APPLICATION OF TISSUE AND PROTOPLASTS CULTURE IN THE GENUS HELIANTHUS L.

N.Bohorova

Bulgarian Academy of Sciences, Institute of Genetics, III3 Sofia Bulgaria

SUMMARY

Growth and differentiation from young leaves and cotyledons pieces, shoot apices, hypocotyl segments from I6 genotypes cultivated, wild and interspecific hybrids of sunflower /Helianthus L./ have been tested. Fast growing calli were obtained on MS medium supplemented with NAA and BAP. Three of the genotypes developed rapid regeneration of meristematic nodulls, roots, many shoot and plants from callus.

These studies form the information of procedures, following induced protoplast isolation, fusion and utilization the in-built visual identification of interspecific fusion products between leaf protoplasts of wild Helianthus species and colorless, etiolated hypocotyl protoplasts of cultivated sunflower H. annuus. Optimal culture conditions were established for the respective protoplast systems. Heterokaryons /H. annuus and H. praecox/ were identified as protoplasts prossessing chloroplasts in a rich cytoplasmic background.

INTRODUCTION

The cultivated sunflower /Helianthus annuus L./ is one of four most important annual crops in the world. The potential agricultural application of plant tissue and cell culture to sunflower improvement requires careful integration of this new biotechnology with existing breeding programs. The in vitro techniques have been developed and promise to play increasingly impotant roles in crop improvement.

MATERIALS AND METHODS

This report summarizes the results of a in vitro Androgenesis and Embryo culture /Bohorova et al I985/ in the genus Helianthus, the use of in vitro methods in interspecific hybridization for obtainig viable plants/Bohorova I982/. The effects of different hormonal balances for growth and differentiation from sunflower tissues were studied /Bohorova, in press/. Optimal culture conditions for protoplast isolation and fusion were established /Bohorova et al.I986, Bohorova,in press/.

RESULTS AND DISCUSSION

The first step towards the use of biotechnology for sunflower improvement is the development of technology for in vitro culture of interspecific hybrids. Embryo rescue should help to obtain hybrids between sexually incompatible species for wider genetic diversity /Bohorova I982, Chandler and Jan I984, Alissa et al. I986/. Interspecific hybrids obtained by crosses of H. annuus with the tetraploid species H. hirsutus, H. decapetalus /Bohorova I977, Georgieva et al.I979/and H. scaberimus /Bohorova I988 /are vigorous and combine the characters of both parent species,

but sterile or the limited seeds were with not fully developed embryos in case of pronouced embryonal incompatibility /Bohorova 1982/. Three hybrid plants were obtained from H.annuus x H.hirsutus /2n=4x=68/ by in vitro germination of seeds, two of them remaining in a rosett form and only one continuing to grow. By in vitro micropropagation and after well-developed shoots, regenerated plantlets were transplanted in the field. The BC_1-$BC_2$with cultivated sunflower restored the chromosome number of H.annuus, but it is possible that some structural chromosomal differences still existed /Bohorova,Georgieva 1987/. Therefore considerable phenotypical diversity was observed among the progeny. Male sterile plants with high fertility were obtained as a results of this hybridization and backcrossing.

Hybridization of the cultivated sunflower with tetraploid species H.scaberimus was successful only if the female parent had the higher ploidy level. The application of in vitro germination to F_1hybrid seeds /Bohorova 1982/ showed that 41,2% of them developed and 25 viable plants were grown, and from F_2 15,9% of the seeds developed and were grown on the experimental field. Populations of the plants were of an intermediate type of inheritance, they differed in height, branchiness, size and shape of leaves and racemes. Bivalents,polyvalents and univalents were observed in pollen mother cells of the F_1-BC_5/P//Bohorova 1988/ hybrids. These populations providing opportunities for the selection of plants possessing valuable characters are obtained as a result of this hybridization. Male sterile plants have been produced and are presented for testing. Plants with interesting economic characters such as with short stems of up to 1m., with diameter of the inforescence 25-28 cm.,early blooming /up to July 15/ and early ripening with high protein content of seeds.

Anther culture is the most recent method for sunflower breeding /Plotnikov 1975,Tzen and Lin 1975, Mix 1985,Alissa et al. 1985, Bohorova et al. 1985/. Although anther culture has proved to be quite efficient for the induction of haploids, it has one main disadvantage: the plants not only originate from pollen but also from various other parts of the anther, beacouse there are a population of plants with various ploidy.Direct shoot formation occurred in anthers of H.divaricatus /one plant/ and H.annuus x H.decapetalus/2n=4x=68/three plants from two anthers/. Cytological analysis of root tips of 125 plants obtaining through the secondary shoot organogenesis during the first 15 subcultures showed that one-third of the plants had 34 chromosomes, while two-thirds had 2n=45 - 51, 68 and 102.Bohorova and Landjeva /1987/ carried out a cytological investigations on the calli from anther of Helianthus species with different ploidy level and established that the callus formed in anther originatedes from sporofitic as well as from somatic anther tissue. Plants regeneranted from F_1 anthers H.annuus x H.decapetalus possessed seed sterility. Recently in vitro development of 5-7 days old embryos give rise to the shoots and plantlets. Experiments for development and cultured these R_0 plants are curently in progress. On the problems of ovule culture as a means aimed at inducing haploid plants worke Mix /1985/, Jang et al/1986/, Gelebart et al./1987/.

The ability to regerate plants from single cells and protoplasts represents a basic step for the genetic improvement of crop plants in vitro. The effects of different hormonal balan-

ces for growth and differentiation from sunflower tissue are rather limited /Pall et al. I98I,Greco et al. I984, Paterson and Everett I985, Puibello and Caso I986, Bohorova et al.I985/very little attention has been given to the culture of sunflower protoplasts /Binding et al. I98I, Lenee and Chupeau I986, Bohorova et al.I986/. Different explants of I6 Helianthus cultivars, wild species and interspecific hybrids were tested for direct shoot formations,callus induction and callus regeneration from tissue and protoplast culture /Bohorova, in press/. Callus induction was obtain on the media supplemented with 2 mg/l NAA and 0,05mg/l BAP related to all genotypes. The best response with callus induction was the young leaves of H.resinosus, H.scaberimus x H.annuus and stem pieces of interspecific hybrids. This explant was suitable for producing friable, light green granular callus. Using the medium with NAA:BAP ratio I:I0, friable callus with numerous green meristematic sections was induced from leaf, hypocotyl, stem segments of H.praecox, H.scaberimus, H.resinosus and the young leaf and stem explants of H.scaberimus x H.annus. As far as the embryogenic tissues are concerned, more than 20 new embryoids per tube were obtained and within 7 days most of these structures developed into small shoots, plantlets and roots. More regeneration was observed when hypocotyl explants were taken from 7 days old seedlings.

Most plant species needed auxins and cytokinins for dedifferentiation and this effect was obtained by NAA and BAP in different sunflower explants. The different hormonal combinations tested, appeared to modify the morphogenetic responses. The presence of NAA and BAP with fixed concentrations claimed an essential components of the culture medium for morphogenetic responses and this regenerational potential was under genetic control / Bohorova, in press/.

Bohorova et al. /I986/ described the protoplasts isolation from seedling roots, hypocotyls and cotyledons of four cultivars of Helianthus annuus and from leaves of axenic shoot cultures of the wild species H.praecox, H.scaberimus and H.rigidus. Optimal culture conditions were established for the respective protoplast systems,using the agarose bead method of culture. Protoplast division was induced for all the species examined. In the case of the cultivars of H.annuus hypocotyl and cotyledon protoplast division was sustained leading to callus formation, which in turn, could be induce roots and organised meristematic regions in the presence of NAA and BAP. These studies form the basis of the development of procedures following induced protoplast fusion and utilising the in built visual identification of interspecific fusion products between leaf protoplasts of H.praecox and colourless etiolated hypocotyl protoplasts of cultivated sunflower. Heterokaryons were recoghnised by the presence of chloroplasts from the mesophyll protoplast and cytoplasmic strans from the etiolated hypocotyl protoplast /Bohorova, in press/. The beads were microscopically examined and those containing a single heterokaryons were recorded. Such experiments are currently in progress.

It has been shown that Agrobacterium tumefaciens T-DNA integrates in to multiple sites of the sunflower crown gall genome /Ursic et al.I983, Matzke et al. I984, Goldsbrough et al. I986, Everett et al I987, Tabata et al. I987) and that T-DNA is transcribed into at least seven polyadenylated RNAs /Murai and Kemp I982/. In the very near future it should be possible to produce transformed sunflower plants.

CONCLUSIONS

During the last decade plant breeders have used the best available technology to develop new varieties with stable yield, high quality and genetic resistance to various stresses. A number of in vitro techniques - tissue culture, cell culture, protoplast fusion and recombinant DNA technology have shown promise as plant breeding tools for creating genetic variability, or increasing selection efficiency. These tools should be used where conventional breeding techniques are less effective or efficient.

REFERENCES

Alissa A., Serieys H., Jonard R., 1985, Sur les possibilites de regeneration d'especes sauvages et d'hybride interspecifiques du genere Helianthus par androgenese in vitro. C.R.Acad.Sc. Paris t.300, Serie III, 25-30.

Alissa A.,R.Jonard, H.Serieys et P.Vincourt,1986, La culture d'embryons isole in vitro dans un programme d'amelioration du tournesol. C.R.Acad.Sc.Paris. t.302, s.III,n.5 161-164.

Binding H.,R.Nehls, R.Kock, J.Finger, G.Mordhorst, 1981, Comparative studies on protoplast regeneration in herbaceous species of the dicotyledonae class. Z.Pflanzenphysiol., 101, 119-130.

Bohorova N., 1977, Study on Helianthus interspecific relations and cytogenetic characteristic of F_I hybrids. Thesis of Ph.D.,Inst.of Gen.,B.A.Sc.,Sofia / in bulg./

Bohorova N., 1982, In vitro plant development of seeds of Helianthus interspecific hybrids. C.R. de Bulg.Acad.Sc. t. 35, n.1, 105-107.

Bohorova N., 1988, Cytogenetical study of F_I-$BC_5(P_2)$ hybrids between H.scaberimus /2n=4x=68/ and H.annuus /2n=2x=34/. Genetics and Breeding n.1 /in bulg/.

Bohorova N.,Georgieva J., 1987, Hybridization between H.annuus /2n=34/ x H.hirsutus /2n=68/ F_I-BC_5. Genetics and Breeding,n.3 /in bulg./

Bohorova N.,Landjeva S., 1987, A cytological study on anther callus culture of Helianthus species in different ploidy level, Genetics and Breeging, n.6 /in bulg/.

Bohorova N., A.Atanassov, J.Georgieva, 1985, In vitro organogenesis,Androgenesis and Embryo culture in the genus Helianthus L., Z.Pflanzenzuchtg. 95, 35-44.

Bohorova N.,C.Cocking, B.Power, 1986, Isolation,culture and callus regeneration of protoplasts of wild and cultivated Helianthus species. Plant Cell Reports 5, 256-258.

Gelebart P.,San L.,1987, Obtention de plantes haploides par culture in vitro d'ovaires et d'ovules non fecondes de tournesol/Helianthus annuus L./Agronomie,7 (2), 81-86.

Chandler J.,1979, Sunflower interspecific hybridization using embryo cultur.MS Thesis, Univ.Cal.

Chandler J.,Beard B.,1983, Embryo culture of Helianthus hybrids Crop Science 23, 1004-1007.

Chandler J., Jan C.,1984, Interspecific hybridization via embryo culture.Col.Eval.andconserv. of wild species and their use in sunf.br.prog.July, Novi Sad.,24-27.

Everett N., K.Robinson and D.Mascarenhas, 1987, Genetic engineering of sunflower /Helianthus annuus L./Biotechnology, v.5 november, 1201-1204.

Georgieva J.,N.Bohorova, 1979.Studies on the chromosomes of the two Helianthus L. tetraploid species,Caryologia, v.32, n.3 335-347.

Goldsbrough P., Gelvin S.,Larkins B., 1986, Expression of maize genes in transformed sunflower cells.,Mol.Gen.Genet., 202, 374-381.

Greco B., G.Tanzarella, G.Carrozzo, A.Blanco, 1984, Callus induction and shoot regeneration in sunflower /Helianthus annuus L./. Plant Sci.Lett. 36, 73-77.

Lenee P.,J.Chupeau, 1986, Callus formation from sunflower /Helianthus annuus L./protoplast. Plant Sci. 43: 69-75.

Mix G.,1985 ,Antheren- und Ovarienkutur von Sonnenblumen /Heli - anthus L./Landbauforsch Volkenrode, 35, n.3

Murai N., J.Kemp,1982, T-DNA of p Ti - 15955 from Agrobacterium tumefaciens is transcribed into a minimum of seven polyaden ylated RNAs in a sunflower crown gall tumor. Nucleic Acids Res.,10, 1679-1689.

Pall H., E.Kurnik, L.Szobo, 1981, Napraforgo noveny-regeneracio in vitro hajtascscustenyeszetbol. Novenytermeles,30,201-209

Paterson K.,Everett N., 1986. Sunflower /Helianthus annuus L./ Biotechnology of Plant Improvement Spr.Verl. ed.Y.P.Bajaj.

Piubello S., O.Caso, 1986, In vitro culture of sunflower /Helianthus annuus L./ tissues. Pyton 46 (2), 131-137.

Plotnikov V.,1975, Production of sunflower sprouts in a generative tissue culture,Severo-kavk.naukyiiTsentr.Vys,3,79-81.

Tabata T., C.Terayama,K.Mikami.,H.Uchimija and M.Iwabuchi, 1987, An Accurate Transcription of Wheat Histone Genes in Sun - flower Cells. Plant Cell Physiol. 28 (1),73-82.

Tzen M.,Lon C., 1975, Influence of concentration of IAA, 2,4-D and kinetin on the in vitro anther cultures of radish broccoli and sunflower.Chung hua Nung Hsuch Hui Pao Tai.II,25-31

Ursic D.,J.Slighton, J.Kemp.,1983, Agrobacterium tumefaciens t-DNA interates into multiple sites of the sunflower crown gall genome. Mol.Gen.Genet. 190, 494-504.