Indian J. Genet., **56** (4): 458–461 (1996)

STUDIES ON CALLUS GROWTH AND DIFFERENTIATION IN SAFFLOWER

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(Received: July 15, 1994; accepted: August 23, 1994)

ABSTRACT

Tissue culture studies were carried out to standardise hormonal requirements and to determine the best source of explant (cotyledonary leaf, hypocotyl and root) for callus induction, growth and differentiation in two safflower genotypes MS-105 (Male sterile) line and Manjira. Murashige–Skoog medium supplemented with 5.0 mg 1^{-1} naphthalene acetic acid (NAA) and 0.25 mg 1^{-1} 6 benzyl aminopurine (BAP) induced maximum callus (65.4%) irrespective of genotype and explant. Cotyledonary leaf was found to be efficient for callus induction, growth and differentiation. MS medium supplemented with 2.0 mg 1^{-1} NAA was significantly superior to other hormonal combinations in terms for differentiation. Frequency of direct rhizogenesis and caulogenesis was maximum from both cotyledonary leaf and hypocotyl explants cultured on MS medium supplemented with 5.0 mg 1_{-1} NAA and 0.1 mg 1^{-1} NAA + 0.25 mg 1^{-1} BAP, respectively.

Key words: Safflower, callus, differentiation, caulogenesis, rhizogenesis.

The current interest in safflower in India and elsewhere stems from the recognition of it's therapeutic value consequent to high degree of polyunsaturation in form of linoleic acid. Extensive evidence now exists which demonstrates that tissue culture induces genetic variability [1]. Tissue culture studies and somaclonal variants in safflower may complement the conventional breeding techniques for widening genetic base for improvement of economically important traits in the existing genotypes. The present investigation aims to determine the best source of explants for maximum callus induction and regeneration so that the technique can be employed for micropropagation of male sterile lines and isolation of somaclonal variants.

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MATERIAL AND METHODS

The material for the present investigation comprised seed of two safflower genotypes, namely MS-105 (male sterile) line and Manjira. Seeds were sterilized with 0.1% mercuric chloride for three minutes, and were then thoroughly washed with sterile water and germinated aseptically. Different explants such as cotyledonary leaves, hypocotyl and roots were excised from 7-day-old seedlings and inoculated on the Murashige–Skoog (MS) medium [2] supplemented with different hormonal concentrations (Table 1).

Callus initiation was observed within 1–2 weeks after inoculation, and the callus was subcultured regularly at 5-week intervals on the original callus inducing medium. Four to five weeks old primary calli were transferred to differentiating medium (MS medium supplemented with 2.0 and 4.0 mg l^{-1} 6- benzylamino purine or kinetin in combination with 0.5 mg l^{-1} naphthalene acetic acid.

Frequency of callus induction, rhizogenesis and caulogenesis was measured as percentage of the cultures responding among the total number of cultures. Statistical analysis was carried out using mixed factorial (2 x 3 x 16) experiment laid out in completely randomized design [3] with 2 genotypes, 3 explants and 16 hormonal treatments to determine the significance of differences between them.

RESULTS AND DISCUSSION

Callus initiation was observed from the cut ends of the explants that were in contact with the medium. Frequency of callus induction was recorded at the end of the fourth week from the date of inoculation. Irrespective of explants and hormonal combinations MS-105 line recorded significantly higher callus induction frequency (31.5%) than Manjira (28.6%). Seeta [4] reported that the variety Manjira gives better response for callus induction (69–96%) among the five genotypes tested (Manjira, A-1, Sagarmuthyalu, Co-1 and S4). These results suggest that there is a marked influence of genotype on callusing efficiency.

Among the explants studied, the cotyledonary leaf recorded the highest callus induction frequency (36.6%) followed by hypocotyl (33.0%) and root (21.2%) and the difference from other explants was significant. Seeta [4] also reported that in safflower cotyledon was efficient in callus induction and growth.

Among the various hormonal concentrations, MS Medium supplemented with 5.0 mg l^{-1} NAA + 0.25 mg l^{-1} BAP was found to be significantly superior over other treatments for callus induction (65.4%) irrespective of the genotype and explant studied (Table 1). In general percentage of callus initiation increased with increasing concentration of NAA in

Table 1. Effect of MS medium with different hormonal concentrations on callus induction frequency from different explants of two safflower genotypes

Hormonal concentration, mg l^{-1}	Mean callus induction frequency (%) of different explants					
	MS-105 line			Manjira		
	cotyledonary leaf	hypocotyl	root	cotyledonary leaf	hypocotyl	root
Control	20.0	14.2	10.0	15.1	13.0	10.0
MS + 0.1 NAA	23.3	23.2	13.0	20.0	20.0	13.0
MS + 0.5 NAA	26.6	26.6	16.4	23.2	23.2	16.4
MS + 1.0 NAA	33.3	30.0	23.2	30.0	23.2	20.0
MS + 2.0 NAA	43.3	40.0	26.6	40.0	36.6	26.6
MS + 5.0 NAA	63.4	53.4	33.3	60.0	53.4	30.0
MS + 0.1 NAA + 0.25 BAP	20.0	16.4	13.0	16.4	16.4	13.0
MS + 0.5 NAA + 0.25 BAP	33.3	26.6	20.0	26.5	23.2	20.0
MS + 1.0 NAA + 0.25 BAP	36.6	26.6	20.0	26.5	23.2	20.0
MS + 2.0 NAA + 0.25 BAP	53.4	43.3	30.2	46.6	50.0	30.2
MS + 5.0 NAA + 0.25 BAP	83.7	76.8	40.6	76.8	70.0	40.2
MS + 0.1 NAA + 0.25 Kn	13.0	10.3	10.0	13.0	13.0	10.2
MS + 0.5 NAA + 0.25 Kn	30.0	23.2	16.4	23.2	23.2	13.0
MS + 1.0 NAA + 0.25 Kn	33.3	30.0	23.2	30.0	26.6	15.1
MS + 2.0 NAA + 0.25 Kn	43.3	36.6	26.6	40.0	36.6	23.2
MS + 5.0 NAA + 0.25 Kn	74.9	80.3	36.6	66.0	60.0	33.3
Mean	38.9	34.3	21.9	34.3	31.7	20.5
	Genotypes		**Explants**		**Hormonal concentration**	
SE	0.3890		0.4765		1.1003	
C.D. (0.05)	0.7625		0.2339		2.1567	

all the hormonal combinations. Greco *et al.* [5] explained that one hormone enhances the level of another by promotion of biosynthesis or inhibition of degenerative metabolism. However, NAA and BAP combinations were superior to NAA and kinetic combinations with respect to callus initiation. Lupi et al. [6] reported no callus induction from cotyledonary leaf explant of sunflower at different levels (0.1, 0.5 and 1.0 mg l^{-1}) of Kn. In the present investigation, the inhibitory effect of Kn on callus induction may have been compensated by NAA in the NAA + Kn combinations.

Based on the amount of callus in the cultures, the callus growth was graded as good, medium and poor. In general, MS Medium supplemented with 5.0 mg l^{-1} NAA + 0.25 mg l^{-1} BAP was the best combination for callus growth.

Morphogenesis in the form of rhizogenesis and caulogenesis was observed from the cotyledonary leaf cultures of both genotypes on the callus inducing medium itself within 30–35 days from the date of inoculation. Frequency of rhizogenesis was significantly higher (47%) on MS Medium supplemented with 5.0 mg l^{-1} NAA, whereas the highest frequency of caulogenesis was observed with 0.1 mg l^{-1} NAA + 0.25 mg l^{-1} BAP in the medium.

The technique of direct morphogenesis without callus formation can be utilized for multiplication of male sterile lines and hybrids in vitro without impairing their genetic constitution.

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