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RESEARCH ARTICLE

IN VITRO CALLOGENESIS AND PROLIFERATION FROM DIFFERENT EXPLANTS OF GARDEN CRESS (*LEPIDIUM SATIVUM*. LINN)

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ABSTRACT

The effects of plant growth regulators and explants on callus induction were investigated for 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 suplemented with 2.0 mg/L 2, 4-D in combination with 3.0 mg/L BA. 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.

INTRODUCTION

The World Health Organization (WHO) has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs (Farnsworth *et al.,* 1985). *Lepidium sativum* (Garden Cress, Fam: Cruciferae) is an annual erect herbaceous plant, growing up to 30 cm. It is a well known culinary herb and the leaves are widely used as a garnish and are consumed raw in salads. The plant is known to possess varied medicinal properties. Leaves of this plant are diuretic and gently stimulant. The seeds are aperient, diuretic, tonic, demulcent, aphrodisiac, carminative, galactagogue and emmenagogue (Nadkarni, 1954). The seeds are rubefacient and are applied as a poultice for hurts and sprains (Chopra *et al.,* 1986). The plant also shows teratogenic effect (Nath *et al.,* 1992) and antiovulatory properties (Jamwal and Anand, 1962; Mulhi and Trivedi, 1972; Kamboj and Dhawan, 1982; Satyavati, 1982). The root is used in the treatment of secondary syphilis and tenesmus (Chopra *et al.,* 1986). A preliminary pharmacological study on seeds of *L. sativum* has suggested the presence of cardioactive substance and is shown to have probable action through adrenergic mechanisms (Vohora and Khan, 1977).

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Preliminary phytochemical study of *L. sativum* with standard procedures showed that it contains flavonoids, coumarins, sulphur glycosides, triterpenes, sterols and various imidazole alkaloids (Radwan *et al.,* 2007). The use of plant extract for medical treatments is enjoying great popularity since 1990s when people realized that the effective life span of antibiotic is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance (Eisenberg *et al.,* 1993) The main goal of this study was to develop an efficient and reproducible protocol for callus induction from Garden cress *L. sativum* a- potent African medicinal plant for phytochemical studies.

MATERIALS AND METHODS

This study was carried out in the Laboratory of Plant Cell and Tissue Culture, Commission for Biotechnology and Genetic Engineering, National Center for Research. Khartoum, Sudan.

Plant Material

Seeds of *L. sativum* used in this study were obtained from the Medicinal and Aromatic Plant Research Institute, National Center for Research, Khartoum, Sudan

Surface sterilization

Seeds were washed by continuously 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. After surface sterilization, 100 seeds were directly inoculated in full strength MS medium

(Murashige and Skoog, 1962) in culture bottles and incubated for 7 days at 25°C ±2 with a 16 h photoperiod. The experiment was repeated three times and data on germination percentages were recorded after 7 days of inoculation.

Explant preparation

In vitro produced seedling 7 days – old (Fig 1.A) were used as a source of explants, Leaves, roots and hypocotyls were used for callus induction (Fig 1.B, C, D).

Effect of auxins on callus induction

For assessing the effect of auxins on callus induction, explants (Leaves , roots and hypocotyls) were cultured in culture bottles containing MS basal media supplemented with different levels (0.5, 1.0, 2.0, 3.0 and 5.0 mg/ L) of Naphthalene Acetic Acid (NAA), and 2,4-dichloro-phenoxyacetic acid (2,4-D).

Callus proliferation

In vitro developed callus that obtained in best callus induction media and (2,4-D 2.0 mg/ L. from leaves explants) were culture in MS basal media supplemented with different concentrations (0.5, 1.0, 2.0, 3.0, 5.0 mg/L) of Benzyl adenine (BA) and Thidiazuron (TDZ).

Statistical analysis

All parameters were collected after 6 weeks of incubation then standard error was calculated by Excel computer program.

RESULTS

The leaves, hypocotyls and root explants of *L. sativum* cultured on MS basal medium without growth regulator did not show morphogenetic responses and eventually died. However, when the three explants cultured on MS containing different concentration of auxins (2, 4-D and NAA) callus has been induced. The highest callusing rate and best callus appearance were obtained on MS media supplemented with 2,4-D, compared to NAA. (Table 1). Within different concentrations of 2, 4-D, 2.0 mg/l promoted rapid growth and produced the highest callusing weight (2.13 mg) from leaves explants. (Table 1). To assess the effect of cytokinins on callus proliferation, BA and TDZ on different concentrations were added to the callus induction media (MS supplemented with 2-4, D 2.0 mg/L). Addition of cytokinins to callus induction media enhanced the callus growth weight as well as callus growth index. The maximum callus weight 4.45 gm was obtained when 5.0 mg/L of BA were added to the callus induction media (Table 2) (Fig. 2). Deepshikha *et al.* 2002 reported similar results in *Lepidium. sativum*. This protocol will pave the way for the development of *in vitro* regeneration system for this multipurpose ethno pharmaceutical plant and consequently will promote the application of plant tissue culture technology in the area of drug production.

DISCUSSSION

Different concentrations of sodium hypochlorite solution were tested for seed surface sterilization. The results showed that 100 % surface disinfection was obtained by using sodium hypochlorite at 10% concentration. Usage of sodium hypochlorite in *L. sativum* surface sterilization was already reported by (Hisashi and

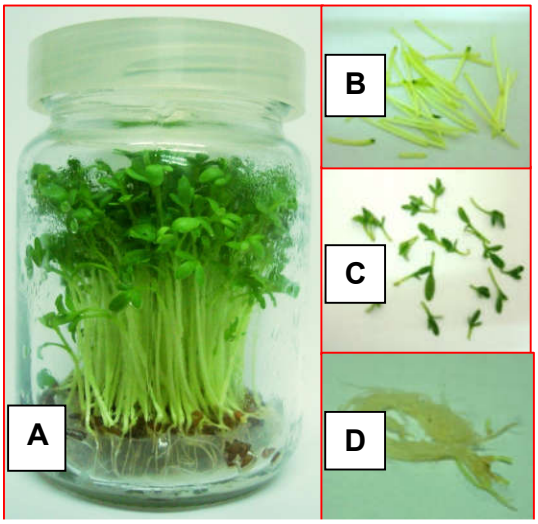

Figure 1. *In vitro* germinated seedlings of *Lepidium sativum* and explants

A. 7- days old in vitro grown seedlings germinated on MS medium B. Leaves explants C. Hypocotyl explants D. Roots explants

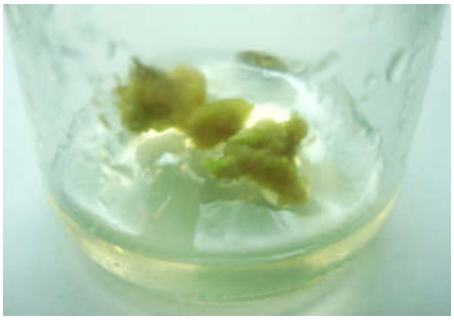

Figure 3. Nonembryogenic callus from leave explant of *L. sativum* cultured on MS media supplemented with 2.0 mg/L 2, 4-D after 6 weeks of culture

Figure 4. Proliferated callus from leave explant of *L. sativum* cultured on MS media supplemented with 2.0 mg/L 2, 4-D in combination with BA at 3.0 mg/L after 6 weeks of culture

Table 1. Effects of different concentrations of (2,4-D) and (NAA)on callus induction from Leaves, hypocotyls and roots explants after 6 weeks of culture

Auxin mg/L		Explants								
		Hypocotyl			Leaves			Roots		
NAA	2,4-D	Callusing (%)	Weight (gm) (Mean±SE)	Callus index	Callusing (%)	Weight (gm) (Mean±SE)	Callus index	Callusing (%)	Weight (gm) (Mean±SE)	Callus index
0	0	-	-	-	-	-	-	-	-	-
0.5	0	80	0.22±0.01	+	80	1.23±0.2	+	100	0.38±0.01	+
1.0	0	100	0.27±0.02	++	100	1.30±0.2	++	100	0.64±0.02	+
2.0	0	100	0.29±0.02	++	100	1.43±0.1	++	100	0.39±0.01	++
3.0	0	100	0.39±0.03	++	100	1.62±0.0	++	100	0.30±0.01	++
5.0	0	100	0.70±0.00	++	100	1.66±0.1	+++	100	0.18±0.02	++
0	0.5	100	0.52±0.01	+	100	1.41±0.1	++	100	0.46±0.00	+
0	1.0	100	0.44±0.02	+	100	1.76±0.1	+++	100	0.43±0.01	++
0	2.0	100	0.40±0.01	++	100	2.13±0.2	++++	100	0.42±0.02	++
0	3.0	100	0.16±0.02	++	100	1.94±0.3	++++	100	0.41±0.03	++
0	5.0	100	0.13±0.03	++	100	1.78±0.1	+++	100	0.29±0.02	+

+ Rare, ++ Good, +++ Very Good, ++++Excellent

Table 2. Effects of addition of (BA) and (TDZ) on callus proliferation of callus induced on MS media containing 2.0 mg/L 2, 4-D after 6 weeks of culture

Cytokinin (mg/L)		Callusing	Weight	Callus
BA	TDZ	(%)	(gm)	index
0	0	100	2.13±0.2	++++
0.5	0	100	2.63±0.2	++++
1.0	0	100	3.82±0.1	++++
2.0	0	100	4.24±0.2	++++
3.0	0	100	4.44±0.0	++++
5.0	0	100	4.58±0.0	++++
0	0.5	100	2.16±0.1	++++
0	1.0	100	2.20±0.1	++++
0	2.0	100	2.35±0.0	++++
0	3.0	100	2.45±0.0	++++
0	5.0	100	3.15±0.1	++++

Francisco, 2006). Among the three explants used, leaves explant was more sensitive and highly responsive in producing callus than hypocotyl and root explants, healthy green yellowish callus was obtained from hypocotyl rather than hypocotyl and roots. Deepshikha *et al.* 2002 obtained high callus rate from hypocotyls explants cultured on NAA supplemented media.

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