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MORPHOGENESIS IN CELL SUSPENSION CULTURES OF CAULIFLOWER

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Numerous somatic embryos developed in the cell suspension cultures of cauliflower, cultured on MS-medium enriched with 0.5- 5.0 mg/l of K or BAP. Alongwith somatic embryos shoot buds also developed on higher concentrations of both K and BAP. Addition of IAA supressed the somatic embryo formation, while 2.4 -D added in induction medium completely checked the embryogenesis and caulogenesis. IAA alone induced rhizogenesis while 2,4-D alone in medium did not evoke any kind of morphogenesis.

Keywords : Morphogenesis ; Embryogenesis; Callus cultures; Suspension cultures.

Introduction

The technique of cell and tissue culture is being largely used in micro-propagation of elite crops (Giles and Sen, 1983). Cauliflower is an important vegetable crop grown throughout the world, so attempts have also been made to propagate it by cell and tissue culture technique. Earlier Pareek and Chandra (1978), got shoot buds and embryoids from the callus cultures in the plant.

The present study deals with the morphogenesis in suspension cultures of cauliflower.

Materials and Methods

Seeds procured from Govt. Research Station, Durgapura, were grown in the experimental beds. Leaves were collected from one month old plants. Mid-rib from the leaf was isolated by scalpal and surface sterilized with 0.1% mercuric chloride solution. After thorough washings with double distilled water, 10 mm long segments of mid-rib were cultured on agarised MS-medium (Murashige and Skoog, 1962) enriched with (Kinetin); BAP (Benzyle aminopurine); and/or IAA (Indole acetic acid); IBA (Indole butyric acid); and 2.4 -D (2,4- dichlorophenoxy acetic acid). Better callus formation was observed on medium having IAA(1.0 mg/1) + K (0.5 mg/1). Fragile whitish callus was augmented on the same medium by monthly sub-culturing.

Suspensions were raised as described earlier (Singh and Chandra, 1986). Experiments were carried out on horizontal shaker fitted in a growth room at 26±2°C temperature and constant illumination of 3000 lux.

Results and Discussion

Establishment of cell suspension culture

Callus growing on agarised MS medium having IAA 1.0 mg/1 and K 0.5 mg/1 was transferred to the medium of similar hormonal and chemical composition except agar. Callus soon dissociated into

Figure 1 - Embryoid formation (1.0 mg/1*K*); Fig.2 - Shoot buds attached with callus (K 1.0-5.0 mg/1); Fig.3-shoot buds.

smaller fragments in liquid medium, experiments on morphogenesis were carried out after three subcultures from liquid to liquid medium. The suspension had a large number of isolated, free floating single cells and a few smaller cell clumps of about 1 mm diameter. Cells were healthy, vacuolated, thin walled and shaped variously, like the suspensions of *Daucus* (Halparin and Wetherell, 1965) and *Brassica campestris* (Singh, *et al.*, 1981, Singh and Chandra,1984).

Effects of medium having IAA or IBA

Suspensions growing on 0.5 -5.0 mg/1 of IAA or IBA became brownish in a month of incubation. Lower concentrations 0.5 -1.0 mg/1 of both the auxins failed in the induction of morphogenesis, while higher concentrations evoked rooting. IBA induced better rooting than IAA as also from the shoots of this variety (Singh and Mathur, 1985) and suspension cultures of *B. campestris* (Singh and Chandra, 1984).

Effects of medium having K or BAP

On K 0.5 -0.1 mg/1, suspension became greenish. Number of single isolated cell decreased, while cell clumps increased in number and size. However, morphogenesis was not observed. On increasing the concentration of K (0.5 mg/1) a few embryoids (which were free floating after globular stage), were observed after 30th day of incubation. Their number increased on 1.0 mg/1 of K but declined on further increase in K level in the medium. Best response was

TABLE 1

EFFECT OF AUXINS AND CYTOKININS USED SINGLY IN MEDIUM ON MORPHOGENESIS IN CALLUS CULTURES OF CAULIFLOWER

MS medium	(mg/l)	Response	% Response
IAA	0.5	NM	-
	1.0	NM	-
	3.0	R + +	100
	5.0	R + + +	100
IBA	0.5	NM	-
	1.0	NM	-
	3.0	R + +	100
	5.0	R + + +	100
K	0.05	NM	-
	0.1	NM	-
	0.5	E + +	100
	1.0	E(maximum)	100
	3.0	E + + S + +	100
	5.0	E + S + + +	100
BAP	0.05	E +	100
	0.1	E + +	100
	0.5	E + + +	100
	1.0	E + +	100
	3.0	E +	100
	5.0	E +	100

E= Embryoid; S= Shoot buds; R= Roots;

NM= No morphogenesis; +,++,+++ = Relative response

TABLE 2.

SHOWING EFFECTS OF COMBINATIONS OF CYTOKININS (K AND BAP) AND AUXINS (IAA AND 2, 4-D) ON MORPHOGENESIS IN CELL SUSPENSION CULTURES OF CAULIFLOWER

MS medium ++		Response	% Response
Cytokinin mg/l	Auxin mg/l		
K 1.0 +	IAA		
	0.1	E + + +	100
	0.25	E + +	100
	0.5	E +	100
	1.0	E + R (few)	100
		R + +	
	2, 4-D		
	0.1	NM	100
	0.25	NM	100
	0.5	NM	100
	1.0	NM	100
BAP +	IAA		
1.0	0.1	E ++	100
	0.25	E +	100
	0.5	E +	100
	1.0	E +	100
	2, 4 D		
	0.1	E +	20
	0.25	NM	100
	0.5	NM	100
	1.0	NM	100

E= Embryo; R= Roots; NM= No morphogenesis;

+,++,+++ = Relative response.

observed on 1.0 mg/l of K (Table -1; Fig. 1). A number of shoot buds also developed along with the embryoids on K 1.0 -5.0 mg/l but they remain attached with the callus (Fig.2). Although numerous shoot buds originated but only a few developed to conspicuous size (Fig.3). Almost similar results were recorded on BAP 0.05 - 5.0 mg/l (Table 1).

Somatic embryogenesis has also been reported in callus culture of this variety(Pareek and Chandra, 1978) but to the best of our knowledge there is no report of somatic embryogenesis in suspension culture, except the preliminary report (Singh and Chandra,1986). Like these findings higher level of K and BAP has also been found to be inhibitory for somatic embryogenesis (Kohlenbach, 1978), while formation of shoot buds on higher levels of cytokinins has also been reported in suspensions of *B. campestris* (Singh, *et al;* 1981 Singh and Chandra, 1984). Minute microscopic observations of suspensions growing on K (1.0 mg/l) revealed that there was no morphogenesis upto the 10th day of incubation. After a fortnight smaller, whitish and semispherical cell clumps developed which became quite conspicuous after 20th day.

One month old clumps of irregular size became compact and greenish. The development of embriyoids was not synchronous, as all the stages of development(i.e. from single cell to torpedo shaped embryoids), were present in a single culture vessel. Similarly, Pareek and Chandra (1978) has also reported formation of embryoids and shoot buds in callus cultures of this variety. In present study, in suspension cultures the frequency of embryoid formation was higher.

Effects of medium having cytokinin *with auxins-* As indicated earlier, maximum number of embryoids were recorded on K (1.0 mg/l). Addition of IAA or 2,4 -D supressed the process (Table 2). Only a few embroyoids were formed on combination of K and IAA, while on substitution of IAA by 2, 4 -D caused total inhibition of embryoid formation. Samilar results were observed in callus and cell suspension cultures of *B. campestris* (Singh *et al;* 1981; Singh and Chandra, 1984) and *Daucus* (Halparin and Wetherell, (1965).

References

Giles K and Sen S K 1983(eds), *Plant cell culture in crop imporvement*, Plenum press, Newyork.

Halparin W and Wetherell D F 1965, *Science* **147** 756

Kohlenbach H W 1978, Comparative somatic embryogenesis

In: Frontiers of plant tissue culture (eds) Thorpe T A

Proceedings of 4th International Congress of plant tissue and cell cultures, Univ. of calgary Alberta Canada. PP 59-66

Murashige T and Skoog F 1962, *Physiol. Plant.* **15** 473

Pareek L K and Chandra N 1978, *Pl.Sci. Lett.* **11** 311

Singh S and Chandra N 1984, *Plant Cell Rep. U S A* **31**

Singh S and Chandra N 1986, *Beitr. Biol. Pflanzen* **60** 191

Singh S and Mathur A 1985, *Curr.Sci.* **54** 381

Singh S and Mathur A 1987, *Acta Bot. Indica* **15** 98

Singh S, Banu S, Pareek L K and Chandra N 1981, *Indian J.Exp. Biol.* **19** 658