USE OF DIRECT ORGANOGENESIS IN VITRO FROM IMMATURE EMBRYOS OF INTERSPECIFIC AND INTERGENERIC HYBRIDS OF Helianthus annuus L.

Encheva,J.,M.Christov, P.Ivanov
Institute for Wheat and Sunflower "Dobroudja"
9520 General Toshevo, Bulgaria

Summary

Immature embryos from sexual hybrids of the crosses: H.mollis x H.annuus; H.annuus x H.salicifolius; H.annuus x H.tuberosus and H.annuus x Verbisinia helianthoides are used for induction of a direct organogenesis. Plants and seeds are obtained from all combinations. The next plant generation is grown under greenhouse conditions. The seeds of F_3 are characterized by cytological and biochemical methods.

Introduction

Sunflower wild species are source of genes carrying a series of valuable characters. The poor crossability and sterility of F_1 hybrid generation appears as a barrier for the use of the genetic potential of the wild species for improvement of the cultivated sunflower. At present the techniques for hybrid embryo rescue undergo considerable improvements. For example Chandler and Beard(1983); Georgieva-Todorova(1984); Bohorova et al(1985) and Kräuter et al(1991) developed through embryo rescue successful combinations, which are not produced according to the conventional methods. Espinasse et al(1991) produced for the first time a successful cross between H.annuus x H. maximiliani by thr use of the embryo rescue technique through ovulo culture. Still the reports for a successful interspecific hybridization with perennial species are rather few.

The aim of this study is to apply the method for a direct somatic organogenesis in hybrid embryos developed abnormal as an approach not applied till now for producing of plants and seeds by some interspecific and intergeneric crosses.

Material and Methods

The sunflower line HA89, Albena commercial hybrid, the wild perennial species: H.molls(2n=34), H.salisifolius(2n=34), H.tuberosus(2n=102) and Verbisinia helianthoides(2n=34) are grown under field conditions at Dobroudja IWS, G.Toshevo.The hybrid embryos are taken between the 4^{th} and 9^{th} day after pollination. They are sterilized,

excised aseptically and cultured in petri dishes. Three different nutrient media are used successively(Table 1). The hybrid embryos are plated on medium I (according to Volin et al 1989) for further embryo development. The culturing conditions are at 26± 1°C, 16/8h photoperiod, 1500 lux cold white flourescent light. After one week the embryos are transferred on medium II for producing of somatic buds (according to Freyssinet and Freyssinet,1988) and cultivated in dark, at 25± 1°C. After 2-3 weeks the somatic buds appeared already are excised and placed on medium III for rooting (according to Wilcox et al,1988) and cultivated in the same conditions as the embryo development. After 2-3 weeks the plants reaching a height of 2-5 mm are transferred in pots containing soil and sand in a ratio 3:1 and grown in a greenhouse. The fertile plants are selfpollinated by hand. The next F_2 generation was grown again in a greenhouse and selfpolinated. The F_3 seeds are subjected to cytological and biochemical studies.

Table 1.Composition of the used media (g/l)

	Media		
	I	II	III
Salts	B_5	MS	MS
Vitamins	MS	MS	-
Inositol	-	3.9	0.1
Thiamine	-	-	0.0004
Supplement	-	+	-
Sucrose	60	90	20
NAA	0.0001	-	-
BAP	-	0.0005	-
IAA	-	-	0.00001
Agar	7	7	6
pH	5.7	5.0	5.7

Supplement : L-Alanine,1; L-Glutamine,0.8; L-Serine,0.16; L-Tryptophane,0.05 and L-Cysteine,0.01

The cytological studies are conducted by a temporary preparation stained by the reagent of Shiff. The storage proteins of the F_3 hybrid seeds and parents are studied electrophoretically in 10% polyacrylamide gel according to Laemmli(1970).

Results and Discussion

Hybrid embryos at 4-9 days are excised aseptically after pollina-

tion and are placed on medium I for further growth(Volin et al,1989) After one week the embryos are transferred on medium II to form a direct organogenesis(Freyssinet and Freyssinet,1988). Between the 6th and 10th day after plating the first somatic buds appear on the hypocotyl part of the embryo and some cases on the cotyledon surface. Formation of an intermediary callus was not observed. After 2-3 weeks the shoots produced are excised and placed on medium III for rooting(Wilcox et al,1988).

Table 2. Responsiveness of the genotypes studied to the conditions of culturing, produced plants and seeds.(in numbers)

Cross	Embryos plated	Embryos survived	Plants produced	Plants survived	Seeds
H.mollis x H.annuus	10	1	4	2	6
hybrid Albena x H.maximiliani	9	1	3	0	0
hybrid Albena x H.salicifolius	10	1	3	2	12
hybrid Albena x H.tuberosus	10	1	5	3	14
HA89 x H.silphioides	10	4	16	0	0
hybrid Albena x Verbisinia helianthoides	9	1	2	2	9

Contrary to the method of Freyssinet and Freyssinet(1988) the medium for further growth of the sometic buds is omitted and the buds are placed on a medium III where they form shoots and roots simultaneously. Table 2 shows that plants produced in all crosses conducted but survivability in some cases is very low.

Table 3. Results from cytological studies.

Mother	Father	Chromosome number
H.mollis	H.annuus	2n=34
hybrid Albena	H.salicifolius	2n=34
hybrid Albena	H.tuberosus	2n=68
hybrid Albena	Verbisinia helianthoides	2n=34

There are several publications for a successful hybridization

through embryo rescue but we are not informed about a direct organogenesis using immature embryos from interspecific and intergeneric hybrids. This is the first report for producing of an intergeneric hybrid in H.annuus x Verbisinia helianthoides.

It is known, that the electrophoretic patterns of the storage proteins are genetically specific and used for a genotypic characterization in many crops including sunflower.(Ivanov et al,1985, Anisimova and Gavrilyuk,1981). The results(Figure 1,2&3) obtained show differences between the species H.annuus (hybrid Albena) and other investigated species. The data about the hybrid seeds show a presence of patterns combining single bands from the two parents. This result can be accepted as an evidence for the hybrid nature of the seeds analyzed. Regarding the differences between the single seeds patterns, the explanation is in the free recombination between the chromosomes from the two genomes and the suggestion that the seed storage protein is controlled by genes localized in more than one chromosome.

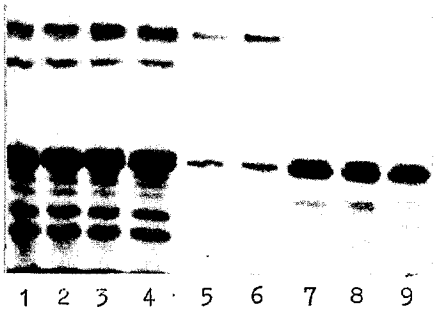

Fig.1 1,2-Albena; 3,4,5,6-F_3;7,8,9-H.salicifolius

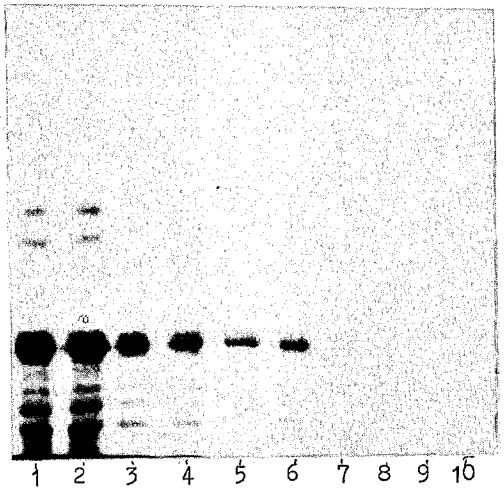

Fig.2 1,2-Albena; 3,4,5,6-F_3; 7,8,9,10-H.tuberosus

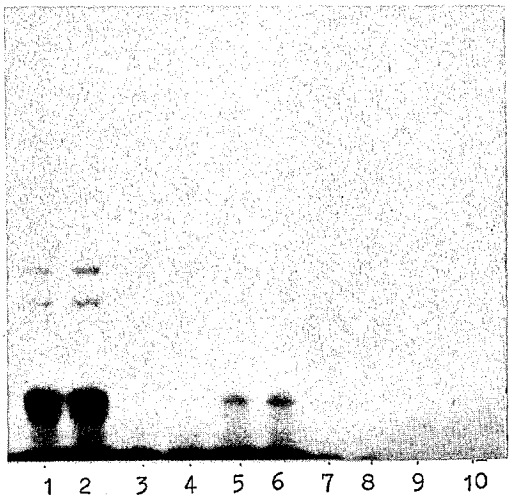

Fig.3 1,2-Albena; 3,4,5,6-F_3; 7,8,9,10-Verbisinia helianthoides

REFERENCES

1. Anisimova,I.N., I.P.Gavrilyuk(1989) "Genetics USSR",25(7),1248-1254
2. Bohorova,N.E., A.Atanassov, J.Georgieva-Todorova(1985) "Z. Pflanzenzuecht",95,34-44
3. Chandler,J.M., B.H.Beard(1983) "Crop Sci.",23,1004-1007
4. Espinasse,A., J.Volin, C.D.Dybining, C.Lay(1991) "Crop Sci.",31 102-108
5. Freyssinet,M., G.Freyssinet(1988) "Plant Sci.",56,177-181
6. Gamborg,O.L., R.A.Miller, K.Ojima(1968) "Exp.Cell Rep.",50,151-158
7. Georgieva-Todorova,J.(1984) "Z.Pflanzenzuecht",93,263-279
8. Ivanov,P., A.Piskov, M.Stoyanova(1985) "Agricultural Science",23 (6),55-60
9. Krauter,R., A.Steinmtz, W.Friedt(1991) "Teor. Appl. Genet.",82 21-523
10. Laemmli,U.K.(1970) "Nature",227,680-685
11. Murashige,T.F., F.Skoog(1962) "Physiol. Plant.",15,473-497
12. Volin,J., A.Espinasse, C.Lay(1989) "Sunflower research workshop" January 9-10
13. Wilcox,A., G.Cooly, J.Van Dreser(1988) "Plant Cell, Tissue and Organ Culture",14,103-110