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Effect of some growth regulators on the initiation and growth of sunflower callus

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ABSTRACT

Callus induction occurred on stem segments of *Helianthus annuus* L. seedlings grown on a modified Murashige & Skoog medium. The medium which promoted the most rapid and sustained callus growth was MS medium containing benzyladenine and indolebutyric acid at concentrations of $0{\cdot}05\,mg\,l^{-1}$ and $1{\cdot}0\,mg\,l^{-1}$ respectively. Addition of activated charcoal to the nutrient media containing various growth regulators was found to retard callus growth considerably.

INTRODUCTION

The elicitation of callus initiation and growth responses through the modification of nutrient media and the manipulation of explants cultured *in vitro* has been demonstrated in many plant species (Murashige 1974; Kartha *et al.* 1976; Street 1978; Mohammad & Raoof 1981). It would seem likely therefore, that physical and chemical conditions, medium strength, sucrose concentration and type of growth regulators are all factors which influence efficient growth and differentiation of the initiated callus of many plants (Takayama & Misawa 1979; Mohammad & Collin 1979; Dodds & Roberts 1984). Viability of callus also varies from one explant to another (Murashige & Skoog 1962; Smith & Street 1974; Anstis & Northcote 1973). Moreover, hormonal control of callus growth and organogenesis in plant tissues and organs cultured *in vitro* has been reported by many workers (see Murashige 1974; Thorpe 1980).

Activated charcoal has also been reported to inhibit callus proliferation, but to increase the amount of organ formation in tissue cultures of different plant species (Fridborg & Eriksson 1975; Fridborg *et al.* 1978; Takayama & Misawa 1980), although responses to activated charcoal appear to be very variable (Mohammad & Yousif 1982). For these reasons we have attempted in this study to examine the effects of charcoal and different growth regulators, namely benzyladenine, kinetin, 2,4-dichlorophenoxyacetic acid, naphthylacetic acid, indole-3-acetic acid, indolebutyric acid and gibberellic acid on the initiation, response and growth of sunflower (*Helianthus annuus* L.) callus.

MATERIAL AND METHODS

Pure-bred seeds of sunflower (*Helianthus annuus* L. var. *sativus*) were used throughout this investigation. Seeds were germinated aseptically in solidified sterilized culture medium as previously described (Mohammad & Raoof 1981). The medium consisted of one-fifth Arnon solution at pH 6·0 (Arnon & Hoagland 1940, 1944) solidified with 0·7% oxoid agar No. 3. Hypocotyls 0·5 cm long (0·045 g) were excised aseptically and placed in McCartney vials containing 10 cm^3 modified Murashige & Skoog (MS) medium (Murashige & Skoog 1962). The vials were kept in a growth chamber at 25 ± 1°C under fluorescent light on a 16-h diurnal cycle.

The growth regulators used in this investigation were kinetin (K), benzyladenine (BA) at concentration of 0·05 mg l^{-1} and 2,4-dichlorophenoxyacetic acid (2,4-D), naphthylacetic acid (NAA), indole-3 acetic acid (IAA), indolebutyric acid (IBA) and gibberellic acid (GA3) at concentration of 1·0 mg l^{-1}. The media used for callus initiation and growth contained the basal medium supplemented with different growth regulators at the same concentration of 2,4-D and K as previously reported (Mohammad & Raoof 1981). In some instances, K at the same concentration was used to replace BA. In addition, all media were used either without charcoal or supplemented with 0·5% activated charcoal plus 4% sucrose. The isolated callus was normally subcultured every 3 weeks to avoid excessive pigmentation.

RESULTS

CALLUS INITIATION TIME

The number of days required for callus initiation was determined from samples of 10 explants. The minimum time required for callus initiation was 2 days and occurred

Table 1. Time in days needed for callus initiation and response to different combinations of growth regulators. Each value indicates the mean of ten replicates. See the text for more detail. + Low, + + moderate, + + + high, + + + + very high. BA = benzyladenine; IAA = indole-3-acetic acid; NAA = naphthylacetic acid; 2,4-D = 2,4-dichlorophenoxyacetic acid; IBA = indolebutyric acid; GA3 = gibberellic acid

Growth regulators	Response of explant to growth regulators	Time in days
BA + IAA	+ + + +	2
BA + NAA	+ + + +	3
BA + 2,4-D	+ + +	4
BA + IBA	+ +	5
BA + GA3	+ +	5
K + IAA	+ + +	4
K + NAA	+ +	5
K + 2,4-D	+	6
K + IBA	+ +	5
K + GA3	+ +	5

on hypocotyl segments grown in MS medium containing BA and IAA (Table 1). The days required for callus initiation with other types of growth regulators, however, varied between 3 and 6 days depending on the combination of either BA or K with auxin source. In all cases, callus grew vigorously with the passage of time but the response of explants to different growth regulators varied. The greatest response was in a medium containing BA and IAA (Table 1), while the smallest responses were recorded in explants grown in a medium containing K and 2,4-D.

GROWTH OF CALLUS CULTURES

The weights of callus grown on media containing different concentrations of growth regulators with or without charcoal were determined at 3, 6 and 9 week intervals. The mean fresh weights of the callus which developed in a particular medium after the above periods are given in Tables 2 and 3. It can be seen that callus growth differed markedly with the type of medium used. Callus grew well in a medium containing BA and IBA without charcoal, reaching 7·98 g in weight during a period of 9 weeks. The callus produced on this medium was compact, soft and only slightly coloured (Fig. 1). Minimum callus growth over the same period was obtained on a medium containing K and IAA (0·894 g), the callus produced being harder and more intensely coloured (Fig. 2). No significant differences were noticed in callus fresh weight grown on media containing BA with either IAA, NAA or GA. Replacing BA by K in the above media did not always have a significant effect on callus fresh weight. Moreover, shoots and roots were produced only in a medium containing K and NAA (Fig. 3). The plantlets were isolated from the callus, subcultured on hormone-free medium and finally

Table 2. Mean fresh weight (g) of callus plus original explant after 9 weeks of incubation on media without charcoal, containing varying combinations of K or BA at a concentration of 0·05 mg l^{-1} and auxins at a concentration of 1·0 mg l^{-1}. Each value represents the mean of ten replicates ± S.E. (standard error of the mean). F.wt. = fresh weight; BA = benzyladenine; IBA = indolebutyric acid; 2,4-D = 2,4-dichlorophenoxyacetic acid; IAA = indole-3-acetic acid; NAA = naphthylacetic acid; GA3 = gibberellic acid

Growth regulators	Age of callus in weeks					
	3		6		9	
	F.wt.	S.E.	F.wt.	S.E.	F.wt.	S.E.
BA + IBA	1·33	±0·53	3·53	±1·22	7·99	±1·42
BA + 2,4-D	0·48	±0·08	2·15	±0·63	3·69	±0·72
BA + IAA	0·66	±0·22	1·99	±0·34	5·14	±0·64
BA + NAA	0·43	±0·13	3·16	±0·84	5·27	±0·94
BA + GA3	0·69	±0·42	1·96	±0·14	5·39	±0·19
K + IBA	0·61	±0·28	2·46	±1·42	3·09	±0·99
K + 2,4-D	0·39	±0·22	2·76	±1·62	4·29	±1·08
K + IAA	0·47	±0·24	0·65	±0·13	0·89	±0·25
K + NAA	0·46	±0·11	1·42	±0·29	1·99	±0·34
K + GA3	0·42	±0·24	2·18	±0·73	3·02	±0·52

Fig. 1. Sunflower callus showing extensive growth after 6 weeks on medium containing benzyladenine and indolebutyric acid.

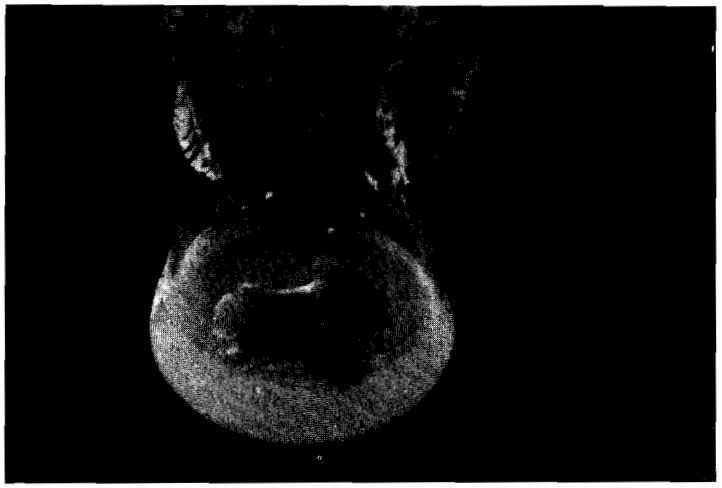

Fig. 2. Sunflower callus grown on medium containing kinetin and indole-3-acetic acid after 6 weeks.

transferred to the soil under greenhouse conditions according to the method of Williams & Collin (1976).

In contrast, callus growth was greatly reduced by the addition of charcoal. Maximum callus fresh weight was attained in a medium containing BA and NAA (Table 3). No significant differences were noticed between the fresh weights of callus grown on most of the media used (e.g. BA & IBA, BA & 2,4-D, K & IBA, etc.).

DISCUSSION

The medium used to initiate and maintain the sunflower callus was a modification of the Murashige and Skoog medium (1962). For the most rapid callus initiation and

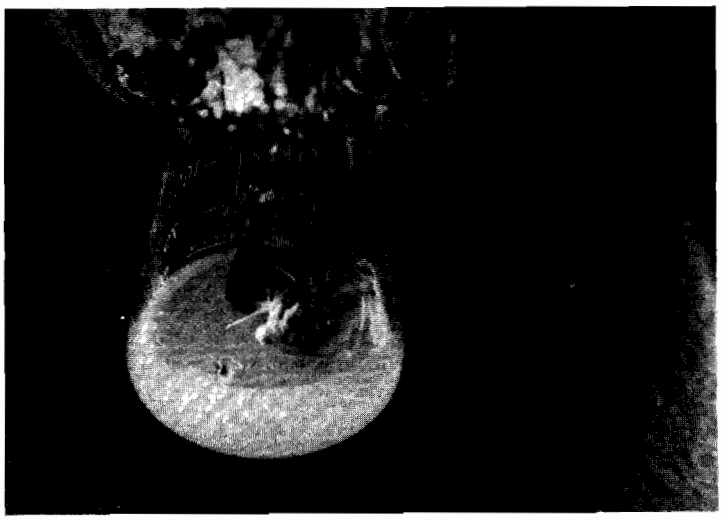

Fig. 3. Sunflower callus showing roots and supporting a number of shoots after 9 weeks on media containing kinetin and naphthylacetic acid.

growth BA and IBA were required and the presence of NAA instead of IBA stimulated root and shoot formation. It has been reported that addition of both auxin and cytokinin to tissue cultures of many plants is necessary to obtain optimal growth. Murashige & Skoog (1962), Okazawa *et al.* (1967) and Lavee & Messer (1969) have shown that certain combinations of IAA and kinetin promote the growth of callus

Table 3. Mean fresh weight (g) of callus plus original explant after 9 weeks of incubation on media with charcoal, containing varying combinations of K or BA at a concentration of 0·05 mg l^{-1} and auxins at a concentration of 1·0 mg l^{-1}. Each value represents the mean of ten replicates ± S.E. (standard error of the mean). F.wt. = fresh weight; BA = benzyladenine; IBA = indolebutyric acid; 2,4-D = 2,4-dichlorophenoxyacetic acid; IAA = indole-3-acetic acid; NAA = naphthylacetic acid; GA3 = gibberellic acid

Growth regulators	Age of callus in weeks					
	3		6		9	
	F.wt.	S.E.	F.wt.	S.E.	F.wt.	S.E.
BA + IBA	3·39	±0·35	3·44	±0·92	3·81	±0·94
BA + 2,4-D	2·41	±0·38	3·58	±0·42	3·71	±0·56
BA + IAA	2·79	±0·49	2·89	±0·63	2·99	±0·84
BA + NAA	3·05	±1·23	3·93	±0·94	4·01	±1·09
BA + GA3	3·21	±0·48	3·37	±0·53	3·52	±0·64
K + IBA	1·60	±0·43	2·99	±0·56	3·73	±0·74
K + 2,4-D	1·92	±0·12	1·98	±0·24	2·04	±0·64
K + IAA	1·05	±0·33	1·17	±0·41	1·24	±0·52
K + NAA	2·39	±0·83	2·46	±0·92	2·49	±0·85
K + GA3	2·11	±0·23	2·49	±0·52	2·59	±0·62

cultures of tobacco, potato and olive plants. Mohammad & Raoof (1981) found that optimal growth of sunflower callus was achieved with 2,4-D and K at concentrations of 0·05 mg l^{-1} and 1·0 mg l^{-1}, respectively. The present work showed that the type of growth regulators used affected greatly both callus formation and growth. The response of explant tissues to callus formation was varied depending on type of growth regulators (Table 1). Similar observations have been reported for other species (Yokoyama & Takeuchi 1976; Street 1978; Nickerson 1980; Amin 1985).

Although tissue cultures of sunflower were not examined specifically for their morphogenetic potential, some indication of the requirements of root and shoot production were established. Fonnesbech (1972), Reinert *et al.* (1978), and Tanimoto & Harda (1982) initiated both root and shoot production in their cultures in media containing both NAA and K. The same was true of the callus examined here, since morphogenesis was only evident in the above medium.

It has been reported that BA is more effective than K in stimulating callus growth, particularly in shoot formation and development (Fonnesbech 1972). Barghchi & Alderson (1983) have shown that BA is better than K in increasing callus growth and morphogenesis in *Pistacia vera* L.

The results obtained here show that callus growth (fresh weight) in media containing BA with all auxins used, was more than that in the other media containing K (Table 2). This is possibly due to an increase in the auxins absorption rate in the presence of BA. Dodds & Roberts (1984) reported that BA enhances the rate of auxin absorption and therefore leads to an increase in the callus fresh weight.

It has been reported that addition of activated charcoal to the culture medium promotes callus growth, root formation and embryogenesis in different plant tissue cultures (Anagostakis 1974; Fridborg *et al.* 1978). It is possible that activated charcoal adsorbs substances excreted into the medium and which either alone or in combination may be active inhibitors of callus growth, root formation and embryogenesis (Letham 1974; Fridborg & Eriksson 1975; Weatherhead *et al.* 1978). One such substance is likely to be ethylene. Ethylene production has been suggested as one of the properties of meristematic tissues and its production is related to cell division (Burg & Burg 1968). The results obtained here, however, show that callus fresh weight was significantly reduced by the addition of charcoal to all media used except those containing K and IAA (Table 3). The decrease in callus fresh weight in the presence of charcoal may be due to adsorption of K or BA by the activated charcoal as reported by Letham (1974) and Weatherhead *et al.* (1978). Moreover, lower levels of K have been shown to reduce greatly sunflower callus fresh and dry weights (Mohammad & Raoof 1981).

It can be concluded from the results that callus initiation and growth was greatly affected by nutrient medium constituents particularly auxin, kinetin and benzyladenine. Addition of charcoal to the culture medium greatly decreases callus fresh weight.

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تأثير بعض منظمات النمو على استحداث ونمو الكالاس في نبات عباد الشمس

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خلاصة

في هذه الدراسة ، تم استحداث الكالاس في قطع سوق بادرات نبات عباد الشمس النامية في وسط موراشيج وسكوج المحوّر . وقد وجد ان أفضل وسط يستحث نمو الكالاس وتواصله هو وسط موارشيج وسكوج المضاف إليه بنزايل أدينين بتركيز ٠,٠٥ مليجـرام في اللتر ، وإندول حمض البوتيريك بتركيز ١,٠ مليجرام في اللتر . كما لوحظ أن إضافة الفحم المنشط إلى الوسط الغذائي الحاوي لمنظمات النمو المختلفة يؤخر نمو الكالاس بدرجة كبيرة .