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ANTHER CULTURE REGENERATION FROM SOME WILD *Helianthus* SPECIES

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SUMMARY

Twenty perennial and annual wild species from *Helianthus* genus were included in the research. Thirteen of them were diploid, three were tetraploid and four were hexaploid. Among the diploid species, four were annuals and nine were perennials.

Four types of nutrient media with different NAA and BAP concentrations were used for callus induction. In the diploid species, callus formation varied from 0.6 to 48.9%, in the tetraploid from 16.7 to 38.3%, and in the hexaploid from 0.1 to 33.3%.

The variation in the perennial diploid species was from 0.6 to 48.9%, and in the annual from 17.2 to 26.7%.

Two variants of nutrient media were used for regeneration from the obtained embriogenic calli - a medium with growth regulators and a medium without hormones. For primary regeneration, the medium with growth regulators gave a better response, while for the secondary regeneration reaction was positive on the hormone-free medium.

Regeneration was obtained in four of the studied species - *H.mollis* (M-020), *H.mollis* (M-034), *H.salicifolius* (M-045) and *H.smithii* (M-008). The initial reaction in the species M-020 has begun from 3 embryogenic calli, in M-034 from 2 calli, in M-045 from 3 calli, and in M-008 from 5 calli, the numbers of obtained plants being 28, 38, 49 and 51, respectively.

The ploidy level of the obtained regenerants was determined flowcytometrically.

Key words: **anther culture, embryogenic callus, regeneration, species, sunflower**

INTRODUCTION

Studies of genetic variability of the cultivated sunflower (*Helianthus annuus* L.) have shown that the cultivated forms could not be a gene source for resistance to diseases, for high protein content, as well as for some other economically important characters. Therefore, a number of studies aim at examining the genetic potential of

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wild species from the genus *Helianthus* to assess possibilities for their use as sources of desirable genes and to assess their applicability in sunflower breeding programs.

Up to now, in interspecific hybridization, the cultivated sunflower has been represented by varieties and lines, and the second partner - the species from the genus *Helianthus* - have been heterozygous populations. Therefore, the obtained hybrid populations were strongly heterozygous, making it difficult or even impossible to efficiently select and stabilize desired genotypes combining the valuable qualities of both parents. The use of isogenic lines from the genus *Helianthus* for hybridization with cultivated sunflower lines would allow a relatively more easy, fast and precise transfer of these characters into the desirable genotypes. To achieve this purpose, methods of anther culture are widely applied in a number of cultures. In sunflower this method still needs a lot of experimental research work. Only a few publications have reported successful regeneration from anther culture from wild species up to now. Bohorova *et al.* (1985) obtained only one regenerant from the species *Helianthus decapetalus*. Alissa *et al.* (1985) obtained regeneration in the species *H.tuberosus*, *H.occidentalis*, *H.resinosus* and *H.rigidus*.

The present study aims at investigating the response of 20 *Helianthus* species to androgensis development; these species could be included in sunflower breeding programs due to their useful characters.

MATERIALS AND METHODS

The following *Helianthus* species were included in this study:

diploid species (2n = 34): *H.praecox* (E-029), *H.mollis* (M-020), *H.mollis* (M-034), *H.salicifolius* (M-045), *H.smithii* (M-008), *H.giganteus* (M-030), *H.decapetalus* (M-043), *H.divaricatus* (M-044), *H.glaucophyllus* (M-012), *H.debilis* (E-089), *H.petiolaris* (E-037), *H.nuttallii* (M-021), *H.argophyllus* (M-081);

tetraploid species (2n = 68); *H.hirsutus* (M-007), *H.laevigatus* (M-016), *H.strumosus* (M-126);

hexaploid species (2n = 102): *H.rigidus* (M-028), *H.eggertii* (M-001), *H.resinosus* (M-046), *H.tuberosus* (M-039).

M stands for the species with the perennial cycle of development, and **E** - for the annual species. The accession numbers belonging to IWS are presented in FAO.

Approximately 20 days after being plated, the embryogenic callus was separated from the non-embriogenic one and placed in a medium for regeneration (medium 3 and medium B_5, Gamborg *et al.*, 1968) with a reduced content of sucrose and without supplementation of hormones.

The anthers, calli and regenerants obtained were stored at 26±1°C, relative air humidity 70%, 2000 lux illumination and a photoperiod of 16/8 h.

Medium B_5 was also used for rooting.

The ploidy level of regenerants was determined at the stage of 2-3 leaves by using a flowcytometer (Partec CA II). A small piece from a leaf was used for this purpose.

RESULTS AND DISCUSSION

CALLUSOGENESIS

Diploid species from the genus *Helianthus*

The study included 12 diploids species, 4 of them annual and 8 perennial. From the 4 tested variants of nutrient media for callus induction from anthers, all tested diploid species produced the highest percentages of embryogenic calli on medium 3 (Figure 1). Only in the species *H.giganteus* (M-030) was high response obtained in medium 4 (4.9%). The species *H.glaucophyllus* (M-012) and *H.divaricatus* (M-044) showed the weakest response to callus induction from the isolated anthers, 0.6 and 1.1%, respectively. In the perennial species, the highest percentages of embryogenic calli were obtained from the species *H.salicifolius* (M-045) and *H.decapetalus* (M-043) - 48.9 and 38.9%, respectively; among the annual species, the percentages were highest in *H.petiolaris* (E-037) and *H.praecox* (E-029) - 26.7 and 22.7%, respectively. The perennial species were more responsive to callusogenesis than the annual ones.

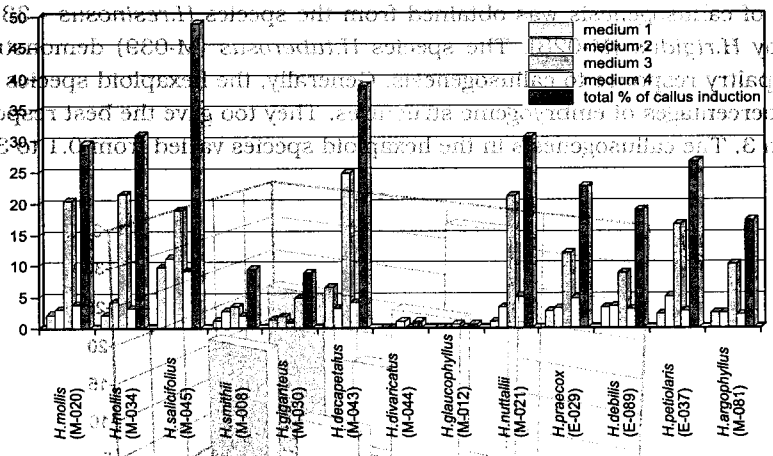

Figure 1: Effect of medium composition on callus induction from anthers of annual and perennial diploid Helianthus species.

Tetraploid species from the genus *Helianthus*

The following three tetraploid species were included in the research: *H.hirsutus* (M-007), *H.leavigatus* (M-016) and *H.strumosus* (M-126). The species *H.strumosus* gave the best response - 38.3% (Figure 2). All three species gave the highest percent-

ages of embryogenous callus on medium 3. The callusogenesis in the tetraploid species varied from 16.7 to 38.3%.

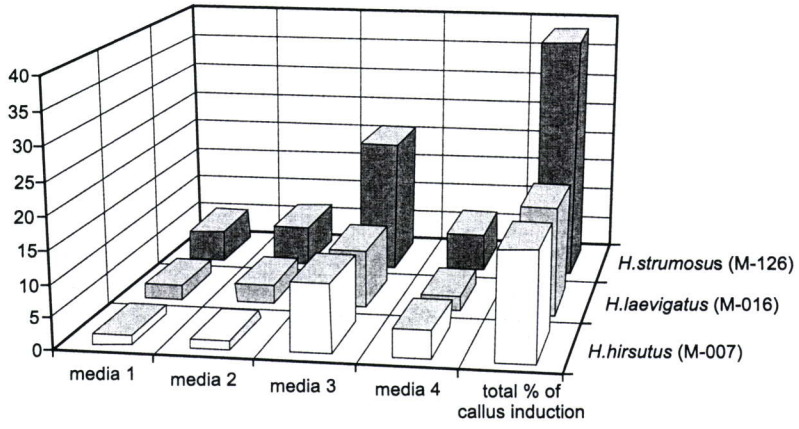

Figure 2: Effect of medium on callus induction from anthers of tetraploid Helianthus species.

Hexaploid species from the genus *Helianthus*

Four hexaploid species were included in the study (Figure 3). The highest percentage of callusogenesis was obtained from the species *H.resinosus* - 33.3%, followed by *H.rigidus* (M-028). The species *H.tuberosus* (M-039) demonstrated an almost paltry response to callusogenesis. Generally, the hexaploid species gave the lowest percentages of embryogenic structures. They too gave the best responses on medium 3. The callusogenesis in the hexaploid species varied from 0.1 to 33.3%.

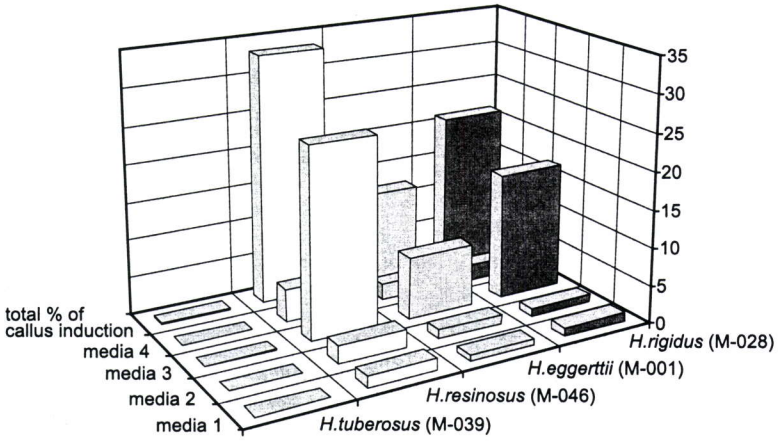

Figure 3: Effect of medium on callus induction from anthers of hexaploid Helianthus species.

REGENERATION

Two nutrient media were used for regeneration - medium 3 and medium B5 with a reduced content of sucrose.

Regeneration was obtained from 4 diploid perennial species with the chromosome number 2n = 34: *H.mollis* (M-020), *H.mollis* (M-034), *H.salicifolius* (M-045) and *H.smithii* (M-008) (Figure 4). In the species *H.mollis* (M-020), the primary regeneration began from 3 embryogenic calli, which were plated on medium B5. The other calli from this species which were on medium 3 did not develop their embryogenic structures into normal plants. The total number of regenerants obtained was 28. They had the diploid chromosome number. Half of them were grown under greenhouse conditions and were self-pollinated. The other half were transferred to the field and were further grown without being isolated. No seeds were obtained from either variant. The plants were successfully propagated vegetatively both under *in vitro* conditions and in the field.

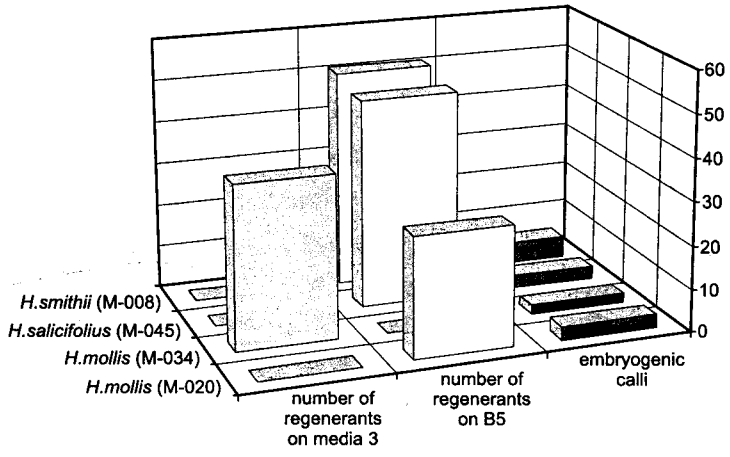

Figure 4: Primary regeneration from anthers of wild Helianthus species.

In the species *H.mollis* (M-034), the primary regeneration began from two embryogenic calli on medium 3. The obtained regenerants were 38 in total. In 11 of the produced regenerants the chromosome number was haploid before sowing in soil. Two weeks later their chromosome number was diploid without applying colchicine. This variation is most probably due to spontaneous diploidization, the reasons for which are difficult to explain at the present stage. No seeds from the regenerants of the species were obtained, a fact that cannot be explained either.

In the species *H.salicifolius* (M-045), 49 regenerants were obtained from 3 embryogenic calli plated on medium B5. All regenerants had the diploid chromosome number.

The species *H.smithii* (M-008) produced the highest number of regenerants. The primary reaction began from 5 calli with embryogenic structure and 51 regenerants were produced. All five calli formed shoots on the regeneration medium B5. Their chromosome number varied. Five of the regenerants had the haploid chromosome number, 39 had the diploid chromosome number and the remaining 9 - the tetraploid chromosome number. The explanation for the tetraploid chromosome number of these plants again could be reduced to spontaneous diploidization or polyploidization. Since these two phenomena are characteristic for the sunflower, we are unable for the time being to give a clear answer to the question about the origin of these diploid regenerants. Further molecular study could prove whether they originated from the somatic tissue of the anther or directly from pollen.

CONCLUSIONS

The following two important conclusions can be drawn from our study.

Twenty species from the genus *Helianthus* were tested for androgenic development. Regeneration was obtained only in four of them.

A medium without hormones allowed to develop the obtained embryos into plants.

In the species *H. smithii* (M-008), polyploidization was established unequivocally. At this stage it is difficult to prove the origin of the obtained regenerants.

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REGENERACIÓN DE UNAS ESPECIES SILVESTRES DEL GÉNERO *Helianthus* POR MEDIO DEL CULTIVO DE ANTERA

RESUMEN

Veinte especies del género *Helianthus* de varios años y de un año han sido estudiadas. Trece especies eran diploides, las tres eran tetraploides, y las cuatro eran hexaploides. Entre las especies diploides, cuatro eran de un año, y nueve de varios años.

El callo empezaba a formarse por cuatro especies de medios nutritivos que tenian varias concentraciones de NAA y BAP. La formación de callo variaba de 0.6 a 48.9% en las especies diploides, de 16.7 a 38.3% en la especies tetraploides, y de 0.1 a 33.3 en las especies hexaploides.

La variación era de 0.6 a 48.9% en las especies diploides de varios años, y de 17.2 a 26.7% en la especies diploides de un año.

Dos variantes de medios nutritivos eran utilizadas para la regeneración de los callos embriogénicos obtenidos - un medio con reguladores del crecimiento y otro sin hormonas. El medio con reguladores se mostró mejor para la regeneración primaria, y el medio sin hormonas fué mejor para la regeneración secundaria.

La regeneración era eficaz en cuatro especies investigadas - *H.mollis* (M-020), *H.mollis* (M-034), *H.salicifolius* (M-045) y *H.smithii* (M-008). La reacción inicial en la especie M-020 fué causada con suceso en tres callos embriogénicos, en la especie M-034 en dos callos, en la especie M-045 en tres callos y en la especie M-008 en cinco callos. El número de plantas obtenidas por especie era de 28, 38, 49 y 51.

El nivel de ploidez de los regenerantes obtenidos fué determinado fluidocitometricamente.

RÉGÉNÉRATION DE QUELQUES ESPÈCES SAUVAGES DE l'*Helianthus* À L'AIDE DE LA CULTURE DE L'ANTHÈRE

RÉSUMÉ

Vingt espèces vivaces et annuelles de l'*Helianthus* ont été observées. Treize d'entre elles étaient diploïdes, trois tétraploïdes, et quatre hexaploïdes. Parmi les espèces diploïdes, quatre étaient annuelles et neuf vivaces.

Quatre types de soutien nutritif ayant différentes concentrations de NAA et BAP ont été utilisés pour induire le callus. La formation du callus allait de 0.6 à 48.9% dans les espèces diploïdes, de 16.7 à 38.3% dans les espèces tétraploïdes et de 0.1 à 33.3% dans les espèces hexaploïdes.

La variation dans les espèces vivaces diploïdes était de 0.6 à 48.9% et de 17.2 à 26.7% dans les espèces diploïdes annuelles.

Deux variantes du soutien nutritif ont été utilisées pour la régénération du callus embryogénique - un soutien avec des régulateurs de croissance et un soutien sans hormones. Le soutien avec régulateurs de croissance a donné de meilleurs résultats dans la régénération primaire et le soutien sans hormones a donné de meilleurs résultats dans la régénération secondaire.

La régénération a été obtenue dans quatre des espèces étudiées - *H.mollis* (M-020), *H.mollis* (M-034), *H.salicifolius* (M-045) et *H.smithii* (M-008). La réaction initiale de l'espèce M-20 a commencé à partir de 3 calli embryogéniques, celle de l'espèce M-034 à partir de 2 calli, de M-45 de 3 calli et de M-008 de 5 calli, les plantes obtenues étant 28, 38, 49 et 59, respectivement.

Le niveau de ploïdité des régénérants obtenus a été déterminé cytométriquement.