New Interspecific Hybrids of Sunflower

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Summary

Wild *Helianthus* species represent an important gene source for various characteristics like disease resistance or cytoplasmic male sterility (CMS). For this reason, interspecific hybridization is performed to transfer the respective genes into the domesticated sunflower.
Different origins of the selected wild *Helianthus* species *H. tuberosus, H. mollis, H. laetiflorus, H. rigidus, H. resinosus, H. strumosus* were used as pollen donors for hybridizations with the line 'HA89' (cms). In order to find new sources of CMS, interspecific bastards with different ecotypes of *H. maximiliani* as female parent were produced.
As interspecific bastards usually abort within the first two weeks of their development, the 'embryo rescue' method was used to guarantee a greater number of hybrid plants. In the disease resistance program 139 promising hybrids have been gained, while 5 new hybrids have been obtained in the CMS program.
By applying isozyme electrophoresis it was possible to confirm the hybrid character of 98% of the investigated bastards. The highest degree of polymorphism was found with the isozymes glucosephosphate isomerase (GPI) and phosphoglucomutase (PGM). With these two isozymes alone it was possible to prove unequivocally the hybrid character of the analyzed plants.

Introduction

The production of sunflowers in Central Europe is particularly endangered by various fungal pathogens such as *Sclerotinia sclerotiorum, Botrytis cinerea* or *Verticillium dahliae*. So far, there is no phytosanitary measure to suppress or even avoid calamities in highly productive areas. However, wild *Helianthus* species are reported to possess resistances to the aforementioned diseases and interspecific hybrids have been reported to be in various cases an interesting basis for resistance breeding in sunflower (HAMMANN and FRIEDT, 1992). Besides, interspecific hybrids are used to recover new systems of cytoplasmic male sterility for sunflower breeding programs.
In several cases interspecific pollination results in seeds and subsequently in vital plants. But generally, postzygotic incompatibility between the interspecific embryo and the surrounding endosperm prevents the natural development and leads to an early abortion within the first two weeks after fertilization. To overcome these problems and to increase the yield of interspecific hybrid plants, the 'embryo rescue' technique was found to be a helpful and easy tool.

Normally, bastard plants can be identified easily by morphological characteristics. However, for borderline cases and in further backcross generations, isozyme starch gel electrophoresis could be established to follow up parental relations.

Material and Methods

a) Interspecific Hybridization and Embryo Rescue

Pollen of selected wild *Helianthus* species (Tab. 1) was collected and stored in liquid nitrogen until pollination. For hybridizations, plants of the cytoplasmic male sterile sunflower line 'HA89' were pollinated when the first flower circles of a head started flowering. Within 6 to 8 days after pollination achenes where fertilization and embryo development had taken place were collected. Embryos were isolated and placed in petridishes with MS_1 medium (Tab. 2). For the further development embryos stayed under a 16 h day at 25°C continuously for at least

Table 1: Promising wild *Helianthus* species used for interspecific hybridization

Helianthus species	Ecotypes or origins	Chromosome number
Helianthus tuberosus	TUB 5, TUB 1705	2n = 102
Helianthus mollis	MOL-RH, MOL-Vienna, MOL 1948	2n = 34
Helianthus strumosus	STR 1974	2n = 68, 102
Helianthus rigidus	RIG 1848	2n = 102
Helianthus resinosus	RES 1545	2n = 102
Helianthus laetiflorus	LAET-Hungary	2n = 102

Table 2: MS_1-medium for embryo culture

Component	conc. mg/l
NH_4NO_3	165
KNO_3	190
$CaCl_2 \times 2\ H_2O$	440
$MgSO_4 \times 7\ H_2O$	370
KH_2PO_4	170
$MnSO_4 \times 4\ H_2O$	16.9
$ZnSO_4 \times 4$ H	8.6
H_3BO_4	6.2
KJ	0.83
$CuSO_4 \times 5\ H_2O$	0.025
$Na_2MoO_4 \times 2\ H_2O$	0.25
Na_2-EDTA	37.25
$FeSO_4 \times 7\ H_2O$	27.9
Myo-Inosit	100
Saccharose	10 g
Agar-Agar	9 g
pH	5.6

two weeks. After rooting, plantlets of about 5 to 8 cm height could be transferred into soil and slowly adapted to greenhouse conditions.

In order to search for new cytoplasmic male sterile plants, different origins of the wild species *Helianthus maximiliani* (MAX 30, MAX 42, and MAX 44) were crossed with high-oleic sunflower restorer lines HO 2916-11 and R24GG (sf3983). Interspecific embryos were treated according to the aforementioned protocol.

b) Starch Gel Electrophoresis

Leave tissue from individual interspecific hybrids (F_1) as well as from their wild and domesticated parents was collected. Isozymes were separated by horizontal starch gel electrophoresis at a concentration of 12%. The samples were homogenized with 100 µl extraction buffer (pH 7.5) according to SOLTIS *et al.* (1983) containing mercaptoethanol. The methods of electrophoresis and staining of the enzymes employed have been described in detail by ALTER (1991).

Two different buffer systems according to STUBER *et al.* (1977) and CARDY *et al.* (1983) were used to assay the following three isozymes: glucosephosphate isomerase (GPI), phosphoglucomutase (PGM) and 6-phosphogluconate dehydrogenase (PGD).

After running electrophoresis the gels were cut into 3 horizontal slices, each of which was stained for a different enzyme system. The enzymes GPI and PGM were stained in the buffer system of STUBER *et al.* (1977), whereas that of CARDY *et al.* (1983) was used to stain PGD. The staining solutions were similar to those described by VALLEJOS (1983).

Results and Discussion

a) Interspecific Hybridization

For the breeding program for disease resistance, 139 intact plants could be produced from 274 cultivated embryos, i.e. a regeneration rate of about 51% (Tab. 3). From the crosses with different *H. maximiliani* ecotypes, 5 plants have been regenerated (Tab. 4). An increased embryo yield could be achieved by decapitating the pistils prior to pollination and by applying the pollen on the wounded surface.

Table 3: New interspecific hybrids with cms line 'HA89'

Pollen donor	number of isolated embryos	regenerated plants
TUB 5	45	30
TUB 1705	115	50
LAET-Hungary	60	25
RES 1545	38	23
STR 1974	7	4
MOL-Vienna	6	4
MOL-RH	2	2
MOL 1948	1	1
RIG 1848	0	0
Σ	274	139

Table 4: New interspecific hybrids with different origins of *H. maximiliani*

Cross combination	Number of isolated embryos	regenerated to plants
MAX 30 x HO 2916-11	7	1
MAX 42 x HO 2916-11	18	1
MAX 44 x HO 2916-11	9	0
MAX 44 x R24GG sf3983	10	3
Σ	44	5

Plants for the disease resistance program were transferred to the field for propagation. Pollen of each male fertile hybrid was collected and stored in liquid nitrogen for further breeding activities. Male sterile plants were used as female parent in sib-crosses. A first selection took place with regard to resistance against fungal diseases, but apart from a severe mildew infection no other diseases could be observed in 1992.
All of the selected interspecific hybrid progeny are currently involved in selfing or backcrossing programs, but up to now sexual progeny have been obtained only in a few cases.

b) Starch Gel Electrophoresis

Genotypic identification of the aforementioned interspecific hybrids, as well as of their wild and domesticated parents was accomplished by staining the isozymes GPI, PGM and PGD. Results for 8 different wild species and the line 'HA89' (cms) are shown in figure 1. All enzyme systems studied were found to be polymorphic. The largest variation exists for GPI and PGM; by means of these two isozymes alone all wild species can be discriminated.
The hybrid character of 40 interspecific hybrids was confirmed by using the isozyme systems GPI, PGM and PGD; the overall identification rate of the available hybrids was 98%. Figure 2 demonstrates the isozyme patterns of F_1 progeny of a cross between *H. annuus* ('HA89') and *H. laetiflorus*. In these F_1 plants the PGM isozymes both of the female and the male parents are expressed. Consequently, the progenies could all be made out as interspecific hybrids. In addition, the 19 progenies from a cross between *H. annuus* and *H. tuberosus* also proved to be hybrids by using the PGM isoenzyme system.
Correspondingly, the progenies of crosses between *H. annuus* and other wild species were identified as interspecific hybrids by using different isozyme systems.

Conclusions

1. Interspecific hybridization has been found to be a helpful tool to broaden the genetic variability in sunflower. With regard to disease resistance and CMS systems, new valuable genotypes have been obtained. The

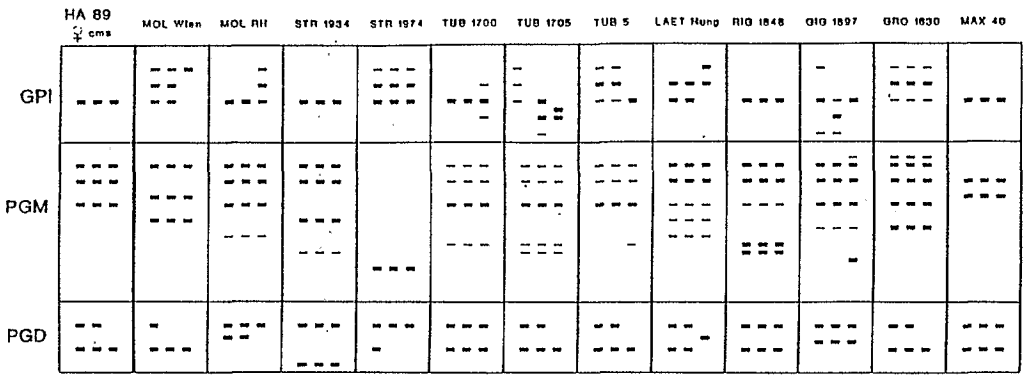

Figure 1: GPI, PGM and PGD isozymes "fingerprint" of the line 'HA89' (cms) and 8 different wild species. Several genotypes of *H. mollis, H. strumosus* and *H. tuberosus* were investigated.

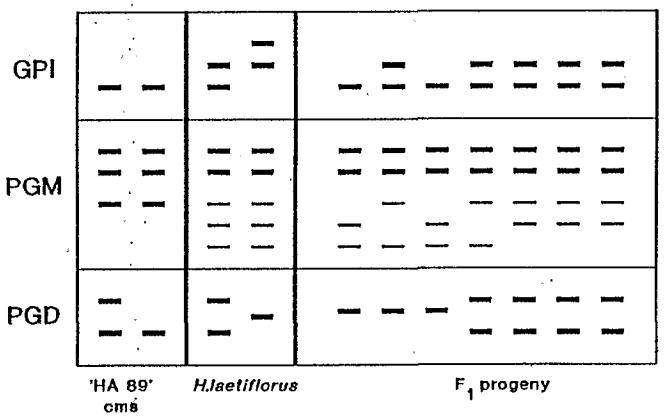

Figure 2: GPI, PGM and PGD patterns of the line 'HA89' (cms), *H. laetiflorus* and their F_1 progeny.

interspecific hybrids behave like their male wild parents as selfing or backcrossing is rendered more difficult by self incompatibility barriers. For further breeding, sib-crosses, decapitation of pistils as well as embryo rescue serve as helpful tools.

2. Starch gel electrophoresis could be established as an easy and inexpensive method for the rapid identification of wild *Helianthus* species, sunflower lines and interspecific hybrids. The isozymes PGM, GPI and PGD gave sufficient polymorphism to distinguish parents and F_1 hybrids. Consequently, this technique can be successfully applied in further backcross generations, inbred progenies or in other steps of a breeding program.

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