J. Phytol. Res. **8** (1):57-61, 1995

IN VITRO STUDIES ON CALLUS INITIATION AND ORGANOGENESIS IN *ALLIUM CEPA* VAR. PUSA RED

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The seeds of *Allium cepa* var. Pusa red when cultured on Murashige and Skoog's (MS) medium supplemented with various amounts and combinations of growth regulators, variable responces on growth and morphogenetic behavior was observed. Auxins induced callusing and organogenesis while cytokinin induced rooting. NAA in different combinationwith Kinetin induced both shoot and root at various levels. Kinetin induced maximum rooting in callus which could be dedifferentiated on 2,4-D containing medium and *vice versa*. Combination of NAA and 2,4-D supported maximum growth of undifferentiated callus cultures.

Keywords: *Allium cepa;* Callus culure; Differentiation; Growth regulators; Morphogenesis.

Introduction

Plant tissue cultures frcm several dicotyledons and monocotyledons have been employed to study the organogenesis.[1] *In vitro* propagation of a number of bulbous plants have been done[2-4]. A method of continuous production of adventitious shoots applicable to a wide range of bulbous species was described by Hussey[5].

Calllus cultures of *Allium cepa* have been raised to study the flavour components[6]. Fridborg[7] studied the growth and organogenesis in cultures of *Allium cepa* var. *proliferum* with special reference to root formation. Studies have also been carried out on effect of physical factors on growth and differentiation of *Allium cepa* tissue cultures[1,8]. Present investigations were undertaken to study the callus initiation and its organeogenesis from seeds of *Allium cepavar.* Pusa red.

Material and Methods

The seeds of *Allium cepa* variety Pusa red were surface sterilized with 0.1 percent mercuric chloride and cultured on Murashige and Skoog's (MS)[9] medium supplemented with various growth regulators viz. L-Naphathalene acetic acid (NAA), Kinetin (K) and 2,4-Dichlorophenoxy acetic acid (2,4-D) in different concentrations and combination. The cultures were incubated at 26±1°C in continuous diffuse light of approximately 200 lux.

The callus thus obtained was subcultured after 6 to 8 week according to growth. The observations were taken at weekly interval.

Results and Discussion

The seeds germinated and the callus was produced from the radicle portion on touching the media. The seed germination and callus formation was influenced by concentration and combination of growth regulators present in the medium.

1. L-Naphthalene acetic acid (NAA): Seed germinated by emergence of radicle and upon contact with the medium, produced callus mass. Among different concentrations of NAA (2 to 20 mg/1) incorporated singly

into the medium, 15mg/1 of NAA supported maximum callusing (Table 1). Callus was also associated with root primordia and rootlets in all the NAA concentrations. But shoot buds were observed at hihgher concentrations only.

Root and shoot differentiation was observed in 30% replicates from 15.0 mg/1 to 17.5mg/1 concentration of NAA, after 15 to 21 days of first subculture. However, subsequent subcultures gradually suppressed the morphogenesis.

2. *Kinetin (K):* Among different kinetin concentrations (0.01 to 0.1 mg/1) used in the medium, none could induce callusing. Seeds germinated normally to produce seedlings (Table 1).

3. *NAA (10 mg/1) + K:* Callus formation was observed at only 0.04 mg/l concentration of kinetin, while none of the other concentrations (0.06 to 0.14 mg/l) could induce callusing (Table 1).

4. *Kinetin (0.04 mg/1) + NAA:* The callus was produced on all the concentrations of NAA (10-20 mg/1) associated with rootlets and root primordia. Maximum callus growth was observed on NAA (15 mg/1) (Table 1). Shoot differentiation was also observed at 15 and 17.5 mg/l of NAA (Fig.1). In general, the results were quite similar to NAA alone in the media (Table 1).

Prolonged culturing of callus for about 2-3 months on NAA (10 mg/l) induced organogenesis in 20 percent replicates forming root and shoot both. Morphogenesis was suppressed by subsequent subcultures.

The callus mass obtained after second subculture on10 mg/l of NAA, was subjected to different combination of growth regulators, and variable responses were observed. The results are given as below :-

(i) MS without growth regulators: Callus subcultured on growth regulator free medium produced roots from almost entire surface of the callus (Fig.2).

(ii) MS + NAA (10 mg/1): It induced rapid callus growth with rooting in 20 percent replicates after 15 days of subculture.

(iii) MS + K (0.04 mg/1): Vigorous rooting was observed in all the replicates and almost whole of the callus mass was converted into rootlets.

This callus with root differentiation, when transferred to medium containing 2,4-D (0.5 mg/1), dedifferentiated into mass of callus tissue (Fig.3), whihch again reverted back to rootlets on kinetin.

(iv) MS + NAA (10 mg/1) + K (0.04 mg/1): Only callus growth was observed in 60 percent replicates.Among the remaining, rooting (20 percent) or shoot formation (10 percent), or both (10 percent) was observed.

(v) MS + NAA (10 mg/1) + 2,4-D (0.5 mg/1): The medium favoured vigorous growth of undifferentiated callus mass.

5. *NAA (10 mg/1) + K (0.04 mg/1) + 2,4-D:* The seeds produced friable callus on all the concentrations of 2,4-D (0.2 to 4.0 mg/1) in combination with all NAA and K. However 2,4-D (0.5 mg/1) favoured maximum callus growth (Table 1).

Few roots were observed alongwith the callus. Higher doses of 2,4-D (1.0 and 4.0 mg/1) were lethal for callus

Table 1. Effect of growth regulators on callus initiation and organogenesis from seeds.

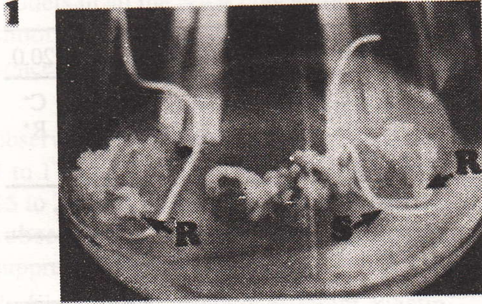
Growth regulators in medium (mg/l)	Variable content (mg/l)	Concentration of growth regulators (mg/1) and results.								
NAA	NAA	2.0	4.0	6.0	8.0	10.0	12.0	15.0	17.0	20.0
	(2-20)	NS	NS	C^{+} R^{+}	C^{+} R^{+}	C^{++} R^{++}	C^{+++} R^{++}	C^{++++} R^{+++} S^{+}	C^{+++} R^{+++} S^{+}	C^{+} R^{+}
K	K	0.01	0.02	0.04	0.08	0.1				
	(0.01-0.1)	NS	NS	NS	NS	NS				
NAA (10) + K	K	0.04	0.06	0.08	0.12	0.14				
	(0.04-0.14)	C^{+} R^{+}	NS	NS	NS	NS				
K (0.04)	NAA	10	12.5	15.0	17.5	20.0				
	(10-20)	C^{+} R^{++}	C^{++} R^{++}	C^{++++} R^{+++} S^{+}	C^{+++} R^{+++} S^{++}	C^{+} R^{+}				
NAA (10) + K (0.04)	2, 4-D	0.2	0.5	0.75	1.0	4.0				
+ 2,4-D	(0.2-4.0)	C^{++} R^{++}	C^{++++} R^{+}	C^{+++} R^{+}	—	—				

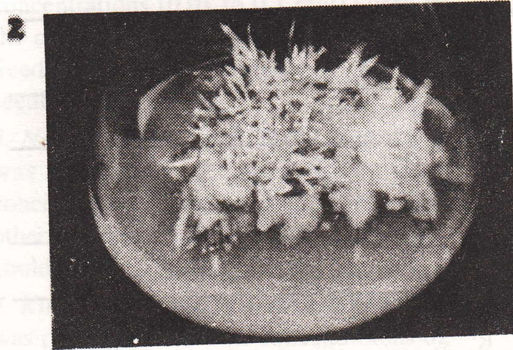
NS : Normal seedling; C : Callus; R : Root; S: Shoot; + : Poor; ++: Moderate; +++: Good; ++++: Excellent.

formation and even seed germination.

The induction of differentiation and the regeneration of complete plants from cultured cells depend on being able to manipulate the process of determination. Plant regeneration from explants or callus may proceed via organogenesis or embryogenesis[10]. Among many factors that influence organogenesis *in vitro* e.g. the source of nitrogen and carbon,pH of the medium and physical conditions of culture, the most important single factor seems to be the plant growth regulators[11,12]. In general the monocotyledons do not show a pronounced response to cytokinins and require high concentrations of potent auxins such as 2,4-D to achieve changes in the development of cultured tissues[12].

During present investigations although absence of growth regulators induced rooting but addition of kinetin had promotive effect on rooting. NAA and kinetin both were required for differentiation into root and shoot. Thus the root, shoot





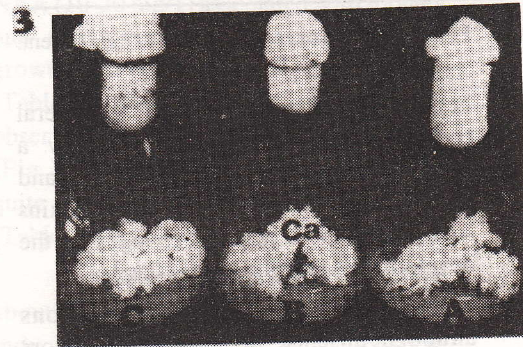


Fig. 1 Organogenesis of callus into root and shoot on kinetin and NAA.
R-Root, S-Shoot.

Fig. 2. Formation of roots on entire callus surface.

Fig. 3. Conversion of rooted callus, produced on kinetin into undifferentiated mass of callus on 2,4-D containing media.
A-Rooted callus on kinetin
B-Partially converted rootlets into callus on 2,4-D (Ca-Callus) C-Undifferentiated mass of callus on 2,4-D.

differentiation is a function of quantitative interaction between auxin and cytokinin specific to particular plant species.

According to Yeoman[1] the culture established on 2,4-D containing media from most of the plant parts consists of what appears to be root primordia arrested at different stages of development. These root types cultures can be serially propagated and form roots when 2,4-D is deleted from the culture medium. This obsevation has been expressed by the present cultures also, in which arrested root type cultures produced on 2,4-D transformed into mass of rootlets when transferred to Kinetin containing media; which dedifferentiated again in to callus on 2,4-D, These two growth regulators reacted as switching mechanism for redifferentiation and dedifferentiation. However the auxin NAA at higher levels induced both root, as well as, shoot formation.

Fridborg[7] reported that kinetin stimulated shoot formation in callus cultures isolated from bulb scales of *Allium cepa* var. *proliferum,* while in the present study contrasting results were recorded on the callus obtained from hypocotyl. The callus raised on NAA, transformed into roots on kinetin. This difference in callus behaviour

may be due to presence of endogenous level of auxin in the callus cells, which was produced on high auxin media.

The morphogenetic potential diminished in subsequent subculturing thereby reducing the differential expression of genetic material of the callus cells.

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