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EFFECT OF AUXIN AND CYTOKININ ON THE BRITTLENESS OF CHICKPEA CALLUS

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ABSTRACT

Chickpea (*Cicer arietinum* L.) var. 6163 nodes were cultured on supplemented B-5 medium. The combination of auxin (3 mg NAA) and cytokinin (0.6 mg BAP) gave calli of approrpiate degree of brittleness and fresh weight. These calli yielded more (9×10^2) free single cells in suspension as compared to compact calli (2×10^2).

INTRODUCTION

The necessity of exogenously supplied auxin for *in vitro* production of callus from an explant was reported by Earle and Torrey (1965). Explants from almost any part of the plant, such as seeds, stems, roots, leaves or fruits can develop a callus by culturing on the surface of the medium (Street, 1977). Continued sub-culture of these callus masses can continue for long periods of time (Earle, 1974; Hartmann and Kester, 1983).

Although callus tissue culture may appear to be a uniform mass of cells, but in reality its structure is relatively complex with considerable morphological, physiological and genetic variation within the callus (Street, 1977; Sarwar, 1984). A number of different culture media has been used by various research workers to grow callus i e., MS, RM, SH, etc. These media contain macroelements, particularly nitrogen as nitrate (NO_3) and ammonium ions (NH_4), sucrose and certain vitamins but initiation of cell division and callus production require both a cytokinin and an auxin supplied in the proper proportion (Skoog and Miller, 1957). Auxin, as IAA, NAA or 2,4-D is the hormone primarily used at a high concentration to produce callus. Cytokinin as kinetin or 6-benzylaminopurine (BAP) is supplied in a small amount on the basis of significant effect of auxin and cytokinin on callus development (Hartmann and Kester, 1983).

In the present investigation, B-5 medium was tried to supplement with

various combinations of auxin and cytokinin for the development of brittle callus with appropriate size to increase the number of free single cells in suspension for cell culture (Gamborg *et al.*, 1968).

MATERIALS AND METHODS

Seeds of chickpea (Cicer arietinum L.) var. 6153, obtained from Nuclear Institute for Agriculture and Biology, Faisalabad, were washed with 1% detergent (Zip) in a beaker and rewashed three times with distilled water. For surface sterilization, they were treated with 20% sodium hypochlorite solution for 10 minutes at room temperature, then rinsed five times in autoclaved distilled water in aseptic conditions to remove the sodium hypochlorite completely.

Surface sterilized seeds were dried on autoclaved filter paper and cultured in test tubes containing autoclaved 9 to 10 ml of B-5 inorganic salts medium for raising the seedlings. Then, the test tubes were maintained at 25-27°C under 1000 lux alternating 12h light and dark period. Nodes of the 3 to 4 weeks old seedlings were used as a source of explant and they were cultured in test tubes containing 15 ml callus inducing medium under the already specified conditions. For cell suspension, calli were put into the same liquid medium and agitated on a gyratory shaker.

After 15 days of culture, the effect of various combinations of auxin and cytokinin was observed on the degree of brittleness by visual study, determined the fresh weight and counted the number of free single cells in suspension per ml released by the brittle and compact calli with a haemocytometer.

RESULTS

The combined effect of different combinations of auxin (NAA) and cytokinin (BAP) on 15 days old culture are shown in Table 1. The different hormone combinations for callus induction produced variable results. All the explants (Nodes) produced callus but did not show equal developing efficiency both in degree of brittleness and in fresh weight. Data showed that combinations III, II and I (with auxin concentration of 2,1 and 0.5mg respectively) developed calli which were hard in texture and low in fresh weight. With gradual increase in auxin (3, 4 and 5 mg) in the combinations IV, V and VI, there was a significant increase in the degree of brittleness but the weight of calli did not show any

significant increase. The combination VII, having higher (1.0 mg) concentration of cytokinin as compared to the rest of the combinations (0.5 mg), gave results which were similar to combination III.

As influenced by texture (brittle or comapct) of calli, the brittle calli released 9 x 10^2 free single cells per ml in suspension, while the release from compact calli was 2 x 10^2 cells per ml, after two hours agitation.

Table 1. *Effect of auxin and cytokinin on the brittleness and fresh weight of chickpea node callus*

Combinatinos of auxin and cytokinin		Concentration (mg/l)	Fresh weight (mg)
I	NAA	0.5	123
	BAP	0.5	
II	NAA	1.0	160
	BAP	0.5	
III	NAA	2.0	161
	BAP	0.5	
IV	NAA	3.0	189
	BAP	0.5	
V	NAA	4.0	190
	BAP	0.5	
VI	NAA	5.0	192
	BAP	0.5	
VII	NAA	3.0	159
	BAP	1.0	

Degree of brittleness

Auxin: $I < II < III < IV \leqslant V = VI > VII$

Cytokinin: $III = VII$

The Combination of auxin (NAA) 3 mg and cytokinin (BAP) 0.5 mg appeared highly suitable to produce brittle callus with appropriate fresh weight of 189 mg, after 15 days of culture.

DISCUSSION

Nodes of chickpea var. 6153, produced brittle calli with appropriate degree of brittleness and yield by culturing on modified B-5 medium, supplemented with auxin (3 0 mg NAA) and cytokinin (0.5 mg BAP) From the results of different experiments, it was obvious that the combined effect of auxin and cytokinin produced brittle calli and increased fresh weight (189 mg) upto certain limit (3.0 mg NAA and 0.5 mg BAP). Higher concentration of auxin did not show any marked improvement in the degree of brittleness and fresh weight (190 and 192 mg) of callus, whereas an increase in the concentration of cytokinin (1.0 mg) did not show any increase in the degree of brittleness and fresh weight. It was also observed that concentration higher than 3 mg NAA after 25 to 30 days of culture, enhanced root initiation and showed a significant decrease in fresh weight (data not presented). It could be concluded that in chickpea both auxin and cytokinin were necessary for the development of calli of appropriate brittleness and fresh weight. Similar observations were reported by Skoog and Miller (1957), Earle (1974) and Hartmann and Kester (1983).

It is clear from the present results that possible increase in auxin with small amount of cytokinin developed more brittleness in the texture of chickpea calli and these brittle calli released much more (9×10^2) free single cells per ml in suspension as compared to compact calli. Thus it may be stated that brittle calli are more suitable for the source single cell inocula in cell culture.

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