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Callogenic Studies of *Achyranthes aspera* Leaf Explant at Different Hormonal Combinations

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Abstract: With the objective to promote *in vitro* callus induction, leaf segments of *Achyranthes aspera* were inoculated on basal MS medium supplemented with 3.0% sucrose and 0.8% agar with different concentrations of 2,4-D alone and in combination with NAA, BAP, IAA, IBA and Zeatin. The explants were maintained in growth room at 25±1°C and 16 h light cycle. The best callus induction was obtained with 2,4-D (1.0 and 2.0 mg L^{-1}) in combination with NAA (0.5 mg L^{-1}). Callus induction and good texture from leaf explant was also observed at 2,4-D with BAP. On these combinations morphologically, light green, soft, compact and non-embryogenic callus (Type III callus) was observed. While morphology of callus and callogenic response was poor at 2,4-D alone or in combination with other hormones at different concentrations.

Key words: *Achyranthes aspera*, Putkanda, growth regulator, callus induction

INTRODUCTION

In Pakistan, medicinal plants and their extracts are widely used in traditional medicines. *Achyranthes aspera* (Putkanda), belonging to the family Amaranthaceae is traditionally used to treat a number of diseases including diabetes mellitus, renal and cardiac dropsy. A number of valuable secondary metabolites such as betaine, achranthine and quinolizidine alkaloids are also produced.

Production of calli form from fragments of stems, leaves and roots is mainly carried out to determine the culture conditions required by the explants to survive and grow (Samoylov *et al*., 1998), to study cell development (Little *et al*., 2000), to exploit products coming from the primary and secondary metabolism and to obtain cell suspension in propagation (Rout *et al*., 2000). The nutritional requirements for the growth under *in vitro* conditions vary for different species, variety and even between different parts of the plant. So, it is therefore, necessary to optimize the culture conditions (Nagao *et al*., 1994). However, MS medium (Murashige and Skoog, 1962), or its modifications, has produced satisfactory results for several species (Rout and Das, 1997). The production of callus mainly depends upon determination of an adequate balance of growth regulators. However, this balance varies largely in relation to the explant type and to the plant species under investigation. The manipulation of the appropriate levels of auxins and cytokinins is crucial to define the balance of growth regulators so that there is induction of callus formation in the different types of explants (Franklin and Dixon, 1994).

Tissue culture of *A. aspera* has not previously been studies. This study reports on investigation on the production of *in vitro* callus of *A. aspera*, using segments of leaf as explants.

MATERIALS AND METHODS

A. aspera material was collected from the locality of Quaid-i-Azam University, Islamabad, Pakistan. The leaves were cut and dipped in distilled water. Healthy, soft and green leaves were selected as an explant source. The leaves were sterilized by immersion in 0.1% mercuric chloride solution for 2.0-3.0 min and washed 3-4 times with sterilized distilled water in laminar flow cabinet. Cut leaf segments (3-4 mm) were used as explants. The basal MS medium (Murashige and Skoog, 1962) with 3% sucrose solidified with 0.8% agar was used. Aqueous solutions of growth regulators (2,4-Dicholorophenoxy acetic acid (2,4-D), Indol-3-butyric acid (IBA), α-naphthalene acetic acid (NAA), 6-benzylamino purine (BAP) and Zeatin (Zea)) were added in required concentration. The medium was heated to dissolve the agar and then dispensed in either test tubes or flasks and autoclaved. The cultures were incubated at 25±1°C with 16 h day light cycle. After 30 days, the cultures were visual examined for formation of callus, texture and coloration.

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RESULTS AND DISCUSSION

Callogenic response from leaf explant of *A. aspera* was observed at all concentrations and combination of growth hormones. The texture, morphology and colour of callus varied due to the synergetic effect of hormone either singly or in combination. It was observed that at concentrations of growth regulators type III callus was produced. Such type of callus shows non distinct somatic embryos (non-embryogenic) and easily dispersible. But to be compact and soft is the properties of type I and type II calluses. These variations may be due to influence of exogenous as well as endogenous concentration of growth hormones.

2,4-D, at 1.0 and 2.0 mg L^{-1} produced light green and soft callus (Table 1) but at lower (0.5 mg L^{-1}) and higher concentrations (3.0 and 4.0 mg L^{-1}), the callus morphology and callus mass changed. Similar callogenic response has been to 2,4-D for other genera was reported by Wakhlu and Sharma (1998). Also the new cells produced in the callus would probably use minerals and other source of carbon other then sucrose, contained in the basal medium (Franklin and Dixon, 1994).

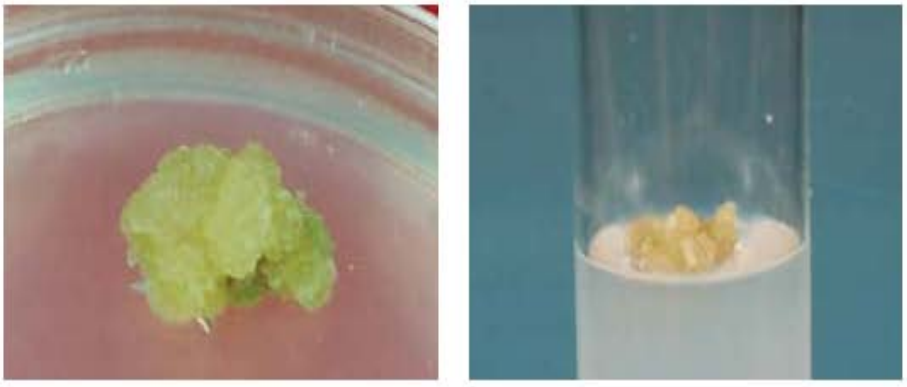
In spite of 2,4-D, be one of the most important factor in the process of stimulating the callus, it is also important to observe the interactive effect that can happen with other hormones. The morphogenesis induction or cell division differs among genotype and on application of hormone. 2,4-D in combination with NAA in MS medium produced morphologically better callus as well as increased mass of callus (Fig. 1a). Increased amount of 2,4-D with lower amount of NAA has batter effect on callus induction while increased amount of NAA has better effect the development of callus in negative way (Table 1), delaying the growth and promoting the browning of callus. For optimization of basal medium for *in vitro* cultures, several formulations can be made by changing hormonal concentrations (Bertolucci, 2000).

Same results were also observed at BAP with 2,4-D but lower concentration of BAP (0.1 mg L^{-1}) in combination, changed callogenic response from leaf explant and coloration of callus. These results demonstrate that for callus induction not a single combination, but different combinations of hormones at different concentrations may vary the results.

Other treatments of 2,4-D in combination with IAA, IBA and Zea presented disappointing results because callus induction was slow with decreased friability consistency and diameter (Fig. 1b). The intensive effect in yield optimization of callus induction consequently

Table 1: Callogenic response of *A. aspera* leaf explants at different concentration/combinations of hormones

Growth regulator	Conc. (mg L^{-1})	Callogenic response	Morphological characters
2,4-D	0.5	++	Green, soft, non-embryogenic, compact
	1.0	+++	Light green, soft, non-embryogenic, compact
	2.0	+++	Green, soft, non-embryogenic, compact
	3.0	++	Light brown, soft, non-embryogenic, compact
	4.0	++	Yellowish brown, compact, soft, non-embryogenic
2,4-D/ BAP	0.5/0.5	+++	Light green, soft, non-embryogenic, friable
	1.0/0.5	+++	Light brown, soft, non-embryogenic, compact
	2.0/0.5	++++	Light brown, soft, non-embryogenic, compact, friable
	3.0/0.5	++++	Light brown, soft, non-embryogenic, compact
	4.0/0.5	+++	Brown, compact, hard, non-embryogenic
	1.0/0.1	++	Light brown, soft, non-embryogenic, compact
	2.0/0.1	++	Light brown, soft, non-embryogenic, compact
	3.0/0.1	+++	Brown, soft, non-embryogenic, compact
	4.0/0.1	++	Dark brown, soft, non-embryogenic
2,4-D/NAA	1.0/0.5	++++	Green, soft, non-embryogenic, compact, friable
	2.0/0.5	++++	Light green, soft, non-embryogenic, compact
	3.0/0.5	+++	Green, soft, non-embryogenic, compact
	4.0/0.5	+++	Greenish brown, soft, non-embryogenic,
	1.0/1.5	++	Light green, soft, non-embryogenic, compact
	2.0/1.5	++	Light green, soft, non-embryogenic
	3.0/1.5	++	Light green, soft, non-embryogenic, friable
	4.0/1.5	++	Greenish brown, green, soft, non-embryogenic
2,4-D/IAA	1.0/0.5	+++	Light green, soft, non-embryogenic, compact
	3.0/0.5	++	Brownish yellow, soft, non-embryogenic
	4.0/0.5	++	Dark brown, non-embryogenic
2,4-D/IBA	1.0/0.5	++	Light green, soft, non-embryogenic, compact
	2.0/0.5	+++	Light green, soft, non-embryogenic
	3.0/0.5	++	Yellowish green, soft, non-embryogenic
	4.0/0.5	++	Brown, non-embryogenic, soft
2,4-D/Zea	1.0/0.5	++	Yellowish green, soft, non-embryogenic
	2.0/0.5	++	Brown, soft, non-embryogenic, compact
	3.0/0.5	+	Brown, compact
	4.0/0.5	-	Swelling of leaf



(a) (b)

Fig. 1: Callogenesis from leaf explant (a) 2,4-D (3.0 mg L^{-1}) + NAA (0.5 mg L^{-1}); (b) 2,4-D (2.0 mg L^{-1}) + Zea (0.5 mg L^{-1})

increases, changing the concentration and type of hormone (De-Silva *et al*., 2003). Different other factors may be involved, that may be hormonal combination, age of explant because varying the age hardens the leaves. These results may further be exploited for production of secondary metabolites in cell suspension culture and may also be useful for other tissue culture techniques.

It can be concluded that appropriate level of 2,4-D, either isolated or interaction with BAP and NAA are able to induce the formation of calli from leaf explants of *A. aspera*.

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