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Establishment of Tissue Culture Protocol in *Brassica* (*B. napus* L.)

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Abstract: Hypocotyl segments of rapeseed cultivar "Rainbow" were cultured on B5 medium supplemented with several concentrations of 2,4-Dichlorophenoxy Acetic Acid (2,4-D) ranging from 0.0-4.0 mg l^{-1}. Response to callus induction differed according to the levels of hormonal treatments. 2,4-D @1.5 mg l^{-1} induced growth of compact, green callus whereas other levels of 2,4-D produced poorly developed calli. Embryogenic calli were transferred to the medium supplemented with different combinations of Benzylaminopurine (BAP) and Naphthalene Acetic Acid (NAA) at levels of 0.0/0.0, 0.1/0.5, 1.0/0.5, 2.5/1.0, 5.0/1.0 and 5.0/2.0 mg l^{-1}, respectively. More than 50% of the calli cultured on the medium supplemented with BAP@ 5.0 mg l^{-1} with NAA 1.0 mg l^{-1} regenerated into plantlets.

Key words: Calli, regeneration, *Brassica napus,* tissue culture, hormonal treatments

INTRODUCTION

The establishment of an efficient plant tissue culture technique represents a basic step in non-conventional improvement of crop plants. *Brassica napus* is one of the world's most important source of vegetable oil and protein meal[1]. Productivity in *Brassica napus* is low in Pakistan due to biotic and abiotic factors. These factors include insects/pests, diseases, drought and other environmental stresses. Thus conventional breeding programme has to be supplemented by non-conventional approaches in order to cope with these stresses and to increase the productivity of this crop.

Biotechnological techniques such as genetic engineering require reliable and efficient tissue culture protocol. This area needs to be researched for a high frequency regenerative system because it is a preliminary step towards advanced non-conventional techniques such as genetic transformation[2, 3]. Present study is aimed at protocol standardization for callus induction and plant regeneration as a preliminary step towards transformation of canola (*B. napus* L.).

MATERIALS AND METHODS

The study was conducted at Agricultural Biotechnology Programme (ABP), National Agricultural Research Centre, Islamabad. Seeds of rapeseed variety "Rainbow" were soaked in 50% ethanol for 2 min. then surface sterilized for 15 min. in 50% commercial sodium hypochlorite solution. Finally, seeds were rinsed 3 times in sterilized distilled water and were germinated in aseptic conditions on filter papers using plain medium having no growth regulators. Sucrose (3%) was used as a carbon source and pH of the medium was adjusted to 5.8 before solidifying it with 0.8% agar.

Cultures were kept at a light intensity of 500 lux for three days followed by higher light intensity of 2000 lux for additional ten days. Hypocotyls from 10-14 days old plants were cut in 5-10 mm segments and were inoculated on the callus induction medium B5[4] basal medium having different levels of 2,4-D (Table 1). Regenerative calli were transferred to the shoot regeneration medium (B5 salts and vitamins) supplemented with different combinations of BAP and NAA. Data was recorded by counting plantlets formed/total number of calli.

RESULTS AND DISCUSSION

The response of explants was dependent on the type of culture media used (Table 1) as reported by Walters and Earle[8]. It was noted that callus proliferation started from the cut ends of hypocotyl on B5 medium enriched with 0-4 mg l^{-1} 2,4-D. Callus induction was observed as 90% in the medium supplemented with 2,4-D @ 1.5 mg l^{-1} while callus induction was lower (30-50%), at other levels of 2,4-D. Increasing 2,4-D level beyond 1.5 mg l^{-1} suppressed callus induction in this study. Similar results were also achieved by others[5, 6]. Response to callus induction of 2,4-D was found to be the best for fast and granular callus formation after 6 weeks. Calli which were greenish and modulated in texture were maintained by

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Table 1: Effect of different concentrations of 2,4-D on callogenesis from hypocotyl explants of 12 days old seedlings of *Brasica napus* L.

2,4-D mg l^{-1}	Callogenesis response			General observations
0.00	-	-	-	No callus
1.00	-	-	+	Brown callus with slow growth rate
2.00	-	+	++	Green callus with slow growth rate
3.00	-	-	++	Brown callus with slow growth rate
1.5	+	++	+++	Green callus with moderate growth rate
2.5	+	++	++	Green callus with slow growth rate
3.4	+	+	++	Brown callus with slow growth rate
4.00	+	+	+	Brown callus with slow growth rate

No callus
\+ Inconspicuous ++ Average +++ Good

Table 2: Callus regeneration using B5 medium with various levels of BAP and NAA in *Brassica napus* L.

Concentrations of BAP and NAA (mg l^{-1})	Total number of calli	Calli produced shoots	Calli produced plantlets	Plantlet formation (%)
0.00/0.00	24	0	0	0.0
0.1/0.5	24	2	0	0.0
1.0/0.5	24	5	0	0.0
2.5/1.0	24	7	1	4.2
5.0/1.0	24	1	13	54.6
5.0/2.0	24	3	7	29.4

transferring them on the same but fresh medium every three weeks.

Lower levels of hormones had an adverse effect on shoot formation and the process was extremely slow as reported by Narasimhulu and Chopra[7]. BAP @ 5 mg l^{-1} with NAA 1 mg l^{-1} showed not only rapid shoot and root formation but the regenerated plans were healthier and greenish. The success rate of plantlet formation was more than 50% (Table 2).

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