

EFFICIENT INTERSPECIFIC HYBRIDIZATION VIA EMBRYO RESCUS FOR APPLICATION IN SUNFLOWER BREEDING

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SUMMARY

The paper presents preliminary results of a crossing program followed by embryo culture in vitro. The program included the female line HA89 (cms) and various American wild species (mostly perennials). Thirty-four interspecific hybrids have been made and 481 plants successfully regenerated, i. e., the average regeneration rate of 54.3% has been achieved. Presently, the hybrid progenies are being identified morphologically, cytologically, by isozyme electrophoresis and/or by RFLP analysis. They will be backcrossed and tested against available restorers. When new CMS materials are made, they will be screened for resistance to *Sclerotinia sclerotiorum* and *Botrytis cinerea*.

INTRODUCTION

Breeding and production of commercial sunflower hybrids is at present exclusively based on cytoplasmic male sterility (CMS) derived from *Helianthus petiolaris* by LECLERCQ (1). In order to avoid the potential occurrence of diseases associated with the cytoplasm, additional wild *Helianthus* species have to be exploited as new CMS sources. These sources can be transferred into the cultivated sunflower by wide hybridization with application of the "embryo rescue" technique. Such wide crosses are also highly valuable for the transfer of resistance genes from wild species to the cultivated sunflower.

MATERIALS AND METHODS

The female line HA89 (cms) has been pollinated by various American wild species (mainly perennials, which are characterized by different chromosome numbers and other distinct characters). In addition, male fertile wild species were used as female parents and pollinated by HA89 (maintainer). Embryo rescue in vitro was carried out 4-7 days after fertilization as described by ESPINASSE et al. (2). The majority of embryos were cultured in a "heart-shape stage" (cf. Fig. 1a), which corresponds to a diameter of 0.3-0.7 mm. Backcrosses of the F₁ hybrids were obtained using pollen of HA89 preserved in liquid nitrogen.

RESULTS AND DISCUSSION

As an example, the results and efficiency of selected interspecific hybridizations and embryo culture experiments are given in Table 1.

Table 1. Regeneration of interspecific hybrids (F₁) via embryo rescue (3)

Cross combinations	46	
- without success	15	(33.0%)
- successful	31	(67.0%)
In vitro culture of embryos	384	
- globular stage	9	(2.4%)
- young heart stage	214	(55.7%)
- differentiated stage	162	(41.9%)
Regenerated plants (rate)	163	(42.0%)

Meanwhile, a total of 34 different interspecific hybrids could be successfully raised via embryo culture *in vitro* (cf. Fig. 1b).

Altogether 481 plants were regenerated from 886 embryos cultured, which corresponds to an average regeneration rate of 54.3% (4). A number of combinations including HA89 as a female or male parent, respectively, are summarized in Table 2.

Table 2. Regenerated interspecific hybrids and F₁ fertility (4)

Female		Male	Hybrid fertility (%)
HA89 (cms)	x	XH. angustifolius	+
HA89 (cms)	x	H. argophyllus (n = 17)	+
HA89 (cms)	x	H. bolanderi (n = 17)	*
HA89 (cms)	x	H. debilis (n = 17)	-
HA89 (cms)	x	H. decapetalus (m = 17, 34)	-
HA89 (cms)	x	H. decapetalus/Dijon (m = 17, 34)	+
HA89 (cms)	x	XII. laetiflorus/558	+
HA89 (cms)	x	H. nuttallii/239 (n = 17)	-
HA89 (cms)	x	H. nuttallii/329 (n = 17)	-
HA89 (cms)	x	H. nuttallii/103 (n = 17)	+
HA89 (cms)	x	XH. originalis	+
HA89 (cms)	x	H. resinosus	+
HA89 (cms)	x	H. strumosus (n = 34, 51)	+
HA89 (cms)	x	H. tuberosus (n = 51)	+
H. argophyllus	x	HA89	+
H. bolanderi	x	HA89	*
H. debilis	x	HA89	+
XH. laetiflorus	x	HA89	+
H. nuttallii	x	HA89	+
H. nuttallii	x	HA89	+

* extinct

It is well established, now, that interspecific hybrids in the genus *Helianthus* can be regenerated efficiently by application of *in vitro* techniques. Even hybrid combinations including perennial species, which are rarely realized without the help of embryo rescue (5), could be raised now. Restoration of male fertility was expressed in some of the F₁ hybrids with HA89 (cms) as a female parent.

Hybrid progenies are now being identified morphologically (e.g. Fig. 1c, d), cytologically, by isozyme electrophoresis and/or by RFLP-analysis. Subsequently, the



Fig. 1. Steps of interspecific hybridization via embryo rescue: a) embryo in a heart-shape stage, b) plantlet from a cross *Hellianthus annuus* x *H. decapetalus*-B regenerated *in vitro*, c, d) leaf morphology of parental species and their hybrids - left to right: c) *H. annuus* HA89 (cms), hybrid, *H. laetiflorus* (558), d) *H. annuus* HA89 (cms), hybrid, *H. strumosus*

progenies will be used for the development of new CMS materials by backcrossing and by testing against available restorers or maintainers for the LECLERCQ-cytoplasm. Furthermore, this valuable material will further be screened for resistances to major diseases like *Sclerotinia sclerotiorum* and *Botrytis cinerea*. Relevant populations or lines will be propagated and maintained by self-pollination (where applicable) and/or by backcrossing.

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HYBRIDISATION INTERSPECIFIQUE EFFICACE GRACE A LA CULTURE D'EMBRYONS, METHODE APPLIQUE LA SELECTION DU TOURNESOL

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Cette publication présente les résultats préliminaires d'un programme de croisements suivi de cultures in vitro d'embryons. Ce programme inclue la lignée femelle HA-89 (cms) et différentes espèces sauvages* p. 265. Les d'Amérique (la plupart pérennes). 34 hybrides interspécifiques ont été réalisés et 481 plantes obtenues avec succès soit un taux de régénération de 54,3%. Actuellement, la progéniture de ces hybrides a été identifiée morphologiquement, cytologiquement par électrophorèse ou/et par analyse RFLP. Ils vont subir des backcross et de nouveaux tests pour déterminer la présence de restaureurs. Quand de nouvelles CMS seront obtenues, elles seront testées pour leur résistance au *Sclerotinia sclerotiorum* et *Botrytis cinerea*.

EFICACIA DE LA HIBRIDACION INTERSPECIFICA VIA RESCATE DE EMBRIONES PARA SER APLICADA EN LA MEJORA DEL GIRASOL

Regina Krauter y W. Friedt

Esta publicación presenta los resultados preliminares de un programa de cruzamiento seguido por cultivo de embriones in vitro. El programa incluyó la línea hembra audioestériles (CMS) y varias especies silvestres / americanas (mayormente perennes). Se han realizado treinta y cuatro híbridos interspécificos y se ha regenerado con éxito 481 plantas, lo que significa que se ha alcanzado una media de regeneración del 54,3%. Actualmente las descendencias de los híbridos están siendo identificadas morfológicamente, citológicamente por electroforesis de isoenzimas y/o por análisis de polimorfismos de enzimas de restricción (RFLP). Cuando se obtengan los nuevos materiales androestériles serán cribados para resistencia a *Sclerotinia sclerotiorum* y *Botrytis cinerea*.