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Somatic embryogenesis in peanut: Influence of growth regulators and sugars

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Abstract

Somatic embryogenesis and plant regeneration were induced from immature embryonal axes and immature cotyledons of peanut (*Arachis hypogaea* L. fastigata type cv JLM-1). Influence of different auxins, cytokinins and sugars on somatic embryogenesis from immature cotyledon explants was also investigated. Among the different auxins tested, 2,4-dichlorophenoxyacetic acid (2,4-D) was most effective, producing the highest frequency of responding cultures and highest average number of somatic embryos per responding culture, while dicamba, picloram, indolepropionic acid, α-naphthaleneacetic acid, 2,4,5-trichlorophenoxypropionic acid and α-naphthoxyacetic acid were also effective for embryogenesis. Indolebutyric acid, indoleacetic acid, p-chlorophenoxyacetic acid and trichlorophenoxyacetic acid were not beneficial. Among the four cytokinins tested, zeatin slightly enhanced the frequency of somatic embryogenesis, while kinetin, 6-γ-γ-dimethylallylaminopurine and benzyladenine were relatively inhibitory. Among the different carbon sources tested, sucrose was the best for embryo induction and at 6% sucrose the highest frequency of responding cultures and average number of somatic embryos per explant were obtained. For inducing embryogenesis from embryonal axes, 2,4-D was more effective than picloram. Highest plant conversion frequency from somatic embryos was obtained in presence of dicamba or NAA and using cotyledon explants.

Abbreviations: BA – benzyladenine, 2,4-D -2,4-dichlorophenoxy-acetic acid, IAA – indoleacetic acid, IBA – indolebutyric acid, IPA – 3-indolepropionic acid, 2iP-6-γ-γ-dimethylallylaminopurine, Dicamba – 3,6-dichloro-O-anisic acid, pCPA – p-chlorophenoxyacetic acid, NAA – α-naphthaleneacetic acid, NOA – α-naphthoxyacetic acid, 2,4,5-T – 2,4,5-trichlorophenoxyacetic acid, 2,4,5-TP – 2,4,5-trichlorophenoxypropionic acid

Introduction

Basal medium supplemented with auxins alone induced somatic embryos from embryonal explants of leguminous crop plants such as soybean (Lazzeri et al. 1987), pea (Kysely & Jacobsen 1990), peanut (Ozias-Akins 1989; Hazra et al. 1989) and white clover (Parrott 1991) while cytokinins were required for somatic embryogenesis in *Trifolium* (Maheswaran & Williams 1986) and *Phaseolus* species (Malik & Saxena 1992). Another important component of the medium known to influence somatic embryo induction is the concentration and type of sugar present (Lazzeri et al. 1988; Strickland et al. 1987). In peanut, the most important oil-seed crop of semi-arid tropics and a source of high quality cooking oil and protein, auxin-stimulated

somatic embryogenesis has been reported (Ozias-Akins 1989; Hazra et al. 1989; McKently 1991).

In this communication, we present the effect of different auxins, cytokinins and sugars on somatic embryogenesis in peanut.

Materials and methods

Peanut (*Arachis hypogaea* L. fastigata type) cv JLM-1 (obtained from Nuclear Agriculture Division, BARC, Bombay) was the source material. Immature cotyledons and embryonal axes excised from immature seeds were used for the experiments. The pods (just prior to maturity) collected from field-grown plants were disinfested by 70% ethanol (v/v) for 1 min followed by 0.1% $HgCl_2$ (w/v) for 10 min. They were washed five times with sterile water and cut open to remove the seeds under aseptic conditions. The immature embryonal axes and cotyledons were separated and used for culture.

The culture medium used for somatic embryo induction consisted of L-6 salts (Kumar et al. 1988), B_5 vitamins (Gamborg et al. 1968) 200 mg l^{-1} of casein hydrolysate, 1% sorbitol and 6% sucrose. The medium was solidified with 0.6% agar (Hi Media, Bombay) prior to autoclaving. To study the effect of various auxins on embryogenesis, auxins such as 2,4-D, 2,4,5-T, 2,4,5-TP, dicamba, picloram, NAA (22.6, 45.2, 90.2, 135.6 μM), NOA, IPA, IBA, pCPA or IAA (135.6 μM) were added to the medium used for culturing of immature cotyledons. Medium supplemented with 2,4-D or picloram (22.6, 45.2, 90.2, 135.6 μM) was used to culture immature embryonal axes. Two immature embryonal explants or one immature cotyledon were cultured on 20 ml medium in 50 ml glass test tubes, closed with cotton plugs. A minimum of 12 explants were tested per treatment and experiments were repeated. The final observation is based on surviving explants.

Cytokinins such as BA, 2iP, kinetin or zeatin (0.5 μM) were incorporated into the medium supplemented with 2,4-D (45.2 μM) to study their effect on somatic embryogenesis.

The effect of different sugars on somatic embryogenesis was investigated by supplementing the basal medium containing casein hydrolysate and 2,4-D (45.2 μM) with sucrose, glucose, fructose or maltose (0.17 M). To study the effect of different concentrations of sucrose on somatic embryogenesis, sucrose at 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% was incorporated into the medium.

All the somatic embryos obtained in each treatment were transferred first to maturation medium consisting of half strength Murashige and Skoog's (Murashige & Skoog 1962) mineral elements supplemented with 1% mannitol, 0.1% activated charcoal and 3% sucrose. Subsequently they were transferred to shoot induction medium containing MS mineral salts supplemented with BA (5.0 μM) and IAA (0.5 μM). Well-developed shoots were later transferred to rooting medium consisting of half-strength MS mineral salts supplemented with NAA (1.0 μM). Conversion frequency of somatic embryos into plants was calculated based on the total number of well-developed plants obtained in each treatment in relation to the total number of somatic embryos. The cultures were incubated at 25 ± 2°C with an 8-h photoperiod (12.1 μmol m^{-2} s^{-1}). Observations were recorded at the end of 30 days for somatic embryo induction and 150 days for plant differentiation.

Results

The frequency of somatic embryo induction from embryonal axes was higher than that from immature cotyledons.

Auxins

Out of the 11 different auxins tested for somatic embryo induction from immature cotyledons, 2,4-D was found to be the most favourable, 2,4-D at 90.4 μM produced the highest frequency of response and at 45.2 μM produced the highest average number of somatic embryos per responding culture. The frequency of response was lower at 22.6 and 135.6 μM of 2,4-D (Table 1). Dicamba was most effective at 135.6 μM in comparison with other concentrations tested. Picloram, NAA and 2,4,5-TP also favoured somatic embryogenesis, but 2,4,5-T was ineffective (Table 1). Among the other auxins tested,

Table 1. Effect of different auxins on somatic embryogenesis from immature cotyledons of peanut.

Auxin	Concentration (μM)	No. of cotyledons cultured	Cotyledons responding (%)	Average no. of somatic embryos ± SE	Total no. of somatic embryos	*Frequency of plant development (%)
2,4-D	(22.6)	46	4.3	5 ± 1.0	10	20.0
2,4-D	(45.2)	36	22.2	9.4 ± 2.4	74	10.8
2,4-D	(90.4)	45	48.9	9.2 ± 1.0	203	7.3
2,4-D	(135.6)	56	5.4	6.3 ± 2.0	19	5.3
Dicamba	(22.6)	46	4.3	4.0 ± 1.0	8	25.0
Dicamba	(45.2)	42	7.1	2.7 ± 1.2	8	12.5
Dicamba	(90.4)	38	5.2	3.5 ± 1.5	7	14.2
Dicamba	(135.6)	20	35.0	6.4 ± 1.5	45	4.4
Picloram	(22.6)	29	13.7	5.0 ± 0.4	20	10.0
Picloram	(45.2)	48	6.3	5.7 ± 1.2	17	11.7
Picloram	(90.4)	76	21.0	5.1 ± 0.7	81	4.9
Picloram	(135.6)	50	24.0	3.4 ± 0.4	41	2.4
2,4,5-TP	(22.6)	37	2.7	1.0 ± 0	ND	ND
2,4,5-TP	(45.2)	43	2.3	2.0 ± 0	ND	ND
2,4,5-TP	(90.4)	44	2.3	3.0 ± 0	ND	ND
2,4,5-TP	(135.6)	36	2.7	1.0 ± 0	ND	ND
NAA	(22.6)	40	2.5	3.0 ± 0	3.0	2.0
NAA	(45.2)	48	8.3	4.0 ± 0.4	16	25.0
NAA	(90.4)	47	10.6	3.2 ± 0.6	16	18.8
NAA	(135.6)	47	8.5	3.5 ± 0.7	14	14.2
2,4,5-T	(22.6,	—	—	—	—	—
	45.2,	—	—	—	—	—
	90.4,	—	—	—	—	—
	135.6)	—	—	—	—	—
NOA	(135.6)	14	7.1	2.0 ± 0	2	—
IPA	(135.6)	20	35.0	3.6 ± 1.0	25	—
IBA	(135.6)	—	—	—	—	—
IAA	(135.6)	—	—	—	—	—
pCPA	(135.6)	—	—	—	—	—

– Nil; SE – Standard error; ND – Development of plants from somatic embryos not done; * – Data taken at the end of 150 days. All the other data recorded at the end of 30 days.

only IPA and NOA were capable of inducing somatic embryos in low frequency.

Somatic embryos obtained in each treatment were transferred to embryo maturation, shoot induction and root induction medium and plant conversion frequency was determined at the end of 150 days. Somatic embryos initiated from cotyledons in presence of 22.6 μM of dicamba or NAA showed the highest plant conversion frequency (25%) (Table 1). In the presence of 2,4-D (22.6 μM) in the somatic embryo induction medium, only 20% of the somatic embryos were converted into plants. Conversion frequency was lower at corresponding concentrations of picloram. Plants were not obtained when NOA and IPA were used in the initial embryo induction medium. In general, lower concentration of auxins in the embryo induction medium favoured a higher conversion rate into plants.

Immature embryonal axes were more responsive than immature cotyledons with regard to the frequency of embryogenesis, 2,4,-D at 22.6, 45.2 and 90.4 μM elicited embryogenesis in all explants while at 135.6 μM, the frequency was slightly less (Table 2). Picloram was most favourable at 22.6 μM, while at higher concentrations,

Table 2. Effect of different auxins on somatic embryogenesis from embryonal axes of peanut cv. JLM-1.

Auxin	Conc. (μM)	No. of embryonal axes cultured	Embryonal axes responding (%)	Average no. of somatic embryos ± SE	Total no. of somatic embryos	*% of plant development (%)
2,4-D	(22.6)	13	100	9.7 ± 1.3	127	9.4
2,4-D	(45.2)	48	100	10.2 ± 1.0	490	6.3
2,4-D	(90.4)	20	100	7.3 ± 0.9	146	4.8
2,4-D	(135.6)	17	85	6.4 ± 0.9	90	5.4
Picloram	(22.6)	10	100	4.8 ± 0.7	48	16.6
Picloram	(45.2)	12	91.6	3.7 ± 0.5	41	17.0
Picloram	(90.4)	13	84.6	4.2 ± 0.7	46	17.3
Picloram	(135.6)	10	70.0	3.6 ± 0.8	25	16.0

SE – Standard error.
* – Observation of whole plants taken at the end of 150 days. All the other data taken at the end of 30 days.

a decrease in the frequency of embryogenesis was observed. The average number of somatic embryos per culture was more with 2,4-D than with picloram.

When somatic embryos from embryonal explants were tested for plant conversion frequency, picloram at all concentrations favoured higher plant differentiation in comparison with 2,4-D.

Cytokinins

Cytokinins (0.5 μM) when incorporated into the medium supplemented with 45.2 μM 2,4-D, zeatin slightly enhanced the frequency of responding cultures (Fig. 1). Kinetin, 2iP and BA reduced the frequency of response. There was a decrease in the number of somatic embryos per explant in presence of all cytokinins.

Sugars

Among the different sugars, sucrose was most favourable for somatic embryogenesis producing the highest frequency and average number of somatic embryos. In the presence of glucose and fructose, the frequency was reduced, while maltose was completely ineffective for somatic embryogenesis (Table 3).

Sucrose at 6% gave the best response, while at lower concentrations, the frequency of response and average number of somatic embryos per responding culture was less. However, higher

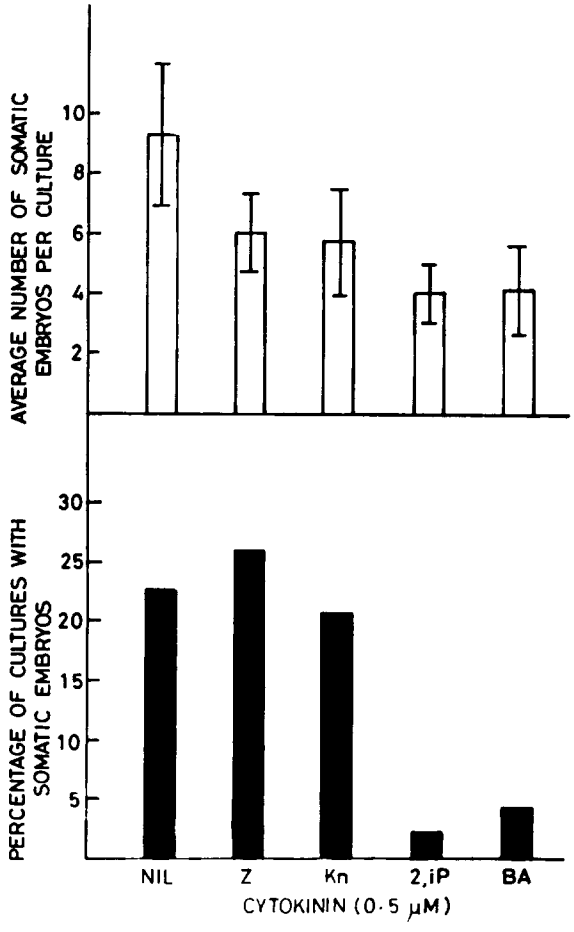

Fig. 1. Effect of different cytokinins on frequency of somatic embryogenesis and average number of somatic embryos per culture (z – zeatin, Kn – kinetin).

Table 3. Effect of different sugars on somatic embryogenesis in peanut cotyledons cultured on BM supplemented with 45.2 μM 2,4-D.

Sugars (0.17 M)	n	Responding cultures (%)	Average no. of somatic embryos/responding cultures ± S.E.
Sucrose	37	22.2	9.3 ± 2.4
Fructose	32	15.6	6.2 ± 1.6
Glucose	48	2.1	4.0 ± 0
Maltose	45	0	0

SE – Standard error.
n – Number of cotyledons cultured.

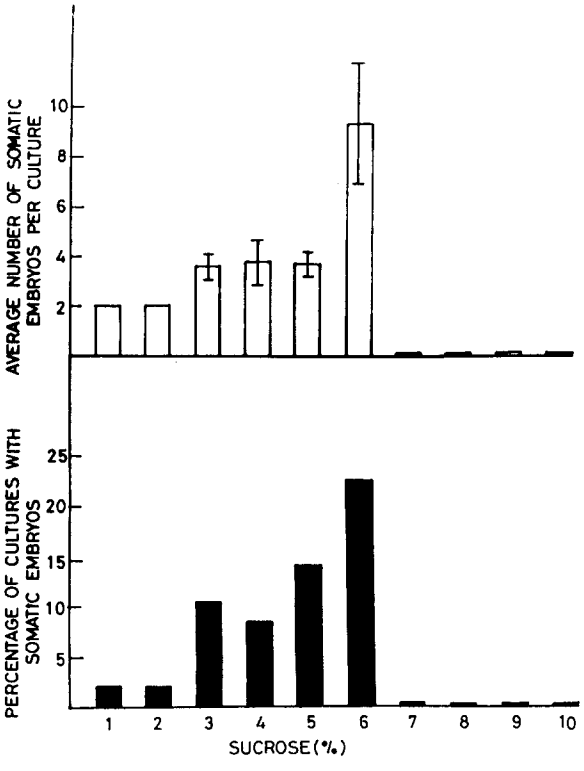

Fig. 2. Effect of different concentrations of sucrose on frequency of cultures with somatic embryos and average number of somatic embryos per culture.

concentrations of sucrose (7, 8, 9 and 10%) inhibited embryogenesis (Fig. 2).

About 400 plants obtained from somatic embryos initiated from embryonal explants were transferred to field for agronomic evaluation.

Discussion

These studies have shown that the frequency of somatic embryo induction in peanut was dependent on the type and concentration of auxin used. 2,4-D was found to be the best auxin producing the highest frequency of responding cultures and highest average number of somatic embryos per culture. 2,4-D has been widely used for somatic embryogenesis in peanut (Hazra et al. 1989) and soybean (Lazzeri et al. 1987). Picloram also induced somatic embryogenesis in the present study, which is in agreement with the report of Ozias-Akins et al. (1992). Dicamba, known to induce somatic embryogenesis in *Dactylis glomerata* (Gray & Conger 1985), was effective in peanut as well. Similarly, 2,4,5-TP is another auxin that has been used in cereal cultures (Eapen & Rao 1982) and could be effectively used for somatic embryogenesis in peanut. In the present study, NAA induced somatic embryogenesis and this is in contrast to the report by Hazra et al. (1989). While IPA and NOA favoured embryogenesis in low frequency, IBA, pCPA and IAA were totally ineffective.

In general, addition of cytokinins to the induction medium containing 2,4-D did not enhance the average number of embryos per explant. This is in conformity with the results obtained in pea (Kysely & Jacobsen 1990).

Among the different carbon sources tested for somatic embryogenesis in peanut, sucrose evoked the best response followed by fructose and glucose while maltose was completely ineffective. The most commonly used carbohydrate for plant tissue culture is sucrose. In nature, carbohydrate is transported within plant tissues as sucrose and tissue may have an inherent capacity for uptake, transport and utilization of sucrose. Maltose was not beneficial for somatic embryogenesis in the present study, although in *Medicago sativa*, it induced the highest embryo yield (Strickland et al. 1987). Sucrose at 6% produced the best results in peanut, while in soybean lower concentrations were more beneficial (Lazzeri et al. 1988, Komatsuda et al. 1992).

The present studies have shown that conversion of somatic embryos into plants was dependent on the type and concentration of auxin used in the somatic embryo induction medium. The

best plant conversion frequency (25%) was obtained when dicamba or picloram was used for somatic embryo induction from cotyledons. Sellers et al. (1990) found that an average of 80% of somatic embryos of peanut produced shoots, while an average of 61% produced roots. In the experiments of Ozias-Akins (1989) the conversion frequency ranged from 0–18% over all experiments. We have used higher concentrations of auxins in the somatic embryo induction medium in comparison with previous reports, which probably may be responsible for the low conversion frequency. In soybean although 2,4-D produced larger number of somatic embryos, NAA favoured subsequent conversion into plants (Barwale et al. 1986, Lazzeri et al. 1987).

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