IMPROVEMENT OF SUNFLOWER USING TISSUE CULTURE TECHNIQUES

AKBAR S. MOHMAND AND MASOOD A. RANA

NATIONAL OILSEED DEVELOPMENT PROJECT (NODP)
NATIONAL AGRICULTURAL RESEARCH CENTRE
P.O. NARC, ISLAMABAD, PAKISTAN

ABSTRACT:

DEVELOPING COUNTRIES ARE NATURALLY ATTRACTED TO THE POTENTIAL APPLICATION OF BIOTECHNOLOGICAL RESEARCH IN SOLVING PROBLEMS OF HUNGER, ENERGY SUPPLY AND IMPROVING THE QUALITY OF LIFE. PAKISTAN IS FACED WITH CHRONIC DEFICIT IN EDIBLE OIL PRODUCTION WHICH IS MET THROUGH THE IMPORT COSTING MORE THAN NINE BILLION RUPEES ANNUALLY. SUNFLOWER IS SIGNIFICANT OILSEED CROP RANKING SECOND TO SOYBEAN IN WORLD FOR VEGETABLE OIL PRODUCTION. VERY RECENTLY WE HAVE STARTED WORK ON SUNFLOWER BIOTECHNOLOGY/TISSUE CULTURE FOR THE ESTABLISHMENT OF PROTOCOLS FOR CALLUS INDUCTION AND PLANT REGENERATION. ENCOURAGING RESULTS HAVE BEEN OBTAINED ON CALLUS INDUCTION IN THREE INBREDS/OPEN POLLINATED VARIETIES OF SUNFLOWER USING VARIOUS COMBINATIONS OF CULTURE MEDIA. DIFFERENT EXPLANT SOURCES WERE USED WHICH RESPONDED DIFFERENTLY. ANTHER CULTURE STUDIES WERE ALSO MADE FOR OBTAINING TRUE HOMOZYGOUS LINES. PRELIMINARY STUDIES WERE ENCOURAGING AND GOOD CALLUS INDUCTION FREQUENCY WAS RECORDED IN NOVINKA AND NOOR LINES. THE POTENTIAL APPLICATION OF TISSUE CULTURE FOR THE IMPROVEMENT IS BRIEFLY DISCUSSED.

INTRODUCTION

Pakistan is faced with a chronic deficit in edible oil production which is met through imports of more than nine million rupees annually. Assuming the present population growth rate and the average per capita consumption, our requirements expected to reach 1.7 million tons by the year 2000.

The potential agricultural application of plant tissue and cell culture to sunflower improvement requires careful integration of this new technology with existing breeding programmes. Using conventional methods such as introduction, selection and breeding, high yielding and widely adapted lines have been developed. Plant biotechnology offers the most potent emerging technique for the induction as well as utilization of genetic variability for desirable agronomic traits.

There has been few reports on cultured sunflower tissues capable of regeneration (Trifi et al.,1981; Greco et al.,1984; Bohorova et al.,1985; Georieva-Teodorova et al., 1980; Sadhu, 1974; Paterson and Everett, 1985; Finer, 1987).

The establishment of an efficient plant tissue culture technique represents a basic step in non-conventional improvement of crop plants. *Helianthus annuus* L. is a crop of increasing importance as a source of oil but the number of studies on sunflower tissue culture are still limited. The development of basic tissue culture protocols is a preliminary step towards plant regeneration from callus culture and single cells. In this study we report on callus induction from different explant sources and anthers of different lines of sunflower.

MATERIALS AND METHODS

Seeds (achenes) of the *Helianthus annuus* L. inbreds were obtained from the Cooperative Research Program on Non-Conventional Oil Seed Crops of National Agricultural Research Center (NARC), Islamabad. The seeds were washed in 0.2% benlate and left overnight. The benlate treated seeds were then surface sterilized with 70% ethanol for 10 minutes followed by washing in 50% chlorox (5.25% commercial bleach of sodium hypochlorite with a drop of Tween-20 for 25 minutes. Seeds were aseptically germinated two per tube with 20 ml of Linsmaier and Skoog's (1965) salts, 1% sucrose and 0.8% agar. Seeds were germinated in the dark for the first three days and then kept in the light with 16 hrs photoperiod at 25°C. 21 day old seedings were used as cotyledon and hypocotyl explant source. Hypocotyl and cotyledon segments (3-5 mm) were put into culture tubes containing LS medium supplemented with various concentrations of 2,4-D, BAP and NAA. The cultures were kept in the growth room with 16 hrs photoperiod at 25 + 2 °C. Data was recorded on passage basis (30 days each) for the frequency of callus induction.

For anther culture, inbreds Novinka and Noor were used. Anthers were isolated at the uninucleate stage from the greenhouse grown plants. Capitulum were first washed thoroughly in tape-water using commercial soap for about 10 minutes followed by 5 min rinse in 70% ethanol. This was followed by immersion in 50% chlorox for 10 min. Finally the capitulum was rinsed three times in sterile distilled water and anthers were isolated.

RESULTS AND DISCUSSION

Callus induction:

Eleven growth regulator combinations were used for somatic callus induction in sunflower inbreds Noor and Shams. The response of the explant differed according to their source and the culture media combinations used (Table 1-4).

Table 1: Callus induction on media supplemented with various concentrations of 2,4-D (mg/1) in Noor and Shams.

Explant	2,4-D + 1.0		2,4-D + 2.0		2,4-D + 3.0	
	No. of Explant	Calli %	No. of Explant	Calli %	No. of Explant	Calli %
Cotyledon	29	26.0	30	0.0	31	32.3
Hypocotyl	28	21.4	26	0.0	26	19.2
			SHAMS			
Cotyledon	45	15.3	42	3.5	35	10.5
Hypocotyl	55	23.0	55	5.0	40	11.3

Table 2: Callus induction on media supplemented with various concentrations of B A P (mg/1) in Noor and Shams.

Explant	BAP + 0.5		BAP + 1.0		BAP + 2.0	
	No. of Explant	Calli %	No. of Explant	Calli %	No. of explant	Calli %
Cotyledon	28	60.7	26	3.8	27	0.0
Hypocotyl	27	100.0	29	68.9	27	0.0
			SHAMS			
Cotyledon	40	50.1	45	5.2	45	3.5
Hypocotyl	42	88.2	40	41.2	35	6.8

Table 3: Callus induction on media supplemented with various concentrations of 2,4-D & B A P (mg/1) in Noor and Shams.

	LS + 1.0 2,4-D 0.5 B A P		LS + 2.0 2,4-D 1.0 B A P		LS + 3.0 2,4 -D 2.0 B A P	
	No. of Explant	Calli %	No. of Explant	Calli %	No. of explant	Calli %
Cotyledon	22	13.6	29	68.9	23	17.4
Hypocotyl	30	33.3	21	71.5	26	11.5
			SHAMS			
Cotyledon	60	33.5	45	70.3	48	15.8
Hypocotyl	52	50.8	50	81.5	53	18.6

Table 4: Callus induction on media supplemented with various concentrations of NAA and BAP (mg/l) in Noor and Shams.

Explant	LS + 2.0 NAA + 0.5 B A P		LS + 2.0 NAA + 0.5 B A P	
	No. of Explant	Calli %	No. of Explant	Calli %
Cotyledon	20	95.00	29	86.20
Hypocotyl	27	100.00	29	91.30
		SHAMS		
Cotyledon	55	80.50	50	90.30
Hypocotyl	50	89.60	60	83.80

Table 5. Callus induction frequency from anthers of Noor and Novinka.

S.No.	Medium	No. of anthers cultured	Callus induced	Percentage
1.	LS+1.0 2,4-D + 2.0 Kinetin	350	22	6.29b*
2.	LS+2.0 2,4-D + 0.5 Kinetin	200	00	0.00c
3.	LS+ 1.0 IAA + 1.0 BAP	250	38	15.20a
4.	LS+ 2.0 IAA + 2.0 BAP	260	17	6.54b
5.	LS+ 0.5 IAA + 0.5 BAP	550	46	8.30b
		NOVINKA		
1.	LS+1.0 2,4-D + 2.0 Kinetin	300	19	6.33c*
2.	LS+2.0 2,4-D + 0.5 Kinetin	350	20	5.71c
3.	LS+ 1.0 IAA + 1.0 BAP	270	23	8.51b
4.	LS+ 2.0 IAA + 2.0 BAP	290	25	8.62b
5.	LS+ 0.5 IAA + 0.5 BAP	460	55	11.52b

* Means followed by the same letters do not differ significantly according to DMRT.

After 10 days in culture, callus proliferation started from the cut ends of the hypocotyls on LS medium enriched with 0.5-1.0 mg/1 benzyl aminopurine (BAP) and 2.0 mg/1 naphthalineacetic acid (NAA). 95-100% callus was induced in Noor while this frequency ranged from 80 to 90 in Shams in both the explant. Most of the calli (70%) were green and compact in nature. LS medium supplemented with various levels of 2,4-D yielded 0-33% callus in both the inbreds and explant (Table 1.). BAP at 0.5 mg/1 induced 60.7% callus on cotyledon and 100% on hypocotyl in Noor; Shams responded about the same way yielding 50% and 88% callus in cotyledons and hypocotyls. Increase in BAP (1.0 to 2.0 mg/1) suppressed callus induction in both the explant and

in both the inbreds. 2.0 mg/1 2.4-D in combination with 1.0 mg/l BAP also improved callus induction frequency in both inbreds in both the explant. Higher levels of 2,4-D in combination with higher levels (2.0 mg/1) of BAP proved to be inhibitory for callus induction. Over all, the hypocotyls responded well to about all the combinations of auxins and cytokinins compared to the cotyledons in both the inbreds except two in which none of explant source yielded any callus (Table 2,3).

Plant Regeneration:

The LS medium enriched with 1.0 mg/1 BAP, 0.2 NAA, adenine sulfate, 5mg/1 KNO_3, 500 mg/1 casamino acid and 0.4 mg/1 GA_3 induced strong and prominent embroyids on the calli of both the explant and in both the inbreds but no plants were produced. Similar tendency was also observed in LS medium plus B_5 vitamins, 0.2 mg/1 GA_3, 1.0 mg/1 BAP, 500 mg/1 adenine sulfate and 500 mg/1 casamino acids.In the third combination LS medium fortified with vitamins and amino acids of C17, no embryoids were observed. Only green spots with very low frequency (30%) were seen. Plain LS medium also could not produced any plants except small tiny green spots. experiments are in progress for testing some more combinations of the auxins and cytokinins for regeneration.

ANTHER CULTURE:

Using anther and/or pollen culture, haploids have been obtained in a wide variety of plants (Bohorova et al, 1985). In sunflower very limited work has been done on *in vitro* culturing of excised anthers. For developing homozygous sunflower lines; efforts are required for the production of haploids for utilization in breeding programmes.

Work on the production of haploid in sunflower was initiated at the tissue culture lab. NARC using different inbreds. Preliminary experiments are being conducted on the optimization and standardization of protocols for callus induction and plant regeneration from anthers of sunflower inbreds.

LS medium fortified with various combinations of auxins and cytokinins are presented in Table 5. Out of five different combinations, LS basal medium supplemented with 1.0 mg/1 IAA and 1.0 mg/1 BAP yielded the highest (15.2%) callus induction frequency compared to LS medium plus 2.0 mg/1 2,4-D and 0.5 mg/1 Kinetin where induction frequency was 0% in Noor. The rest of the combinations yielded 6.29 and 6.54% callus. Inbred Novinka responded differently showing on an average 8.13% induction frequency. Similarly Alisa et al. (1985) have reported androgenesis from the cultured anthers of *Helianthus*. Bohorova et al, (1985) have reported best callus formation from the anthers of many species on MS medium with 1 mg/l 2,4-D and 0.2 mg/l kinetin. However, calli thus obtained lacked subsequent plant regeneration. Further studies are in progress for the optimization of these culture conditions.

REFERENCES

Alisa, A., H. Serieys, and R. Jonard.1985. Wild species and interspecific hybrid regeneration in *Helianthus* genus by *in vitro* androgenesis. Compt. Rend. Acad. Sciences. 300: 25-30.

Bohorova, N.E., A. Atanassov, and J. Georgieva-Todorova. 1985. *In vitro* organogenesis, androgenesis and embryo culture in the genus *Helianthus*. Z. Pflanzenzuchtung. 95: 35-44.

Greco, B., O.A. Tanzarella, G. Carrozo, and A. Blanco. 1984. Callus induction and shoot regeneration in *Helianthus annuus* L. Plant Sci. 36: 73-77.

Linsmaier, E.M., and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue culture. Physiol. Plant 18: 100-127.

Georgieva-Todorova, J., N. Bohorova, and A. Atanassov. 1980. Utilization of the method of tissue culture in the interspecific hybrids of *Helianthus*. 9th Intern. Conf. Sunflower, Tormolinas, Spain; 122-128.

Sadhu, N.M.K. 1974. Effect of different auxins on growth and differentiation in callus tissue from sunflower stem pith. Indian J. Expt. Soil. 12: 110-111.

Paterson, K.E., and N.P. Everett. 1985. Regeneration of *Helianthus annuus* inbred plants from callus. Plant Sci. 42: 125-132.

Finer, J.J. 1987. Direct somatic embryogenesis and plant regeneration from immature embryos of hybrid sunflower *Helianthus annuus* L. on a high sucrose containing medium. Plant Cell Reports. 6: 372-374.

Trifi, M., S. Mezghani, and M. Marrakchi. 1984. Vegetative propagation of *Helianthus annuus* L. by *in vitro* culture. Physiol. Veg. 19: 99-102.