Karnataka J. Agric. Sci., 11 (1) : (63-66) 1998

Callus Induction and Morphogenesis from Somatic Tissue cultures of Sunflower (*Helianthus annuus* L.)*

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(Received : September, 1996)

Abstract : Hypocotyl and cotyledon explants of 'BSH-1', 'KBSH-1' and 'Morden' genotypes of sunflower were cultured in *vitro* for callus and shoot bud formation. Hypocotyl segments responded better (94.30%) than cotyledons (83.50%) for callus formation. MS medium supplemented with NAA or 2,4-D alone or with BA induced the highest frequency of callus in all the three genotypes. In many cultures of hypocotyl, cotyledon explants, yellowish-green morphogenic nodular structures were produced in suspension cultures. Limited regeneration of shoot buds from cotyledonary callus of 'Morden' occurred on MS medium with 1.0 mg/1 each of 2,4-D and BA.

Introduction

The major requirement in the application of in *vitro* culture techniques for plant improvement is that the cells and tissues be established in culture with ease and that the plantlets are regenerated after a variety of manipulations.

Though sunflower is one of the important oilseed crops, many *in vitro* culture studies have not resulted in usuable technology. It is one of the many plant species which has been considered as recalcitrant (Patil *et al.*, 1993). Both short and long term sunflower callus cultures can be easily established on a variety media formulations and growth regulators. But plant regeneration from callus cultures is the main problem and it is very poor even in the most responsive genotypes. Successful, yet genotype dependent, plant regeneration has been demonstrated in a few groups of cultivars and wild species (Lupi *et al.*, 1987; Ceriani *et al.*, 1992; Nemeth and Frank, 1992; Barotti *et al.*, 1995) very few attempts have been made in India in this regard. More efforts are needed to obtain high frequency plant regenertion from established cultures of a wide variety of germplasm to enable these technologies to be useful in sunflower improvement. In the present study, callus induction and growth from hypocotyl and cotyledon explants of three popular Indian genotypes, along with the limited plant morphogenesis has been reported.

Material and Medthods

Hypocotyl and cotyledonary leaves were collected from 2-3 days old, aseptically grown seedlings of 'KBSH-1', 'BSH-1' and 'Mordent' genotypes of sunflower. Cotyledonary leaves, cut transversly into two pieces and 5-10 mm hypocotyl segments were cultured on Murashige and Skoog (1962) medium (MS) with auxins and cytokinins. two modifications of the MS medium (MS_1 and MS_2) were also tried for callus induction and plant morphogenesis.

MS_1 : MS + 5.05/1 KNO_3, 500 mg/1 Casein hydrolysate, 20 mg/1 Adenine sulphate and 1 mg/1 Benzyladenine (BA).

MS_2 : MS_1 + 0.1 mg/1 NAA.

For estimating the frequency of callus induction, at least 40 cultures in two replications were considered. The cultures were incubated under light (12 h) in a culture room maintained at 25± 2°C.

Results and Discussion

The genotypes had significant effect on the frequency of callus induction from

* Part of the M.Sc. (Agri) thesis submitted by the senior author to the university of Agricultural Sciences, Dharwad -580 005

cotyledonary leaves. Liu *et al.* (1991) and Nemeth and Frank (1992) also noted genotypic differences for callus induction in *Helianthus* species. Maximum mean callus induction from cotyledons was observed in 'Morden' (88.%) followed by 'KBSH-1' (86.0%) and 'BSH-1' (76.5%). However, the response from hypocotyl segments did not vary significantly among the genotypes. Comparatively, hypocotyls showed better response (94.3%) than cotyledons (83.5%), over all genotypes and growth regulator combinations (Table 1). The studies of Greco *et al.* (1984) and Lupi *et al.* (1987) have also indicated the suitability of hypocotyl explants over cotyledons for high frequency callus induction.

MS medium supplemented with NAA or 2,4-D (1.0 mg/1) alone or combination of NAA (0.3, 0.5 and 1.0) with BA (1.0 mg/1) induced the highest frequency of callus from both explants in all three genotypes. The combination of auxin and cytoxinin for effective callus formation was also observed by many others (Lupi *et al.*, 1987; Ceriani *et al.*, 1992; Barotti *et al.*, 1995). Both explants responded similarly on MS2 medium also. Identical observations have been made with hypocotyl (Paterson and Everett, 1985) and cotyledon (Knittel *et al.*, 1991; Liu *et al.*, 1991 and Ceriani *et. al.*, 1992) explants. Both hypocotyl (Fig. 1) and cotyledon explants produced loose, translucent callus. It is evident that callus induction in sunflower is an simple and easy process.

In the present study, 'Morden' alone was found to produce a few bud initials in cotyledon derived callus on MS medium containing 1 mg each of 2,4-D and BA (Fig. 3). In the other genotypes, there was continued callus proliferaion without any kind of shoot morphogenesis. The fact that morphogenic response is dependent on the genotype and other factors has been well documented (Lupi *et al.*, 1987; Chraibi *et al.*, 1992; Ceriani *et al.*, 1992). On the same medium hypocotyl callus was more compact and dark green without any differentiation. However, root morphogenesis form callus was quite frequent in all the genotypes.

In order to obtain induction of morphogenesis, callus was also cultured in liquid MS medium with BA (1.0mg) alone or with NAA (0.1 mg/1). After four weeks in culture, small, yellowish green nodules were produced but, there was no morphogenesis (Fig. 2). However, Chraibi *et al.*, (1992) demonstrated high frequency of shoot formation using liquid culture system. They recommended the use of liquid medium as it permited better contact between tissue and medium and it was also known to inhibit ethylene production by sunflower cotyledons (Chraibi *et al.*, 1992).

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Table 1. Effect of growth regulators on callus induction (%) from hypocotyl and cotyledon explants of sunflower

Growth regulators	KBSH-1 BSH-1 Morden Mean (%)							
	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon	Hypocotyl ± 1.04	Cotyledon ± 2.79
NAA (1.0)	100.0	100.0	100.0	100.0	100.0	100.0	100.0 (90.00)	100.0 (90.00)
NAA (0.1) + BA (1.0)	93.3	93.3	81.2	85.7	91.0	100.0	88.5 (70.92)	93.0 (81.14)
NAA (0.3) + BA (1.0)	100.0	100.0	100.0	93.7	100.0	100.0	100.0 (90.00)	97.9 (86.55)
NAA (0.5) + BA (1.0)	100.0	100.0	100.0	100.0	100.0	100.0	100.0 (90.00)	100.0 (90.00)
NAA (1.0) + BA (1.0)	100.0	100.0	100.0	100.0	100.0	100.0	100.0 (90.00)	100.0 (90.00)
2,4-D (1.0)	100.0	100.0	100.0	100.0	100.0	100.0	100.0 (90.00)	100.0 (90.00)
2,4-D (2.0)	100.0	100.0	100.0	100.0	100.0	100.0	100.0 (90.00)	100.0 (90.00)
2,4-D (0.1)+BA(1.0)	100.0	66.0	100.0	66.0	100.0	100.0	100.0 (90.00)	77.3 (66.55)
2,4-D (0.5+BA(1.0)	100.0	80.0	100.0	71.4	100.0	100.0	100.0 (90.00)	83.8 (72.02)
2,4-D (1.0)+BA(1.0)	100.0	90.0	100.0	77.8	100.0	100.0	100.0 (90.00)	89.2 (77.31)
MS_1	80.0	87.5	28.5	33.3	100.0	81.8	69.5 (58.96)	67.5 (55.31)
MS_2	100.0	100.0	100.0	90.0	87.5	100.0	95.8 (78.17)	96.6 (85.00)
IAA (1.0)	87.5	62.5	85.7	44.4	62.5	33.3	78.5 (68.95)	46.7 (42.93)
IAA (1.) + BA (1.0)	87.5	25.0	100.0	9.1	80.0	20.0	89.1 (71.88)	18.0 (24.41)
Mean (%) ± 0.4	96.3 (84.07)	86 (75.92)	92.5 (82.45)	76.5 (67.98)	94.3 (82.68)	88.2 (79.39)	94.3 (82.77)	83.5 (74.39)

Figures in paranthesis indicate transformed (are sine) values.

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