

ANTHER CULTURE REGENERATION OF F_1 HYBRIDS OF HELIANTHUS
ANNUUS x HELIANTHUS SMITHII AND HELIANTHUS ANNUUS x
HELIANTHUS EGGERTTII

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SUMMARY

Anthers from F_1 hybrids of *H. annuus* L. (cms HA-89) x *H. smithii* and *H. annuus* (cms HA-89) x *H. eggerttii* in uninucleate microspore stage of the pollen are used for the callus induction and plant regeneration. The anthers are plated on a MS medium added with NAA and BAP. The calluses of the two hybrids differ in colour and structure. Some of the plants produced are included in the collection of "Dobroudja" IWS and others are used for cytological studies.

INTRODUCTION

The use of androgenesis in vitro in different species allows a rapid fixation of uniformed lines. There are few publications concerning the species of the genus *Helianthus*. Bohorova et al. (1980, 1985) worked with F_1 hybrids between *H. annuus* ($2n=34$) x *H. decapetalus* ($2n=68$) and *H. annuus* ($2n=34$) x *H. hirsutus* ($2n=68$). The anthers are isolated and plated in a MS medium with addition of 2,4D (1mg/l) and kin (0,2mg/l). Regeneration is produced in the combination *H. annuus* x *H. decapetalus*. Alissa et al. (1985) had used anthers from the species *H. resinosus*, *H. rigidus*, *H. tuberosus*, *H. occidentalis* plantagineus and F_1 interspecific hybrids *H. annuus* x *H. occidentalis*, *H. annuus* x *H. rigidus*, *H. annuus* x *H. resinosus*, *H. annuus* x *H. tuberosus*, *H. rigidus* x *H. annuus* and *H. tuberosus* x *H. annuus*. The anthers are isolated on a MS media, supplemented by NAA and BAP (0,5mg/l). The highest percentage of regeneration is produced by F_1 hybrids of *H. annuus* x *H. resinosus* (71%) and the species *H. tuberosus* (36,5%) and *H. resinosus* (25%). One of the first reports for regeneration from anthers of the cultivated species *H. annuus* is announced by Mezzarobba and Jonard (1986). They used 3 lines from the

collection of INRA and 5 hybrids. Eleven haploids and diploid plants are obtained as the anthers are treated under 35°C in dark during the first 12 days after isolation from the heads. The aim of this paper is to study the regeneration ability of some interspecific hybrids produced in the IWS near General Toshevo.

MATERIALS AND METHODS

Interspecific hybrids (F_1) of *H. annuus* (cmsHA-89) x *H. smithii* (2n=34) and *H. annuus* (cmsHA-89) x *H. eggertii* (2n=102), are produced searching new restorer genes. In these hybrids female line (cmsHA-89) is characterized with cytoplasmic sterility and consequently there is no doubt about hybrid nature of the seed and the developed plants. The plants are grown under field conditions. The anthers are isolated in uninucleate microspore stage. The sterilisation is conducted by a commercial bleach solution (1,6%). The medium of Murashige and Skoog (MS) is used for callus induction supplemented by NAA and BAP (0,5mg/l). The cultured anthers are stored under 26±1°C relative air humidity 70% light of 2000 lux and photoperiod of 16/8h (Alissa et al, 1985).

For regeneration are used the next media :

AV - MS + 1mg/l BAP + 0,5mg/l IAA

AA - MS + 0,1mg/l BAP + 0,1mg/l NAA + 0,1mg/l GA₃

AL - MS + 0,5mg/l NAA + 0,5mg/l BAP

B₅ - free of hormones and with a reduced content of sucrose (10g/l)

The medium used for rooting is also B₅.

The cytological observation are conducted on root tips from regenerants stained by acetocarmine.

RESULTS AND DISCUSSION

The calli produced by the two interspecific hybrids differed between themselves. The calli of a hybrid *H. annuus* (HA-89) x *H. smithii* exhibited more green zones, more pale, and with a more coarse grainy structure, and the calli of the other hybrid *H. annuus* (cmsHA-89) x *H. eggertii* are yellow and the grainy structure could not be observed. In the Table 1 are shown data about the responsibility of the two genotypes to produce callus. The percentage in both cases is very high.

For the regeneration are used 4 media (Table 2). The best regeneration medium is B₅ with a reduced content of sucrose (10g/l) and free of hormones. According to Chandler et al. (1983) recommended for germi-

duced from the diploid tissue of the sack, but also by a haploid callus produced from pollen grains, followed by a spontaneous diploidization of the cells forming the young regenerants. The twelve observed plants with 68 chromosomes confirm the running spontaneous diploidization. There is no doubt here that there is at least a single double chromosome number. Data for the tendency of the sunflower species to a spontaneous diploidization of their chromosome number are found also by Bohorova et al. (1985). In the cross *H. annuus* ($2n=34$) x *H. eggertii* ($2n=102$), all plants are with 68 chromosome number. In this case for the time being, it is difficult a preference to be given to the two possibilities. The plants originated by the pollen grains. Our studies on the pollen viability through staining by acetocarmine show that its viability is normal. These observations can be explained assuming the Rf genes transfer in the hybrids from the used pollinators (*H. smithii* and *H. eggertii*). That is the self-incompatibility and selectivity in fertilization widely expressed in sunflower should be taken into consideration in explanation of the sterility observed in the produced regenerants. The sterility in the distant hybrids is a common phenomenon and is due to a series of disturbances as a result of to the incompatibility between the cytoplasm and genotype, disturbances in the different chromosome number in the genotype etc. Finding an explanation of these problems is matter of further investigations. The regenerants produced till now are propagated successfully and maintained in a vegetative way the collection of the Institute

CONCLUSION

1. A regeneration from anthers of the interspecific hybrids (F_1) *H. annuus* ($2n=34$) x *H. smithii* ($2n=34$) and *H. annuus* ($2n=34$) x *H. eggertii* ($2n=102$) is produced.
2. Most of the regenerants are produced in the hybrid *H. annuus* x *H. smithii* at the fourth passage, and in the 7th passage in the hybrid *H. annuus* x *H. eggertii*. The regenerative ability of the calli is maintained already two years.
3. Medium B_5 , free of hormones is found as most suitable for regeneration.
4. Data assuming the running of a spontaneous diploidization in regenerants from anthers of the interspecific hybrid *H. annuus* x *H. smithii* are presented.

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