterilized razor into pieces and washed with liquid detergent (Teepol) for 10 minutes. Then they were thoroughly washed with distilled water and transferred to 0.25% $HgCl_2$ solution for 5 minutes for surface sterilization. Finally, these were rinsed with sterile distilled water for three to four times before inoculation. Sterilized explants were inoculated onto three culture media *viz.,* Murashige and skoog's (MS) 1962, Gamborg's B5 1968, and White's 1939, culture medium with various combinations and concentrations of 2,4- Dichloro phenoxy acetic acid (2,4- D), Kinetin (Kn), benzylaminopurine (BAP), Naphthalene acetic acid (NAA). Different phytohormones are added in different media's and pH is adjusted to 5.8. After inoculating the explants in the test tubes, the tubes were tightly plugged with cotton plugs.

Inoculation was done under laminar air flow hood. All cultures were incubated at 25±2^{0}C for a photoperiod of 16 hours per day under fluorescent light (about 1200Lux). The experiment was repeated five times for all cultures. These inoculated explants will result into callus development after 4 weeks of culturing. The callus obtained from the explants were aseptically removed from the culture tubes and then divided into 5mm x 5mm x 5mm size pieces with the help of sterilized razor. Crumbs were then picked up by sterilized forceps and inoculated into the media. The culture tubes were then incubated at 25 ± 2^{0}C and 16 hrs (light) and 8 hrs (dark) photoperiod day^{-1}. Average fresh callus weight and callusing % should be determined. The shoot regeneration capacity of each medium was observed after 25 days. When shoots attained a height of 40-50 mm, they were carefully removed from the shoot regeneration medium and placed on sterilized petridishes. Dry leaves and adhering calli were removed with the help of forceps and scalpel. Shoots were then placed onto different rooting media at the rate of one shoot per tube.The rooting media contains the half strength of salts but double quantity of sucrose. The basal ends of shoots were slightly dipped into media and the cultures were incubated at 25^{0}C under a 16hrs photo period.

OBSERVATIONS AND ANALYSIS

The different combinations and concentrations of growth hormones were tried in Murashige and skoog's (MS), Gamborg's B_5 and White's culture medium.Seven media's were tried under present study (Table 1). Of them, five were of Murashige and skoog's (MS) medium with various concentration and combinations of growth hormones and other two media's were Gamborg's B_5

Table 1: Different media used in experiment — Media protocol

Media	Combinations and concentrations of hormones (mg/l)
M_1	MS +2mg/l 2,4-D
M_2	MS +1mg/l 2,4-D + 3 mg/l Kinetin
M_3	MS+ 2mg/l BAP + 2mg/l Kinetin + 1mg/l IAA and NAA
M_4	MS + 2mg/l Kinetin +1mg/l NAA
M_5	MS + 4mg/lNAA + 2 mg/l BA + 2 mg/l GA_3
B_5	B_5 Salts +0.5mg/l NAA + 2 mg/l BAP
White's	White's salts + 2mg/l NAA + 2mg/l Kinetin + 2mg/l BAP +100 ml/l Coconut water

and White's culture medium. Nodal segment is used as explants. The callus is initiated within four weeks of inoculation of leaf base explants in different culture media's. The callus induction was found in different quantity in different combinations of growth hormones.

The mean value of callusing percentage using leaf base as explants averaged over five replications. The data indicated that average callusing percentage for various media *viz.,* M_1, M_2, M_3, M_4, M_5, B_5 and White's media. The average callusing % of five replications was found to 29.19, 43.37, 70.80, 52.58, 38.20, 20.20 and 12.96%, respectively (Fig. 1). The highest callusing was observed 70.80 % in M_3 medium containing MS+ 2mg/l BAP + 2mg/l Kinetin + 1mg/l IAA and NAA. This combination was found best for induction as well as for growth of calli. The least responding medium was White's medium. These result are supported by Arya *et al.* (2002) using nodal shoot segments as explants for mass/clonal propagation of *Celastrus paniculatus.*

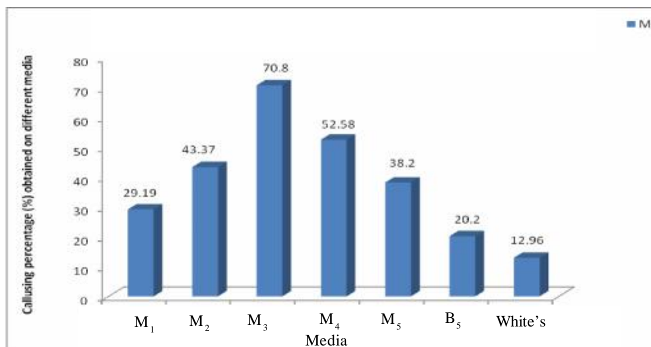

Fig. 1: Callusing percentage (%) obtained from nodal segment in different media

The mean values of fresh weight of callus using nodal segment as explants have been presented in (Fig. 2). This revealed that in M_1, M_2, M_3, M_4, M_5, B_5 and White's media, the average fresh callus weight was found to be 104.05, 133.60, 473.40, 234.20, 114.00, 93.60 and 79.40 mg, respectively. The data in the bar diagram indicates that the maximum callus weight was noted on M_3 medium (473.40 mg), followed by M_4 (234.20 mg) and M_2 (133.60 mg), respectively.

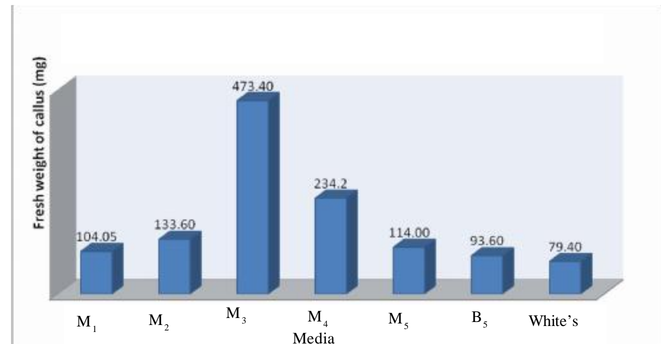

Fig. 2: Averarage fresh callus weight (mg) of nodal segment in different media

a. Callus inducation

b. Multiple shoots initiation from the callus

With regard to callusing percentage and callus growth, some modifications of MS medium gave high callusing efficiency, as compared to other MS combinations. Medium M_3 was the best, which contained MS+ 2mg/l BAP +2mg/l Kinetin +1mg/l IAA and NAA, followed by Medium M_4 (MS + 2mg/l Kinetin + 1mg/l NAA). The callusing has been less in White's medium. The decreasing order of effectiveness of different media tried was $M_3 > M_4 > M_2 > M_5 > M_1 > B_5 >$ White's. The experimental performance of medium M_3 may be attributed to the presences of IAA + Kinetin in the media. They are good growth hormones and stimulant for callus induction. The present findings are in accordance with Bhave *et al*. (2010) and Li Qin and Wang (2006).

Shoot tip were obtained from this source. The sterilized explants were inoculated in MS medium containing different concentrations of auxins and cytokinins. From the various explants, the stem explant gives the excellent result. Maximum callus prolification was observed in 2, 4-D at 2mg/l (92.8%) after 15 days of inoculation. Regeneration of plants from stem callus was obtained after transferring the callus to medium supplemented with 6mg/l 2, 4-D. In case of rooting, high percentage was observed at 2.0mg/l NAA (93.3) and mean number of roots per shoot was 3.6±0.47, after 7days of inoculation.

In the present investigation rooting of individual regenerated shoots was best seen in M_3 medium with 2.6 cm root length after 25 days of inoculation of shoot on rooting media. Out of these seven media M_3 is the best medium for rooting which contained MS (1/2 strength salts) + 2mg/l BAP +2mg/l kinetin+ 1mg/l IAA and NAA. The outstanding performance of M_3 media can be attributed to the presence of IAA, which is an auxin, effective in root induction. Several workers have reported the efficiency of IAA in inducing morphogenesis of callus.

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