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SUNFLOWER INTERSPECIFIC HYBRIDIZATION USING EMBRYO CULTURE

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Abstract

The wild Helianthus species are potential sources of germplasm for improving the cultivated sunflower (Helianthus annuus L.) but many have never been artificially hybridized with the cultivated sunflower. The abortion of hybrid embryos is one of the mechanisms preventing hybridization. Embryo culture techniques were investigated and tested on a wide range of attempted interspecific crosses with H. annuus. The hybridization results ranged from fertilization failure through production of hybrid plants. Hybrids were obtained via embryo culture using the domestic sunflower as the pollen parent and H. angustifolius, H. argophyllus, H. bolanderi (exilis), H. giganteus, H. grosseserratus, H. hirsutus, H. maximiliani and H. petiolaris ssp. fallax as the maternal parents in different crosses.

Introduction

Interspecific hybridization between the cultivated sunflower H. annuus and its close relatives has been the objective of considerable interest and research because it represents a valuable method to develop the immense diversity present in the genus. Many of the species in the genus, particularly the perennials, have never been successfully hybridized with the cultivated sunflower. Abortion of the hybrid embryos during early developmental stages is one important barrier. The classic solution to this problem in other crops has been the use of embryo culture, excising the embryo before it has aborted and placing it on nutrient media to grow in vitro into a seedling capable of supporting itself. Embryo culture has been tried previously with interspecific sunflower hybrids to a limited extent (Whelan, 1976).

The purpose of the research reported here was to find a culture medium which would allow hybrid embryos to survive and develop into plants, and to use a wide range of interspecific crosses to see how useful the medium would be in aiding interspecific hybridization.

Methods and Materials

Embryo Culture

Stock solutions for the embryo culture medium were prepared in advance and frozen until needed. To prepare one liter of medium the agar was mixed with 500

ml of water and sterilized in an autoclave. The proper amounts of the thawed stock solutions and sucrose were mixed with sufficient water to make 500 ml. This fraction was sterilized by filtration. After sterilization, both fractions were mixed and poured into 16 ml vials which were covered with sterile foil and cooled on a slant.

Hybridization

P21, an H. annuus line segregating for genetic male sterility was used as the domestic parent in all crosses. The wild parents were from the sunflower collection at U.S.D.A. Davis, which included the annual species H. anomalus Blake, H. argophyllus Torrey and Gray, H. bolanderi A. Gray, H. niveus ssp. tephrodes (A. Gray) Heiser, and H. petiolaris ssp. fallax Heiser, plus the perennial species H. angustifolius L., H. californicus D.C., H. cusickii A. Gray, H. giganteus L., H. gracilentus A. Gray, H. grosserratus Martens, H. hirsutus Raf., H. maximiliani Schrader, H. microcephalus T. & G., H. salicifolus A. Dietr., H. silphioides Nutt., and H. tuberosus L. Also included were two species from different genera, Phoebanthus and Viguiera.

Individual heads were covered with paper bags to prevent outcrossing and to collect pollen. No consistent attempts were made to emasculate the wild species. Pollen was transferred and applied by hand. Crosses were made in the field during the summer of 1977 between seventeen different Helianthus species and P21 Ms ms, and in seven cases the reciprocal cross with P21 ms ms was also made. The heads were harvested for embryo culture from 3 to 10 days after pollination.

Seeds containing embryos to be cultured were removed from the head and sterilized in a 2% sodium hypochlorite solution for 15 minutes. The embryos were removed with a sharp scalpel under a dissecting microscope and placed on the surface of the medium. The vials were covered with snap caps and placed in a growth cabinet at 27 C under 18 hours of light. Care was taken to see that the root primordia were in contact with the medium to prevent desiccation and death of the root meristem. After roots and shoots developed, the embryos were removed from the vials and transplanted into a sand-peat mixture (1:1 v/v) in the greenhouse. The vials were placed over them to increase the humidity until they developed new root hairs.

Results

Two media were tested for their ability to support H. annuus embryos, one developed by J. Dvorak (personal communication) for use with wheat interspecific hybrids, and Gamborg's B-5 (Gamborg et al, 1968) modified to 9% sucrose. No significant differences were found in survival, although very small embryos initially grew faster on Dvorak's medium. Both supported the excised H. annuus embryos very well, giving about 90% survival of embryos 1 to 3 mm long. This size range gives the maximum survival rate in H. annuus embryos. Younger embryos frequently fail to form roots while older embryos tend to remain dormant on the medium. Various alterations of sucrose and auxin levels in both media were tested. Using 0.05 ppm NAA gave better results than the original 1.0 ppm IAA or no auxin. The optimum sucrose concentration was the original 9% level. Composition of the modified Dvorak's medium is given in Table 1. This formula gives results as good as or better than any other formula tested.

TABLE 1. Modified Dvorak's Medium.

Item	Amount for Stock	Amount of stock to use for one liter of medium
Inorganic Compounds		
Macroelements	1 liter stock	100 ml
$NaH_2PO_4-H_2O$	1500 mg	
KNO_3	25000 mg	
$(NH_4)_2SO_4$	1340 mg	
$MgSO_4-7H_2O$	2500 mg	
Fe EDTA	350 mg	
Microelements	100 ml stock	1.0 ml
$MnSO_4-H_2O$	1000 mg	
H_3BO_3	300 mg	
$ZnSO_4-7H_2O$	200 mg	
$Na_2MoO_4-2H_2O$	25 mg	
$CuSO_4$	2.5 mg	
$CoCl_2-6H_2O$	2.5 mg	
Calcium	100 ml stock	1.0 ml
$CaCl_2-2H_2O$	15000 mg	
Iodine	100 ml stock	1.0 ml
KI	75 mg	
Organic Compounds		
Vitamins	100 ml stock	20 ml
Nicotinic acid	5.0 mg	
Thiamine-Hcl	50 mg	
Pyridoxine-Hcl	5.0 mg	
Inositol	20000 mg	
Amino Acids	1 liter stock	40 ml
Glutamine	20000 mg	
Serine	4000 mg	
Tryptophan	1250 mg	
Cysteine	250 mg	
Alanine	25000 mg	
Phenylalanine	1250 mg	
Hormones	100 ml stock	1.0 ml
NAA	5.0 mg	
Sucrose		90 g
Agar		8 g

The occurrence of fertilization was determined by checking for darkening and enlargement of the achene plus visible growth of the embryo sac. Under this criterion seeds which were fertilized but failed to grow or died before growth was visible would be classified as not fertilized. Fertilization did not occur in the crosses involving V. reticulata, H. gracilentis, H. microcephalus and H. silphioides, but fertilization occurred in all the other combinations in which H. annuus was used as the pollen parent. Embryos from the successful crosses were excised and placed in culture where all but those of H. salicifolius x H. annuus grew for at least a short time. To avoid embryo abortion, the embryos from the H. salicifolius cross had to be excised at an extremely young age (3 days after pollination) at which time they were still in the globular stage before differentiation of the cotyledons was visible. None of the hybrid salicifolius embryos showed any signs of growth in culture despite repeated efforts. At least a few embryos grew in culture from each of the remaining fourteen combinations. The results are summarized in Table 2.

TABLE 2. Species crossed with H. annuus (cv. P21 Ms ms) and Plants Obtained

Crossed with H. annuus	Fertilization	Number of plants obtained through embryo culture	
		Hybrids	Selfs
H. angustifolius	Yes	1	0
H. anomolus	Yes	0	0
H. argophylus	Yes	3	1
H. bolanderi	Yes	1	1
H. californicus	Yes	0	2
H. cusickii	Yes	0	0
H. giganteus	Yes	2	0
H. gracilentis	No	0	0
H. grosseratus	Yes	1	1
H. hirsutus	Yes	2	0
H. maximiliani	Yes	2	1
H. microcephalus	No	0	0
H. niveus	Yes	0	0
H. petiolaris	Yes	7	0
H. salicifolius	Yes	0	0
H. silphioides	No	0	0
H. tuberosis	Yes	0	0
Phoebanthus	Yes	0	5
Viguiera	No	0	0

When placed on the medium, the embryos from four of the crosses developed but failed to produce seedlings which could survive transplanting. Embryos from crosses of H. anomalus, H. niveus and H. tuberosus x H. annuus grew well in vitro but failed to produce a strong root system which would support them upon transfer to soil. H. cusickii x H. annuus produced a single plant with a delicate shoot system which disintegrated during transplanting and the plant failed to survive.

Two crosses produced embryos that survived through the medium state and produced healthy plants but were found to be selfs. Phoebanthus x H. annuus produced five such plants while H. californicus x H. annuus produced two.

The remaining eight crosses produced true hybrids through embryo culture. Selfs were also produced in four cases. Seven of these hybrids have been previously reported by others: H. argophylus x H. annuus, H. bolanderi x H. annuus, H. petiolaris x H. annuus and H. hirsutus x H. annuus (Heiser, et al, 1969), H. giganteus x H. annuus and H. maximiliani x H. annuus (Whelan, 1976), H. grosserratus x H. annuus (Georgieva-Todorova, 1975); the cross H. angustifolius x H. annuus has never been reported to our knowledge. The hybrids were confirmed by morphological comparisons and wherever possible by pollen straining and chromosome studies.

Discussion

The lack of fertilization in some of the crosses presents a serious barrier to hybridization but this could possibly be overcome by using different collections and individuals from the wild species. Observations on two different collections of H. hirsutus showed no fertilization in one collection despite many hybridization attempts; pollination of the other collection produced two to five embryos per head. Two of these were cultured into hybrid plants. Similar differences occurred between two H. petiolaris collections. In addition, differences in fertilization were noticed between individual plants within many of the collections.

Although some of the hybridizations produced embryos which did not develop in virto into plants,this does not mean that these hybridizations cannot be made. Of the five combinations responding this way, three will readily produce hybrids without embryo culture. The main cause for the failure of embryo culture was the lack of root formation in vitro. The four combinations which failed to root in culture were not intensively studied. Only two heads of H. cusickii were crossed with H. annuus, from which two embryos were excised and no plants were obtained.

Selfs occurred in six of the ten hybrid combinations that produced plants. These selfs are probably induced by the addition of the H. annuus pollen which caused a breakdown of the self-incompatibility system. Whelan (1976) noticed a similar phenomenon. Pollen which has been killed by exposure to extreme cold will give similar results. In fact, we used the killed pollen method to induce selfing in the H. petiolaris, H. californicus and H. maximiliani collections in this study. The method failed on the H. tuberosus and H. salicifolius collections. Emasculation of the flowers would eliminate selfing but it is not worth the additional effort since there is usually little problem identifying the selfs and the hybrids by difference in morphology.

The most useful morphological characters for determining whether a plant was a hybrid or not were leaf size and shape. All the wild species had smaller leaves than H. annuus, and with the exception of H. argophylus, all had narrower leaves. All of the hybrid plants had larger and wider leaves than the wild parent.

All the hybrids except those involving H. maximiliani or H. grosserratus flowered in the greenhouse during the winter. Pollen straining ranged from 11% in the perennial x H. annuus hybrids to 80% in one of the H. bolandari x H. annuus hybrids. All of the hybrids between annual species had a higher percent-

age normal straining pollen than did the perennial x H. annuus hybrids. The H. angustifolius x H. annuus hybrid was completely male sterile.

Meiotic chromosome analysis was done on all hybrids that flowered except the H. bolanderi x H. annuus hybrids. The annual species x H. annuus showed good chromosome pairing at metaphase I with several multivalents. Paracentric inversion bridges and fragments were common. The perennial species x H. annuus hybrids showed poor pairing with many univalents and multivalents. Paracentric inversion bridges and fragments were also common.

Literature Cited

1. GAMBORG, O.L., R.A. MILLER, and K. OJIMA, 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158.

2. GEORGIEVA-TODOROVA, I., 1975. A new male sterility form in sunflower. Proc. Sixth Intl. Sunflower Conf., Bucharest, Romania, p. 1.

3. HEISER, C.B., D.M. SMITH, S.B. CLEVENGER, W.C. MARTIN, Jr., 1969. The North American Sunflowers (Helianthus), Memoirs of Torrey Botanical Club, 22(3):1-218.

4. WHELAN, E.D.P., 1976. Sterility problems in interspecific hybridization of Helianthus species. Proc. Second Sunflower Forum, p. 5-7.