RESEARCH ARTICLE

Micropropogation of *Lepidium Sativum*

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Chavhan Arvind

Cite this article as:
Moghe Sandhya, Laud Deepti , Bawankar Manisha, Moghe Ravindra, Joshi Shreepad, Ade Gauri, Bansod Ishani, Hadke Asmit (2016) Micropropogation of *Lepidium Sativum, Int. J. of Life Sciences*, A6: 141-144.

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ABSTRACT

Lepidium sativum is an edible plant. Seeds, leaves and roots of these plants are of economic importance. *Lepidium sativum* is widely used in folk medicine for treatment of asthma, bronchitis, chronic liver enlargement and spleen diseases ect. Leaves of *Lepidum sativum* are considered as a main source of vitamins, minerals and fibres therefore these plant are in-vitro regenerated. The seeds were surface sterilized using various disinfectant like liquid soap, Bavistin and mercuric chloride at appropriate concentration. The seeds of *Lepidium Sativum* were successfully germinated in half MS medium aseptically at pH 6. 10- 15 days old in-vitro germinated seedlings were used for isolation of explants. The explants used for present study was shoot tip. The explants were cut into desirable size and inoculated into regeneration medium consisting of Murashige and Skoog basal combination and various combination of phytohormone such as BAP+ kin and 2,4 D+ kin. The cultures were incubated at 16:8 hrs photoperiod and temperature of 27-2°C. It has been observed that the shoot tip responded and initiate to produce multiple shoots in MS media with growth regulator combinations of BAP+KIN. Callus induction was obtained in medium containing MS + 2, 4 D+ kin. In all, 100% of shoot tips were responded multiple shoot induction on medium containing BAP and kinetin combinations(0.4mg:0.4mg/L) and BAP+KIN (2mg+2mg/L) and 100% explants were responded callus induction in MS medium supplemented with 2,4 D and kinetin (0.3mg+0.3mg/L).The regenerated plant were subjected to hardening.

Keywords: Micropropogation, Multiplication, In-vitro, *Lepidium Sativum*, 2, 4D callus.

INTRODUCTION

Lepidium Sativum known as a pepper cress or Elrashad belongs to the family Brassicaceae (cruciferae) and it is an erect, annual herb grows up to 50 cm height. The leaves are variously lobed, entire, flowers are white small and found in racemes and fruits are obviating pods, about 5mm long, with two seeds per pods. The seeds and leaves contain volatile oils. The plant is eaten and seed's oil is used in treating dysentery and diarrhea and migraine. The plant was found to contain glucosinolate and glucotropaeolin. It is a fast growing edible plant. Seeds, leaves and roots are of economic importance.

The seeds are used in treatment of chronic liver, enlargement and spleen diseases. The bruised seeds mixed with lime juice are applied to relief of local inflammation and rheumatic pains. The leaves are mildly stimulant, diuretic (Maghrani *et al.,* 2005) and serviceable in scorbutic diseases. It is used for significant antimicrobial activity against Staphylococcus aureus (Afolayan *et al.,* 2007). Kambu *et al*. (1982), reported that majority of phytochemicals have been known valuable therapeutic insecticidal activities. It has the hypoglycemic activity and also used for symptoms of asthma and improve lung function in asthmatics. Sahstrabudde & De,1943, reported that supplemented in the diet of lactin of woman to increase the milk secretion during post natal period. Jamwal and Anand, (1962), discovered the antiovulatory property of lepidium sativum plant. Deepshikha *et al*. (2002) had studied tissue culture and developed protocol for in- vitro micropropagation of lepidium sativum linn with yield enhancementof of lepidine.

MATERIAL AND METHODS

Seeds of *Lepidium Sativum* were collected from general stores in Nagpur. The healthy seeds were selected from the common seed lot and before they are used for in-vitro regeneration they were surface sterilize using surface disinfectants. For surface sterilization, seeds were immersed first with 2% Bavistin (antifungal agent) for 60 minutes and then was washed properly 3-4 times with distilled water to remove traces of bavistin. Seeds were then treated with 0.1% $HgCl_2$ for 5-7 minutes, with gentle shaking in clockwise and anticlockwise direction in conical flask and washed with distilled water 3-4 times to remove traces of $HgCl_2$. After this, seeds were removed from conical flask and place in sterile petri plate overloaded with tissue paper. Surface sterilized seeds were inoculated in 250 ml conical flask containing half MS medium. The flasks were incubated at 27.2 ^{o}C under 16:8 hrs photoperiods for germination. The seeds were successfully germinated in half MS medium. Seedlings of 10-15 days old were used for isolation of shoot tip explants. The explants isolated from the *in-vitro* grown seedlings were excised aseptically in laminar air flow and were cultured for shoot regeneration in test tubes (25 mm) containing Murashige and Skoog (MS) medium with different hormone combinations.

For shoot tip culture, different combinations of hormone were used: BAP + Kinetin (0.1mg+0.1mg/l)(0.2mg+0.2mg/l)(0.3mg+0.3mg/l)(0.4mg+0.4mg/l)(1mg+1mg/l)(2mg+2mg/l(3mg+3mg/l) and 2,4D + Kinetin (0.1mg+0.1mg/l)(0.2mg+0.2mg/l) (0.3mg+0.3mg/l) (0.4mg+0.4mg/l) (0.5mg+0.5mg/l). All these explants were inoculated one by one in shoot multiplication medium. After inoculation, culture flask and bottles were incubated in culture room at 27±2 º c under 16:8h photoperiod. Explants were subculture after 20 days on same multiple shoot induction medium containing similar combination of growth regulator for more development of shoot. Sub culturing was done 2 to 3 times for proper growth of shoots.

RESULT & DISCUSSION

The evaluation of seed germination percent was done for *Lepidium Sativum* using healthy seeds. The seeds were inoculated in 10 different lot i.e. 1400 seeds. Out of 1400 seeds 1250 seeds were germinated. In all average seed germination percentage was recorded as 89.28%.

Shoot tip explants from germinated seedlings were further evaluated for shoot induction. Evaluation for shoot induction was done using different combinations of auxin and cytokinin MS medium with phytohormone combination BAP+KIN (0.1mg+0.1 mg/L) 44 shoot tip explants were cultured from 10 days old germinated seedlings. Thirty one explants responded to multiple shooting. The response to multiple shoot induction was 70.45%. In BAP+KIN (0.2mg+0.2mg/L) combination, 52 shoot tip explants were cultured from 10 days old germinated seedlings and 36 explants responded to multiple shooting. Proliferation percentage was found to be 69.23%. In BAP+KIN

Table 1:In-vitro germination of *Lepidium Sativum*

Lot No.	No. of Seeds Inoculated	No. Of Seeds Germinated	Germination Percentage (%)
1	200	190	95
2	100	80	80
3	200	180	90
4	150	130	86
5	100	80	80
6	200	170	85
7	50	40	80
8	200	195	97.5
9	50	45	90
10	150	130	86.6
Total	**1400**	**1250**	**89.28**

Table 2: In-vitro response of shoot tip explants to regeneration in different hormone combination.

Media combination	Replication	No. of explants culture	No. of explants Responded to multiple shoot induction	Proliferation %
MS+BAP+KIN (0.1mg+0.1mg/l)	R1	26	17	
	R2	18	14	
	Total	44	31	70.45%
MS+BAP+KIN (0.2mg+0.2mg/l)	R1	26	21	
	R2	26	15	
	Total	52	36	69.23%
MS+BAP+KIN (0.3mg+0.3mg/l)	R1	26	13	
	R2	26	19	
	Total	52	32	61.53%
MS+BAP+KIN (0.4mg+0.4mg/l)	R1	24	24	
	R2	23	23	
	Total	47	47	100%
MS+BAP+KIN (1mg+1mg/l)	R1	35	31	
	R2	29	24	
	Total	64	55	85.93%
MS+BAP+KIN (2mg+2mg/l)	R1	28	28	
	R2	27	27	
	Total	55	55	100%
MS+BAP+KIN (3mg+3mg/l)	R1	23	16	
	R2	18	15	
	Total	41	31	75.60%

Table 3: In-vitro Response of Callus induction with different hormone combination

Media combination (mg/L)	Replication	No. of explants culture	Total no of explants responded to callus	Percentage of explants responded callusing
MS+2,4D+KIN (0.1:0.1)	I	24	21	
	II	23	20	
	Total	47	41	87.23%
MS+2,4D+KIN (0.2:0.2)	I	35	31	
	II	29	24	
	Total	64	55	85.93%
MS+2,4D+KIN (0.3:0.3)	I	28	28	
	II	27	27	
	Total	55	55	100%
MS+2,4D+KIN (0.4:0.4)	I	23	16	
	II	18	15	
	Total	41	31	75.60%
MS+2,4D+KIN (0.5:0.5)	I	20	17	
	II	16	14	
	Total	36	31	86.11%

(0.3mg+0.3mg/L)combination however 52 shoot tip explants were cultured and 47explants could respond to multiple shoots. The responded explants were 61.53%. (Table 2). In BAP+KIN(1mg+1mg) combination 64 shoot tip explants were cultured from 10 days old in- vitro germinated seedlings, 55 shoot tip explants responded to multiple shooting. The responded explants were 85.93%. In BAP+KIN (2mg+2mg/L) concentration 55 explants were culture from 10 days old germinated seedlings from which 55 explants responded. The responded explants were 100%. In BAP+KIN (3mg+3mg/L) 41 shoot of explants were cultured from 10 days old germinated seedling. From which 31 explants responded. The responded explants were 75.60 %. (table 2) In BAP+KIN with concentration (0.4mg:0.4mg/L)47 shoot tip were cultured from 10-15 days old in-vitro germinated seedlings. From which 47 explants were responded. Proliferation percentage for these combinations was 100%

In-vitro response of shoot tip explants to regeneration in different hormone combination

Callusing was reported 3 to 5 days after inoculation. In 2,4-D+KIN (0.1mg+0.1mg/L) combination 87.23% callusing was observed. In this medium callus

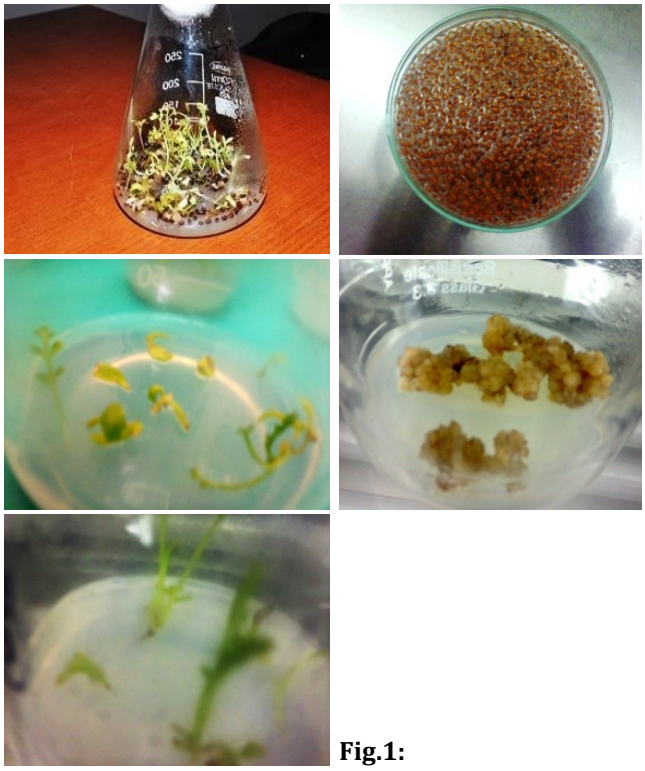

Fig.1:

was greenish and watery. In 2 mg/L each growth regulator combination 85.95% callusing was observed. Callus was yellowish and watery .In 2,4D+KIN (0.3mg+0.3mg/L) combination, callus showed 100% callus. Callus morphology was yellowish and watery. On 2,4 D+KIN (0.4mg+0.4mg/L) media shows 75.60% callus induced and morphology was brownish and compact.2,4D+KIN (0.5mg+0.5mg/L) media shows 86.11% callus. Morphology was green yellowish color and granular (Table 3)

DISCUSSION

In the present study seeds were sterilized using 0.1 % Bavistin solution and 0.1 % mercuric chloride and seeds were inoculated on half MS media for germination. Seed germination percent was found to be 89.28 %.(Eltayb *et al*., 2010) Seeds were washed in running tap water for 15 minutes followed by thorough washing with sterile distilled water. Under laminar flow cabinet seeds were disinfected by 10% (v/v) of Clorox® (0.5 % free chlorine) for 10 mints then rinsed five times with sterile distilled water. For In-vitro response of multiple shoot induction, shoot tip explant was isolated from 10 days old seedling and were inoculated with help of forceps in MS medium aseptically. Such a protocol was also developed by Pande and Malik (2002) in L. Sativum and observed enhancement in the yield of lepidine.

In the present study Shoot tip explants were used for induction of multiple shoots. It has been observed that BAP and kinetin combination at 2 and 4 mg/L each showed 100% shoot induction . For the callus induction MS medium along with different concentration of 2,4, D and Kinetin were used. Eltayb Abdellatef, Ilham A Gadir Hassan *et al*. (2010) report the effects of plant growth regulators and explants with the purpose of developing a protocol for callus induction of garden cress (L. sativum. L). Three explants namely, leaves, hypocotyls and roots obtained from 7 days -old in vitro germinated seedlings were cultured on MS medium supplemented with different concentrations of 2,4-dichloro-phenoxyacetic acid (2,4-D) and α-naphthalene acetic acid (NAA). The highest callus weight (2.13) gm. was obtained when leaves explant was cultured on MS medium containing 2.0 mg/L 2,4-D. Addition of cytokinns Benzyl adenine (BA) and Thiadizuroun (TDZ) enhanced the callus weight as well as callus growth index. The maximum callus weight obtained when leaves explant cultured on MS media supplemented with 2.0 mg/L 2, 4-D in combination with 3.0 mg/L BA

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