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Callogenesis and multiplication of *Lepidium sativum* from shoot tip explants under *in vitro* condition

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Abstract
An experiment was carried out to establish an efficient callus induction system and multiplication of *Lepidium sativum*. Among the different explants derived from leaves, root, hypocotyl and shoot tip. The best callus initiation performance was observed by shoot tip (96%). 15 media combinations of Murashige and Skoog basal media supplemented with varying concentration of cytokinin and auxin were evaluated. All media combination produced calli in varying frequencies. However, culture media combination MS5B1N (90.00%), MS5B1N (81.00%) MS3N (84.50%) was found to be most responsive for callus induction, closely followed by MS5D (78.00%). This procedure can be advantageously of extraction of active ingredient as well as developing of *in vitro* regeneration protocol that can use in genetic improvement of this multipurpose medicinal plant.

Keywords: *Lepidium sativum*, shoot tip, callus induction and regeneration

Introduction
Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Plant tissue culture techniques offer an integrated approach for the production of standardized quality phytopharmaceuticals through mass-production of consistent plant material for physiological characterization, analysis of active ingredients and enhancement of the natural levels of valuable secondary plant products. *L. sativum* (Garden cress, Cruciferae) is a fast-growing, edible herb that is botanically related to watercress and mustard. *L. sativum* is reported to exhibit antihypertensive, diuretic, anti-inflammatory, analgesic, anticoagulant, antirheumatic, hypoglycemic, laxative, prokinetic, antidiarrheal, and antispasmodic properties (Raheman *et al.*, 2012) [8]. *L. sativum* mainly contains alkaloids, saponins, anthracene glycosides, carbohydrates, proteins, amino acids, flavonoids, sterols as chief photochemical constituents. Glutamic acid is the most abundant amino acid; leucine and methionine are the highest and the lowest essential amino acids respectively. *In vitro* propagation of *L. sativum* holds tremendous potential for the production of high-quality plant-based medicine (Murch *et al.*, 2000) [4] but despite its potential therapeutic values, the plant has not received the attention it deserves.

In the present investigation an attempt was made to obtain an efficient protocol for callus induction and multiplication of *Lepidium sativum* which is a potential Asian and African herbal medicine for primary health care. In addition, the effect of auxin/cytokinin interactions on the morphogenic response of hypocotyl, leaf, root and shoot tip explants was monitored to formulate a reliable regeneration protocol. As the plants are annual, *in vitro* propagules could prove fruitful as a continuous source of raw material for valuable medicinal compounds like benzylisothiocynate, benzylcyanide, and an alkaloid (lepidine), which possesses antifertility activity (Pande *et al.*, 1999) [6].

Materials and methods
Sterilization of seeds
The seeds of *L. sativum* collected from Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) were soaked in Tween 20 for 20 minutes. Further sterilization was carried inside the laminar airflow chamber. The seeds were washed thoroughly with autoclaved distilled water for 15 minutes, rinsed with 70 per cent ethyl alcohol for five minutes followed by 0.1 per cent $HgCl_2$ solution for five minutes. Treated seeds were again washed thoroughly with autoclaved

distilled water for eight to ten times to remove traces of $HgCl_2$. After surface sterilization, were directly inoculated on full strength MS medium (Murashige and Skoog, 1962) [3] in culture bottles and incubated for 7 days at 25 °C in culture bottles and incubated for 7 days at 25 °C.

Explant

In vitro produced seven days old seedlings were used as a source of explants. Leaves, roots, shoot tip and hypocotyls were used as explants for callus induction. Explants from different plant parts were cut and placed on medium MS fortified varying concentrations of hormones and 3.0 per cent sucrose as energy source as well as 0.75 per cent agar was used as a solidifying agent and pH was adjusted to 5.8 (Fig 1).

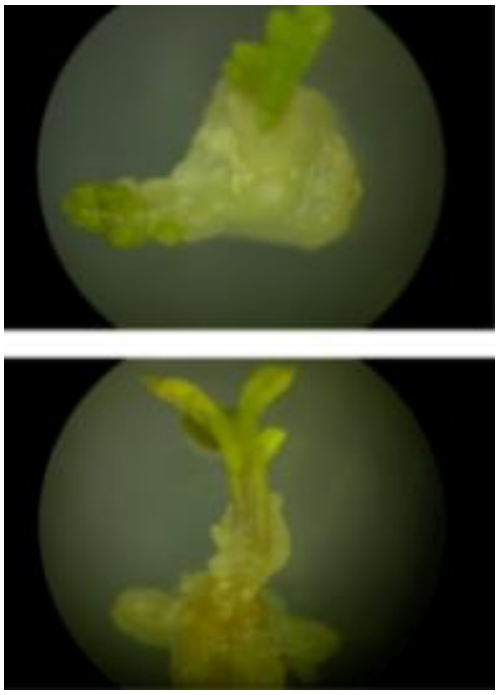

Fig 1: Regentration of shoot tip explants.

Preparation of culture medium for callus formation and regeneration

Basal Murashige and Skoog (MS) medium was prepared and supplemented with growth hormones in varying proportions (Table 1). To all the constituents of medium 30 g/l sucrose was added and the final volume was made to 1000ml and pH was adjusted to 5.8 ± 0.1 with the help of 1N NaOH and 1N HCL.

All the 15 media combinations supplemented with different concentrations alone and in combinations of BAP (Benzyl amino purine), cytokinin, NAA (Napthalene acetic acid) and 2,4-D (2,4-Dichlorophenoxyacetic acid) auxin were used. For regeneration of plantlets addition of plant growth regulators were required.

Table 1: Concentrations of plant growth regulators fortified with MS culture media for callus culture.

S. No.	Culture media	Growth regulators (mg l^{-1})		
		BAP	NAA	2,4-D
1	MS2D	-	-	2.0
2	MS3D	-	-	3.0
3	MS5D	-	-	5.0
4	MS6D	-	-	6.0
5	MS10D	-	-	10.0
6	MS2N	-	2.0	-
7	MS3N	-	3.0	-
8	MS5N	-	5.0	-
9	MS1B5D	1.0	-	5.0
10	MS2B5D	2.0	-	5.0
11	MS3B5D	3.0	-	5.0
12	MS4B5D	4.0	-	5.0
13	MS5B5D	5.0	-	5.0
14	MS1N5B	5.0	1.0	-
15	MS2N5B	5.0	2.0	-

Results

For callus culture in chandrasur several attempts with various explants have been made. Leaves, roots, shoot tip and hypocotyls of *in vitro* produced seven days old seedlings were used as a source of explants for callus induction. The explants of *L. sativum* cultured on MS basal medium without growth regulator did not show any morphogenetic response and eventually died. However, when the four explants cultured on

MS containing different concentration of auxins (2, 4-D and NAA) callus has been induced. Callus culture was successfully obtained from shoot tip of *L. sativum* (96%) on different fortifications of MS medium with different concentrations of auxins and cytokinins. The growth regulators were used alone as well as in combinations.

Explants became swollen and proliferation was not observed during the first week of culture, however, callus initiated during the second week of culture. The callus initiation started from the upper portion of explant usually in contact with the culture media. After 28-35 days of culture, callus initiating explants were counted. The calli generated from various explants on different media combinations, varied in texture and colour. The best results were obtained from Kn of 3mg l^{-1} (a cytokinin) in combination with slight amount of IAA (auxin) at rate 0.5 mg l^{-1} supported plantlet regeneration. MS basal medium supplemented with Kn 0.5 mg l^{-1}, IAA 3 mg l^{-1}, adenine 20 mg l^{-1}, asparagine 100 mg l^{-1}, glutamine 100 mg l^{-1}, CH 1000 mg l^{-1} (Figure 2.). Culture medium fortified with a single cytokinin and auxin responded poorly as compared to culture medium supplemented with different combination.

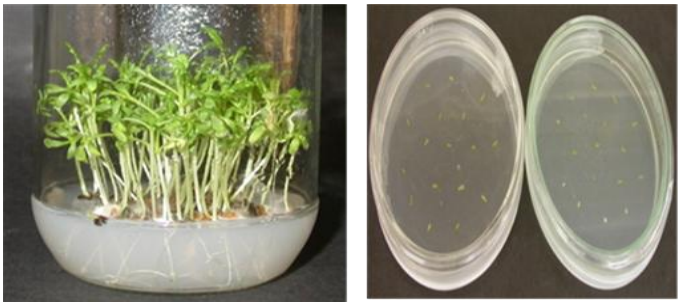

Fig 2: A Seedling develops after seven days of seed planting on ms basal media under in vetro condition, B shoot tip as explant

Callus induction (%)

Among the fifteen media combinations tested for their callus forming ability, all media combinations produced calli in varying frequencies. Culture media combinations MS3B5D (90.00%), MS5B1N (81.00%) and MS3N (84.50%) were found to be most responsive for callus induction closely followed by MS5D (78.00%) (Fig 3). Maximum proliferated callus from shoot tip was weight of 2.96 gm.

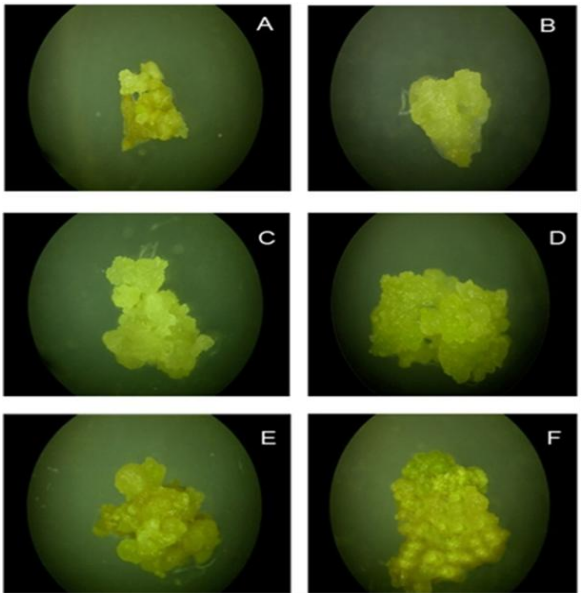

Fig 3: callus culture, Ainitiation, B14 day old culture, C-D 21 day old culture, E-F 28 day old culture,

Regeneration

Kn of 3mgl^{-1} in combination with slight amount of IAA at rate 0.5 mg.l^{-1} supported plantlet regeneration. MS basal medium supplemented with Kn 0.5 mg.l^{-1}, IAA 3 mg.l^{-1}, adenine 20 mg.l^{-1}, asparagine 100 mg.$l^{-1,}$ glutamine 100 mg.$l^{-1,}$ CH 1000 mg.l^{-1} (plate). Shoots of approx of 3 cm were transferred to rooting media MS + Glu [100 mg l^{-1}] to develop root system (Fig 4).

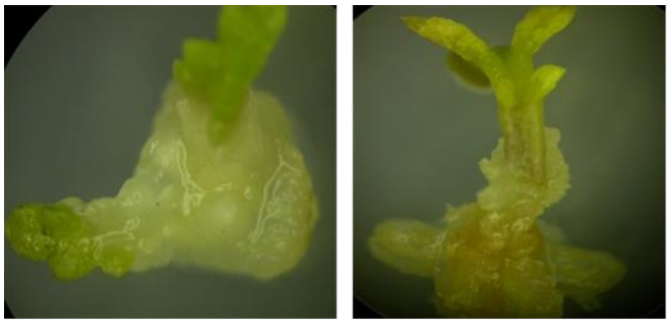

Fig 4: callus showing shooot initiation

Discussion

Among the four explants used, shoot tip explant was highly responsive in producing callus than leaf, hypocotyl and root explants. High callus rate from hypocotyls explants cultured on NAA supplemented media and highest callus proliferation from leaf explants has been observed in earlier attempts (Pande *et al.* 2002 and Eltayb *et al.* 2010) [7, 2].

The percent calli induction in MS3B5D, MS5B1N, MS3N and MS5D were 90%, 81% 84.50% and 78% respectively. Found to be most responsive for callus induction closely followed by (78.00%). On comparing the results with those of Pande *et al.* (2002) [7], wide variation in callus production was observed, when various combinations of NAA and BA were used in the MS medium. Specifically NAA 2.0 mg $l^{-1,}$ BAP 5.0 mg l^{-1}, CH 1000 mg l^{-1} proved best for hypocotyl explants and produced compact, green regenerating callus with several tiny shoot bud-like structures. Eltayb *et al.* (2010) [2] used different concentration of auxins (2, 4-D and NAA) for callus induction. The highest callusing rate and best callus appearance were obtained on MS media supplemented with 2,4-D, compared to NAA. Addition of cytokinin to callus induction media enhanced the callus growth and weight as well as callus growth index. Similar trend of regeneration has been recorded in earlier attempts (Pande *et al.*, 2002 and Tripathi *et al.*, 2008) [7]. Unconditionally, the regeneration of plantlets in *Lepidium sativum* strictly followed a two-step process of shoot differentiation followed by rooting. Thus, micro propagation and culturing of cells and tissues of *L. sativum* will provide a novel means for conserving and rapidly propagating genotype that has tremendous pharmacological importance.

Acknowledgement

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