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IN VITRO APPROACHES ON THE DEVELOPMENT AND PROLIFERATIVE GROWTH OF INDUCING CALLUS FROM SOMATIC EXPLANTS OF HOT CHILI PEPPER (*C. ANNUUM* L. CV. PINTEA AND THE CV. DE CAYENNE)

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Abstract

Starting from the fact that synthesis and accumulation of secondary metabolites can be stimulated by in vitro cell culture from somatic tissues, the purpose of this study was to obtain biologically active substances from callus cell proliferation of varieties of hot chili pepper, like Pintea and De Cayenne genotype (C. annuum L.).
Somatic explants taken after 21 days from the regenerated plantlets from germinated seeds in aseptic in vitro conditions, like hypocotyls, cotyledons, young leaves and apex were inoculated on several variants of hormonal combinations, added to the recipe of basal Murashige and Skoog (1962) culture medium.
For the tested variants we used phytohormones, like auxins (NAA and 2.4-D) and cytokinins (kinetin) in concentrations ranging from 0.5 mg/L for kinetin and 0.3-1.0 mg/L for NAA and 2.4 D. The best results on the active growth of callus, were obtained for Pintea variety when there were utilized the cotyledons and apex (100%) and in the case of young leaves, the result was 58% on media supplemented with kinetin 0.5 mg/L .
Comparing with this genotype, for explants of De Cayenne variety cultivated on the same combination of tested culture medium (MS supplemented with 0.5 mg/L kinetin), the results of 100% were obtained only at the apex level and for the other types of tested somatic explants, the values recorded was 94% in the case of young leaves and only 55% for cotyledons.

Keywords*: pepper, somatic explants*, *callus induction, direct organogenesis.*

INTRODUCTION

Capsicum is a genus of the flowering plant family *Solanaceae*. Its species have been cultivated in America since thousands of years, and are now cultivated worldwide. *Capsicum* consists of approximately 20-27 species from which five are domesticated - *Capsicum annuum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens* and *Capsicum pubescens* (Walsh et al., 2001; Heiser et al., 1969; Bosland, 1994).

The fruits of capsicum have a variety of names like chili pepper, red or green pepper, sweet pepper, bell pepper, miniature paprika, among others.

The various colours exhibited in *Capsicum* are due to mixture of esters of capsorubin, zeaxanthine, crytoxanthine, capsanthin and other carotenoids. These various and extractable colours of *Capsicum* fruits is extensively used in the food processing industry in wide range of products.

Capsicum is an excellent source of vitamins A, B, C and E and also rich in minerals like molybdenum, potassium, manganese and thiamine. β Carotenoids and vitamins C and A are powerful antioxidants that destroy free radicals. The total antioxidants is completed by phenolic compounds, which occur in peppers in connection with sugars.

Even chilli contains seven times more vitamin C than orange. It also contains bioactive nutrients, such as violaxanthin, lutein, β-cryptoxanthin and β-carotene.

The therapeutic properties and pungency exhibited in *Capsicum* contain capsaicinoids ($C_{18}H_{27}NO_3$) alkaloids specific for *Capsicum* genus, which show many pharmacological properties.

As medicine, it is used for neuralgia, rheumatic disorders, non-allergic rhinitis, among others,

thus their importance is widely known as a wellbeing food (Khomendra et al., 2013).
Plant regeneration system by organogenesis in *Capsicum* has been reported from diverse explants (Swamy et al., 2014).
Direct somatic embryogenesis was first described in chilli pepper by Harini and Sita (1993) and in sweet pepper by Binzel et al. (1996). Number of chemical and physical factors like media components, phytohormones, pH, temperature has been extensively studied also for a large number of plant species (Fett-Neto et al., 1995).
The type and concentration of auxin or cytokinin or the auxin/cytokinin ratio alters dramatically both the growth and the product formation in cultured plant cells (Mantell and Smith, 1984).
In this report, an efficient system for inducing of proliferating growth callus from *Capsicum* somatic explants (Pintea and De Cayenne genotype) starting from young leaf, apex, hypocotyls and cotyledons explants using auxins and cytokinins is reported.

MATERIALS AND METHODS

Explant sources: experiments were carried out using as explants sources plantlets having approx. 2cm in height, obtained by germinating chilli pepper seeds in „*in vitro*" culture conditions.
Seeds of two varieties of *C. annuum* L. (cv. Pintea and the cv. De Cayenne) were properly washed in running tap water and sterilized in ethanol (70%) for 5 minutes and sodium hypochlorite (10%) for 10 minutes and then, a final rinse for three times with sterile distilled water. Seeds were inoculated on full-strength Murashige and Skoog (1962) basal medium (MS), with the addition of 3% sucrose, 0.8% agar and with different concentrations and combinations of plant growth regulators, like auxins (NAA and 2.4-D) and cytokinins (kinetin) in concentrations ranging from 0.5 mg/L for kinetin and 0.3-1.0 mg/L for NAA and 2.4 D. Plant growth regulator supplements were added prior to the media autoclaving.
The pH of the culture media was adjusted to 5.5 using NaOH 1N and solidified with 0.8% (v/v) agar before autoclaving at 121°C for 15 min. After inoculation, seeds were maintained in dark for 10 to 12 days for germination, at 25°C and later they were exposed to 16 hours of light followed by 8 hours of darkness photoperiod.
Explants were collected after 21 days from healthy plantlets grown in the culture bottle in the laboratory. Explants used for the experiment included hypocotyls fragments, cotyledons, young leaves and apex. They were excised from seedling and cut into 1cm long segments and explants were inoculated immediately on MS medium, supplemented with 0.5 mg/L NAA and 1 mg/L 2.4 D (V1 variants) and with 0.5 mg/L Kinetin (V2 variants) for each variety.
Morphogenetic culture establishment: the explants were placed on the surface of the culture media variants (Table 1), distributed in 12 cm ø Petri plates (containing 7-10 mL of sterile autoclaved culture medium solidified with 8 g/L agar) and the incubation was performed in the growth chamber, at 25± 2°C, under a 16/8 h photoperiod, with a light intensity of 3000 lux. The periodical transfers on fresh culture media were performed at 3 week intervals.

Table 1. Culture media variants used for inducing of proliferating growth callus from somatic explants of *Capsicum*

Variant	Auxins (mg/L)		Cytokinins (mg/L)	Somatic explant type used
	NAA	2.4D	Kin	
V1	0.5	1	-	hypocotyls, apex, young leaves
V2	-	-	0.5	cotyledons, young-leaves, apex

Legend: NAA = α –naphthalene-acetic acid; 2.4-D = 2.4-dichlorophenoxyacetic acid; Kin = Kinetin.

RESULTS AND DISCUSSIONS

Plant cell culture offers a promising approach for a large scale production of phytochemicals and has several advantages over whole plant production. Callus initiation involves three major considerations: selection of explants, medium and culture conditions (Hall et al., 1988).
In this experiment, explants were collected from the plantlets which were contamination of free „*in vitro*" healthy plants for direct regeneration after 21 days of seed inoculation.

Two different combinations of auxins and cytochinins (V1 and V2) were used in the first stage of callus initiation and for callus culture establishment.
The beginning of callus development was observed within 2 weeks after explants inoculation on the inductive media.
The calli developed on these two variants were yellowish–green, partly friable, with a pronounced propensity to develop roots (Fig. 1, Fig. 2).

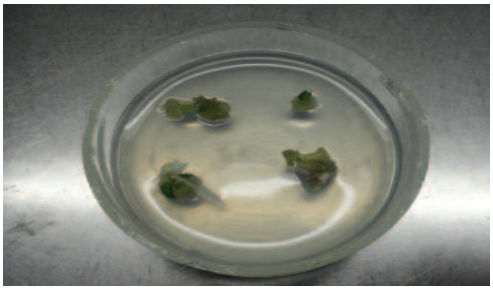


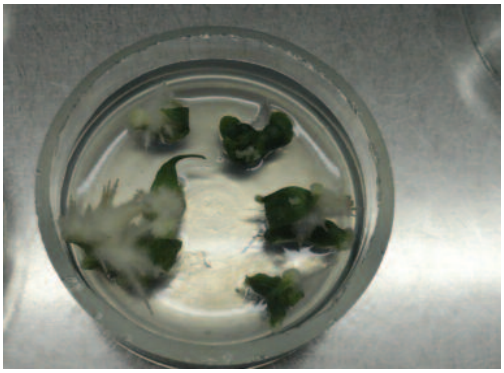
Figure 1. Developing calli induced from apex and explants after 3 weeks since inoculation on 0.5 mg/L NAA and 1 mg/L 2.4 D (Variant 1) of *C. annuum* L. (cv. Pintea)

The transfers were performed on fresh culture media at 3 weeks intervals, and the increase of the callus biomass on every culture media variant was recorded, by weighing their callus biomass from every culture vessel compared with the initial weight of the transferred callus piece.
The average increase of callus biomass /culture dish (4-6 explants inoculated/Petri plate) evaluated after 15 weeks of initiating the experiment with somatic chilli pepper explants cv. Pintea are shown in Table 2 and those for cv. De Cayenne in Table 3.

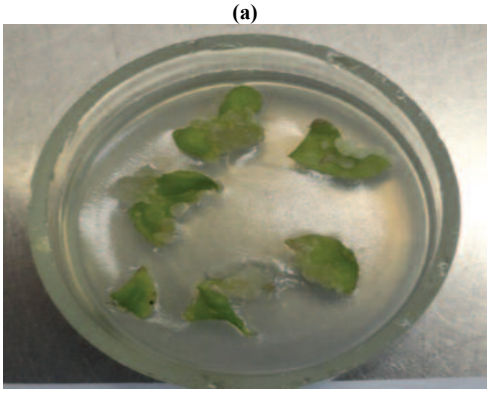
Table 2 Average of callus biomass increase/ culture vessel during a 15 weeks of initiation of the experiment in somatic explants of chilli pepper cv. Pintea. (g)

Variant	Type of used explant	Average of callus biomass increase/culture vessel (g)
V1	hypocotyl	2.74
V1	apex	1.29
V2	cotyledon	3.10
V2	young leaves	3.05
V2	apex	2.92

Legend: V1 = 0.5 mg/L NAA and 1 mg/L 2.4 D; V2 = 0.5 mg/L Kinetin



(a)



(b).

Figure 2. Developing calli induced from cotyledons and young leaves explants after 3 weeks since inoculation on Variant 2 of *C. annuum* L. cv. Pintea(a) and the cv. De Cayenne (b).

Table 3 Average of callus biomass increase/ culture vessel during a 15 weeks of initiation of the experiment in somatic explants of chilli pepper cv. De Cayenne (g)

Variant	Type of used explant	Average of callus biomass increase/culture vessel (g)
V1	hypocotyl	0.86
V1	young leaves	1.63
V2	cotyledon	1.99

Legend: V1 = 0.5 mg/L NAA and 1 mg/L 2.4 D; V2 = 0.5 mg/L Kinetin

The best results on the active growth of callus, were obtained for Pintea variety when it was utilized the hypocotyls fragment explants inoculated on V1 variant supplemented with 0.5 mg/L NAA and 1 mg/L 2.4 D and when utilized the cotyledons cultivated on media supplemented with kinetin 0.5 mg/L.
Comparing with this genotype, for explants of De Cayenne variety cultivated on the same combination of tested culture medium (MS supplemented with 0.5 mg/L kinetin), the results of 64% were obtained only at the

cotyledons level and for the other types of tested somatic explants, also the values recorded was lower than the cv. Pintea explants.

The calli developed from chilli pepper cv. De Cayenne young leaves cultivated on V1 variant supplemented with 0.5 mg/L NAA and 1 mg/L 2.4 D, were yellowish – green, compact with an activ proliferation activities (Fig. 3).

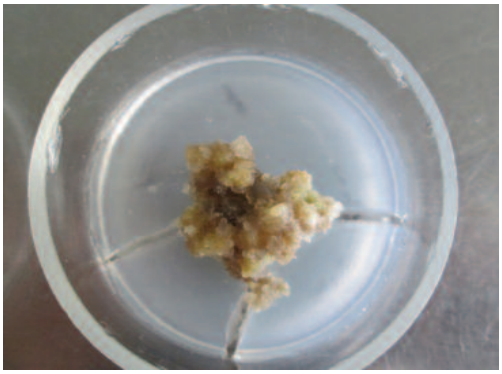


Figure 3. Developing calli induced from young leaves and explants after 15 weeks since inoculation on 0.5 mg/L NAA and 1 mg/L 2.4 D (Variant 1) of *C. annuum* L. (cv.De Cayenne)

Comparing the results for proliferative growth of inducing callus, the most successful explants are often cotyledons for both tested genotype varieties of hot chili pepper, Pintea and De Cayenne.

CONCLUSIONS

Through repeated subculturing at intervals of 3 weeks of various somatic explants for callus proliferation, on the hormonal recipes Variant 1 and Variant 2, it was found after approximately 4 months to the experiments initiation, an increase in biomass of callus culture vessels, that registered average values of 2.62 g / Petri plate culture for Pintea variety, respectively 1.49 g / Petri plate for De Cayenne variety chilli pepper explants.

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