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2,4,5-Trichloro Phenoxy Propionic Acid Induced Rhizogenesis in *Carthamus tinctorius* L.

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Low concentrations of 6-benzylamino purine (BAP) α-naphthalene acetic acid (NAA) induced 10-12 shoot buds per culture of seedling cotyledons of safflower (*Carthamus tinctorius* L.) cultivars A-1 and Manjira. Although A-1 showed a wider plasticity for growth regulator requirement, it showed a slightly lesser degree of shoot bud regeneration than CV Manjira. Conventional chemical treatments failed to induce roots in *in vitro* formed shoots, whereas, 2,4,5-trichlorophenoxy propionic acid (2,4,5 Cl_3pop) at 1 and 2 mg/l induced rooting of the shoots in high frequency, this facilitated the recovery of plantlets.

Key Words: Safflower, Seedling-cotyledon, Culture, Multiple shoots, Rhizogenesis

Introduction

Carthamus tinctorius (Safflower) yields an edible oil of superior quality containing unsaturated fatty acids oleic acid and linoleic acid. Plant breeding programmes aimed at developing high yielding varieties with resistance to diseases and pests have been less successful. Tissue culture methods may serve as an efficient tool in broadening the genetic base for certain important agronomic characters and in isolation of true-to-type somaclones. However, a major limitation in the application of tissue culture methods to safflower is the lack of efficient protocol for plant regeneration.

The present study describes a protocol for easy and efficient regeneration of plantlets with well developed tap root-like system for subsequent tranfer to field.

Material and Methods

Seeds of two local cultivars of safflower, namely A-1 and Manjira were obtained from the Directorate of Oil Seeds, Rajendranagar, Hyderabad, India. Good seeds of uniform size were surface-sterilized with 0.1% mercuric chlroide for 8 min washed thrice with sterile distilled water and germinated on wet filter paper in sterile test tubes under continuous flourecent light of about 800-1000 Lux.

Cotyledons excised from 2 to 3-day-old seedlings were cultured on MS medium (3% sucrose + 0.9% BDH agar) supplimented with BAP, Kn and NAA in concentrations varying from 0.02 to 1 mg/l. Two cotyledons/culture tube were implanted with their cut end slightly depressed into the medium. The shoots obtained in the cultures were

transfered to a medium supplemented with one of the following substances: NAA, IAA, IBA and 2,4,5-Cl_3pop (Concentrations 0-2.0 mg/l) either alone or in combination with BAP or kn for root initiation.

The cultures were incubated under fluorescent light (800-1000 Lux continuously) at 26 ± 1°C. The experiments were replicated three times, each replicate comprised of 20 cultures.

Results and Discussion

Callus formation and root initiation occurred at the cut end of the cotyledons in all the treatments, although the percentage of responsive cultures was only 10-20% in the different treatments..

In general, the cotyledons cultured on MS + kinetin, did not show any shoot bud development (table 1). On MS + 0.2-1 mg/l BAP, along with callus formation shoot buds differentiated from the cut ends of the cotyledons in 20-50% of the cultures within a week. In combination with NAA (0.1 mg/l), BAP (0.5 mg/l) enhanced shoot bud formation occurred up to 87% (table 1). A similar response was reported for cotyledon cultures of thin hull cultivars of safflower (George & Rao 1982). In our study, each responsive explant produced 10-12 shoot buds. However, the frequency of shoot bud regeneration was higher in CV A-1 than in Manjira (table 1).

Shoot buds that attained a height of about 3 cm were separated and cultured on basal MS medium and MS supplimented with 0.1-2 mg/l, NAA or IAA or IBA. Shoots cultured on basal MS medium rooted but the frequency of rooting was practically nil (table 2). Shoots cultured on MS + NAA or IAA or IBA (0.1 to 2.0 mg/l) did not root. Failure of NAA, IAA and IBA to induce roots had been reported for safflower cultivars NP-9 and th-10; in these cultivars root induction occurred in low frequency on basal medium (George & Rao 1982).

Table 1 *Shoot formation in seedling cotyledon cultures of safflower cultivars Manjira and A-1 (3-4 weeks old cultures)*

MS + growth substances (mg/l)	Percentage of shoot forming cultures	
	CV: Manjira	CV: A-1
BAP 0.2	20	20
0.5	50	45
1.0	30	25
Kn 0.2	Nil	Nil
0.5	Nil	25
1.0	Nil	17
BAP 0.05 + NAA 0.01	Nil	Nil
BAP 0.5 + NAA 0.1	87	68
BAP 0.5 + NAA 0.5	50	50
Kn 0.05 + NAA 0.01	Nil	Nil
Kn 0.5 + NAA 0.1	55	43
Kn 0.5 + NAA 0.5	50	30

Table 2 *Rhizogenic response of* ***in vitro*** *formed shoots (about 5-7 weeks old cultures)*

Culture medium (Auxins in mg/l)	Degree of response	
	CV:A-1	CV:Manjira
MS + 3% Sucrose	Nil	Nil
MS + 6% Sucrose	Nil	Nil
MS + 9% Sucrose	+	+
MS + IAA (0.1-1.2)	Nil	Nil
MS + IBA (0.1-2.0)	Nil	Nil
MS + NAA (0.1-2.0)	Nil	Nil
MS + 2,4,5-Cl_3pop (0.5)	+	+
MS + 2,4,51Cl_3pop (1.0)	+ + +	+ + +
MS + 2,4,5-Cl_3pop (2.0)	+ + +	+ + +
MS + 2,4,5-Cl_3pop (2.0) + BAP (0.5)	+ +	+ +
MS + 2,4,5-Cl_3pop (2.0) + Kn (0.5)	Nil	Nil

+ Rotting in less than 10% cultures
+ + Rooting in 10-15% cuiltures
+ + + Rooting in 51-75% cultures

Our attempt to improve the frequency of rooting in the recalcitrant shoots of CV A-1

and Manjira by treatment with safflower embryo extract was in vain. But root initiation occurred in shoots transferred to MS medium supplemented with 2,4,5-Cl_3pop (0.5 to 2.0 mg/l) within 10-15 days from transfer. Addition of BAP decreased and that of kn inhibited the rhizogenic effect of 2,4,5-Cl_3pop (table 2).

Our data suggest that 2,4,5-Cl_3pop may be utilized efficiently to induce rooting of shoots and thus recover plantlets in safflower. The synthetic substance 2,4,5-Cl_3pop has a strong rhizogenic effect (Aberg 1974, Sanchez et al. 1981) and to a lesser extent caulogenic effect also (Susan & Rao 1982).

The present study suggests a clear role for 2,4,5-Cl_3pop in the effecient induction of rooting and an efficient regeneration protocol for safflower. This may in the long run help in improvement of the genetic base of safflower.

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References

Aberg B 1974 Plant growth regulators. XXXII. Some tri- and tetra-substituted phenoxy acetic and related optically active propionic acids; *Swed. J. Agric. Res.* **4** 71-85

George L and Rao P S 1982 *In vitro* multiplication of safflower (*Carthamus tinctorius* L.) through tissue culture; *Proc. Indian natn. Sci. Acad.* **B58** 791-794

Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures; *Physiol. Pl.* **15** 473-497

Sanchez de J E, Albores M and Loyola-Varges V M 1981 Effect of 2,4,-D analogues on the induction and maintenance of callus in maize tissue culture; *Ann. Appl. Bot.* **98** 347-353

Susan E and Rao P S 1982 Plant regeneration from callus cultures of Durum and Emmer Wheat; *Plant cell Reports* **1** 215-218