RESEARCH PAPER

Callus induction and organogenesis in chandrasoor (*Lepidium sativum* L.)

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The experiment was carried out in the Tissue culture laboratory, College of Agriculture, Indore J.N.K.V.V. (M.P.), during the session 2008-2009. Seven media were tried under the study. Of them, five were of Murashige and Skoog's basal medium with various concentrations and combinations of growth hormones and the other two media were Gamborg B_5 and White's media. The three explants used in this investigation were stem disc, leaf base and leaf blade (middle). 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_4 was the best, which contained MS+ 0.2mg/l BAP +1mg/l Kinetin +2mg/l IAA.The callusing was less in medium M_1 (MS +0.5mg/l 2,4-D) followed by Medium M_3 (MS + 2mg/l 2,4-D +1 mg/l Kinetin + 0.5 mg/l BAP) The decreasing order of effectiveness of different media tried was M_4> M_5>M_3> B_5> White's>M_2>M_1. Among the explants used, stem disc was found to be the best explant closely followed by the leaf base. Thus, the present investigation suggested the use of stem disc and leaf base for callusing. Regarding shoot regeneration M_4 medium containing $^1/_2$MS salts + 0.2mg/l BAP + 0.05 mg/l Kinetin + 1mg/l IAA was the best followed by M_5 with MS salts + 1mg/l Kinetin + 2mg/l NAA + 0.5mg/l GA_3. For root regeneration, M_4 medium containing $^1/_2$MS salts + 0.2mg/l BAP + 0.05 mg/l Kinetin + 1mg/l IAA was found to be the best. Darkness was found to be favourable for root regeneration.

Key words **:** Callus induction, Organogenesis, *Lepidium sativum* L.

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INTRODUCTION

Lepidium sativum (2n=16, 24,36) Linn. is a valuable medicinal plant belonging to the family Cruciferae grown in India, Europe and US is an underutilized crop and distributed throughout India, cultivated as well as growing wild. The plant name in different languages is Chandrasoora Chandrika, in Sanskrit, Garden cress in English and Chandrasoor in Hindi. Chandrasoor is an erect annual herb up to 50cm height, leaves variously lobed, entire, leaves in lower part are petiolate, and upper sessile; flowers white, small and found in racemes; fruits ovate pods, about 5mm long, with two seeds per pod. Chandrasoor has some medicinal properties like, to cure vitiated vata, kapha, urinary retention, colic, indigestion, painful dioarrhea, pain, and arthritis. It also works as a stimulant. It induces production of breast milk. The useful plant parts of chandsoor are roots, leaves, and seeds.

RESEARCH METHODOLOGY

The experiments were carried out at Tissue culture Laboratory of College of Agriculture, Indore a constituent campus of Jawaharlal Nehru Krishi Vishwa Vidhyalaya, Jabalpur (M.P.) during the year 2008-2009. The experimental material was *Lepidium sativum* Linn which was subjected to *in vitro* culture using three explants *viz.,* stem disc, leaf discs and cotyledons and design was completely randomized design.

Sterilization of glass wares:

Under *in vitro* conditions, It is necessary to have complete aseptic conditions of culture medium.

Stock solutions:

Three culture media, *viz.,* Murashige and Skooge (MS) (1962), Gamborg's B5 (1968) and White's (1934) were used during investigation. Freshly prepared solution of inositol, sucrose and hormones were added to culture medium during

Different media used in experiment	
Media	Combinations and concentrations of hormones (mg/l)
M_1	MS +0.5mg/l 2,4-D
M_2	MS + 2mg/l 2,4-D +1 mg/l Kinetin
M_3	MS +1mg/l2,4-D +0.1mg/l Kinetin +0.1 BAP
M_4	MS+ 0.2mg/l BAP +1mg/l Kinetin +2mg/l IAA
M_5	MS + 1 mg/l kinetin +2mg/l NAA + 0.5 mg/l GA_3
B_5	B_5 Salts +2mg/l NAA+ 0.2mg/l Kinetin
White's	White's salts + 2mg/l NAA + 0.5 mg/l Kinetin

preparation. Specific quantities of nutrients were weighted on electronic balance and dissolved in autoclaved distill water.

Sterilization and inoculation of explants to culture media :

A solution of 0.25% $HgCl_2$ and liquid detergent was used as a disinfectant during the investigation. 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. Inoculation was done under laminar air flow hood.

Observations:

Observations were recorded on various explants *viz.*, seedling, cotyledon stem disc, and leaf base for induction of callus, and regeneration on different culture media.

The following observation were recorded in the experiment

Determination of average fresh callus weight:

For obtaining average fresh callus weight, the average weight of non-cultured explants was calculated before its inoculation. Mean weight of non cultured explants was subtracted from mean weight of callus samples to obtain average fresh callus weight (mg).

Determination of callusing percentage:

Individual category of explants was inoculated into different media (100 test tubes with one medium), under five replication (*i.e.* total of 500 test tubes per explants per medium), the number of test tubes showing positive response to callus growth was recorded and the callusing percentage was calculated by the formula given below:

$$\text{Callusing percentage (\%)} = \frac{\text{No. of test tubes responded}}{\text{No. of test tubes inoculated}} \times 100$$

Determination of shoot regeneration capacity of callus:

Shoots regeneration capacity of each medium has been expressed in degree as below:

- NR : No response
- P : Poor less than 3 shoots
- F : Fair, between 3-7 shoots
- G : Good, between 8-11 shoots
- VG : Very good, between 12-15 shoots
- E : Excellent, more than 15 shoots per 100 calli transferred to regeneration medium.

Determination of root regeneration capacity:

The root regeneration capacity of all the devised culture media, using their respective rooting medium was tested, reported in cm as the average root length of 25 shoots transferred to the rooting medium.

RESEARCH FINDINGS AND ANALYSIS

The experiment was conducted with the objectives to identify the best explant and medium for callusing and to study shoot and root regeneration capacities of the callus in *Lepidium sativum* Linn. Seven media were tried under the present study. Of them, five were of Murashige and Skoog's basal medium with various concentrations and combinations of growth hormones and the other two media were Gamborg B_5 and White's media. The three explants used in this investigation were stem disc, leaf base and leaf blade (middle). 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_4 was the best, which contained MS+ 0.2mg/l BAP +1mg/l Kinetin +2mg/l IAA (Table 1). The callusing was less in medium M_1 (MS

Table 1: Average fresh weight of explants on M_4 media

Explants	Average callusing percentage*	Average fresh weight (mg)
Stem disc	28	564.6
Leaf base	27.2	483.3
Leaf blade (middle)	16.6	352.2
S.E. ±	1.59	60.79
C.D. (P=(0.05)	4.9	187.31

Table 2: Callusing percentage, fresh weight of callus, shoot regeneration capacity and root regeneration capacity at different media

Media	Callusing percentage			Fresh weight of callus (mg)			Shoot regeneration capacity			Root regeneration capacity	
	Stem disc	Leaf base	Leaf blade (middle)	Stem disc	Leaf base	Leaf blade (middle)	Stem disc	Leaf base	Leaf blade	Average primary root length of 25 shoots (cms)	Remarks
M_1	10.8	10.4	5.2	39.8	32.3	50.2	NR	NR	NR	0	NR
M_2	15	11.2	7	136.7	129.6	73.5	P	NR	NR	0	NR
M_3	17	19	14.6	421.2	361.6	237.1	P	P	NR	0	NR
M_4	28	27.2	16.6	564.6	483.3	352.2	VG	G	F	2.1	Good root regeneration
M_5	17.8	21.4	15.4	439.8	416.9	271.8	F	P	P	1.5	Slow growth
B_5	16	13.6	11.2	323.6	288.5	189.7	P	NR	NR	0	NR
White's	15.6	12.2	9.4	295.2	205.8	121.3	NR	NR	NR	0.5	NR

+0.5mg/l 2,4-D) followed by medium M_3 (MS + 2mg/l 2,4-D +1 mg/l Kinetin + 0.5 mg/l BAP) The decreasing order of effectiveness of different media tried was M_4> M_5>M_3> B_5> White's>M_2>M_1. Among the explants used, stem disc was found to be the best explant closely followed by the leaf base. Thus, the investigation suggested the use of stem disc and leaf base for callusing. Regarding shoot regeneration M_4 medium containing $^1/_2$MS salts + 0.2mg/l BAP + 0.05 mg/l Kinetin + 1mg/l IAA was the best followed by M_5 with MS salts + 1mg/l Kinetin + 2mg/l NAA + 0.5mg/l GA_3. For root regeneration (Table 2) M_4 medium containing $^1/_2$MS salts + 0.2mg/l BAP + 0.05 mg/l Kinetin + 1mg/l IAA was found to be the best. Darkness was found to be favourable for root regeneration. Similar findings were reported by Saba *et al*. (2000), Pande *et al.* (2002), Gaikwad and Prasad (2003), Osuna *et al*. (2006) and Eriksson *et al*. (2009).

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