UTILIZATION OF WILD HELIANTHUS IN GERMPLASM DEVELOPMENT.

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ABSTRACT

In North America it is easy to collect a large number of *Helianthus* species, or accessions of a single species, but evaluation of this material is difficult. Rather than have research programs to test accessions for different characteristics and then attempt to transfer a desirable character into an inbred line, we have chosen a germplasm development program which randomly introgresses the genes from a wild species into a cultivated sunflower background. The germplasm pool is then released to others for selection of characteristics valuable to them. This method minimizes the effort spent in overcoming the barriers to gene flow from the wild into the cultivated, and allows the screening efforts to take place at a facility well equipped for specific testing. Difficulties which must be overcome include seed dormancy, self incompatability, and early shattering of seeds. There are also crossing difficulties, embryo abortion, and sterility barriers in the hybrids and later generations. We are using two techniques for this germplasm development, mass and controlled crossing. We have released seed of two germplasm pools involving wild *H. annuus* and will have pools involving *H. bolanderi* and *H. argophyllus* soon. During the controlled crossing program phylogenetic relationships of many wild species have been determined, based on the chromosome translocation patterns expressed in the F_1 plants.

INTRODUCTION

Wild *Helianthus* species have contributed characteristics of significant importance in the improvement of the sunflower crop, *H. annuus* L. (Beard, 1981a). Even so there has been only limited effort to use most of the available species. Since 1972 a major part of the USDA — University of California sunflower research program has been concerned with the incorporation of genes from the wild species into the domestic germplasm.

We have done only limited testing of the wild species to determine which have valuable characteristics. There are at least four reasons for this attitude. Evaluation of wild species may be useful in detecting pest resistance, but specialized screening methods are often needed. Resistance to pests available at Davis may not be relevant to goals at other locations. Expression of desirable traits may be affected by genetic background so they may "disappear" while being transferred or conversely new valuable traits may appear which were not expected. Some traits in a wild species may not be transferrable without making undesirable changes, i.e., pest resistance found in a species with a woody stem might not be transferrable without changing the morphological structure of the crop plant. By the time initial testing of the wild species has been completed a germplasm pool could have been developed and testing started which would furnish selections of more potential value in a breeding program.

Thus, we are randomly introgressing a large number of accessions of each wild species into a genetic male sterile line. There is a problem of losing valuable characteristics through genetic drift and natural selection, but the use of large populations each generation should minimize this problem. The resulting germplasm pools are released to other sunflower breeders where more ideal facilities or conditions allow better testing of the segregates for characteristics important for their programs.

However, we have made some measurements of the wild species and are using statistical analyses in an effort to make evaluation of accessions less time consuming, (Beard and Williams, in Press).

MATERIALS AND METHODS

We have 409 accessions of wild *H. annuus*, 27 accessions of *H. bolanderi*, 4 accessions of *H. argophyllus*, and 1 or 2 accessions of about 30 other species from the 50 recognized by Heiser *et al.*, (1969). We use P21-VR1 genetic male sterile as the parent whose male fertile progeny will furnish pollen in the next generation, and cms Ha89, cms Ha124, cms Ha232, and other cms inbreds as additional parents in the mass germplasm development program.

Species that are easily crossed to domestic plants are grown in isolation with the male sterile P21 plants. Honeybees *(Apis mellifera)* and other pollinators transfer the pollen from the wild species to the male sterile plants. Progeny from the genetic male sterile line are used as the pollen source for the next generation. The domestic parents in this and future generations include the P21 and the cms parents. This procedure can be repeated for as many cycles as desired.

Species that are most difficult to cross are subjected to a different procedure. Domestic plants are used to pollinate the wild plants either with or without emasculation. The embryos are excised and grown as described by Chandler and Beard (1978, 1980). Detailed morphological, cytological, and agronomic data are recorded for parents, F_1, and BC_1 plants for both mass and controlled crosses.

Another area of research not directly connected with germplasm development is the study of crossability and evolutionary relationships among the wild annual species. These species have been crossed in all possible combinations. Detailed cytological analyses are presently being made of the F_1 plants for each successful cross.

Vital pollen staining was determined by mixing a sample of fresh pollen in a drop of stain containing .1% 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium Bromide and 30% sucrose on a slide, covered with a cover slip and incubated for 24 hours at room temperature. A random sample of 200 to 1000 grains was scored for staining. Stained grains were considered viable.

Analysis of meiosis was on disc florets fixed in Carnoys fluid and stored in 70% ethanol under refrigeration. Temporary slides were prepared by squashing whole florets in a 2% carmine-45% proprionic acid stain. Pollen mother cells in diakinesis and metaphase I were scored for multivalents with particular attention paid to cells with the most complicated multivalent configurations.

RESULTS AND DISCUSSION

Germplasm pools III, and IV involving wild *H. annuus* were released in 1981 (Beard, 1981b, 1981c). Because the number of isolation areas is limited we can develop only two germplasm pools in any season. We have *H. bolanderi* and *H. argophyllus* germplasm pools under development at present. These will be released in 1982.

One concern we had in our mass crossing program was that the alleles from the wild species would be strongly selected against during the backcross process and they would either be eliminated or fall to very low frequencies. To examine what really happens, the segregation of fertility, four quantitative, and four simply inherited traits was examined in a BC_1 population of *(H. niveus* ssp. *tephrodes* x *H. annuus)* x *H. annuus* produced via embryo culture. The four quantitative traits were achene weight, and length to width ratios for leaves, ray florets, and achenes. The four simply inherited traits were branching, purple hypocotyl, self-incompatibility, and pubesence on the achene. The data for fertility and the quantitative traits from 68 BC_1 greenhouse grown plants are shown in Table 1.

Fertility was estimated as percent vital pollen staining, and

percent seedset after selfing or further backcrossing. Viable pollen ranged from 0 to 92%, with a mean of about 32%. Viable pollen staining for *H. niveus* x *H. annuus* F_1's was from 1 to 4%. Seed set of BC_1 plants varied from 0 to 53% but the mean for 58 plants was only 8 to 9%. The correlation coefficient between pollen staining and seed set was 0.476. This is highly significant but not very useful as a predictive tool. As with fertility the quantitative traits segregated continuously in the BC_1, spanning the range from the F_1 to the recurrent parent.

One of the simple traits, branching, fit a 3:1 ratio, indicating two dominant genes from the wild parent. Segregation of the other three simple traits fit a 1:1 ratio, indicating a single dominant gene from the wild species. The segregation ratios gave no evidence of selection against the alleles of the wild species during transmission from the F_1 to BC_1 generations. However, in the BC_1 generation three of the wild alleles were significantly associated with low fertility levels. These associations are probably caused by linkage between the wild alleles and chromosomal sterility factors such as inversions and translocation break points. Such a linkage will cause selection against the alleles from wild species and serve to decrease their frequency in the BC_2 F_1 and BC_1 F_2 generations. Fortunately, the selection pressure is not extreme since for each trait some recombinant plants were found with both the wild allele and above average fertility. None of the quantitative traits were associated with fertility level.

Table 1. Means and standard deviations for the quantitative characters of *H. niveus* x *H. annuus* BC_1 F_1 plants.

Character studied	Number observations	Mean	Standard deviation	Range
Stained pollen %	68	31.7	23.4	0.0 — 91.7
Seedset %	58	8.6	10.9	0.0 — 53.3
Nodes #	68	17.5	4.8	7.0 — 30.0
Leaf width mm	68	58.0	25.8	19.0 — 163.0
Leaf length mm	68	88.3	25.7	37.0 — 170.0
Petiole length mm	68	48.7	26.0	14.0 — 166.0
Ray width mm	62	13.7	3.8	8.0 — 24.0
Ray length mm	62	41.5	10.2	22.0 — 62.0
Achene length mm	46	8.8	1.2	6.2 — 11.0
Achene width mm	46	4.1	0.7	3.0 — 7.2
Achene depth mm	46	3.2	1.1	2.0 — 9.0
Achene weight mg	49	35.9	10.1	17.0 — 58.0
Leaf length/width ratio	68	1.6	0.3	1.0 — 2.3
Ray length/width ratio	62	3.1	0.5	1.6 — 4.1
Achene length/width ratio	46	2.2	0.4	1.3 — 3.1

While these particular traits are of no economic importance, they were randomly chosen so they could easily represent a trait which would be of interest. With this in mind it seems wise to avoid growing unnecessary generations for these wild-species-derived germplasm populations since each generation could decrease the frequency of potentially valuable alleles. A germplasm pool derived from the *H. niveus* BC_1 material used in this study should be available soon.

Table 2. Crossability patterns, and multivalent configurations during meisois in interspecific hybrids of annual Helianthus species.

	H. niveus	*H. debilis*	*H. praecox*	*H. petiolaris*	*H. neglectus*	*H. annuus*	*H. argophyllus*	*H. bolanderi*	*H. deserticola*	*H. anomalus*	*H. paradoxis*
H. niveus		IV*	IV + VI*	+	IV + VI*	IV + XII	2IV + VI	IV	IV	+	+
H. debilis	0		NA	IV + VI	NA	2VI	IV + VI	IV		N.M.	N.M.
H. praecox	+	+		+	VI	IV + VI	IV	+	IV + VI	+	
H. petiolaris	0		IV		+	X	0	IV + VI	IV + VI	2IV	+
H. neglectus	+	+		IV		VIII	IV + VI	IV + VI	IV + VI	+	IV + VI
H. annuus	IV + XII	2VI	0				2IV	IV + 2VI		0	0
H. argophyllus	0					2IV		+			
H. bolanderi	IV	IV	IV + VI		+	IV + 2VI	IV + VI		IV	IV	+
H. deserticola	IV	IV	IV + VI	IV + VI	+	VI + VIII	IV + VI	IV		N.M.	+
H. anomalus	0	0	0		0	NA	0	0			0
H. paradoxis	0	+	0			0	0	0	0	+	

blank = cross not attempted
0 = cross attempted but embryos did not initiate
+ = cross made, embryos initiated but no mature hybrids produced
N.M. = no multivalents
NA = hybrids not presently analyzed
Roman numerals = maximum multivalent configurations in pollen mother cells of F_1 plants. In all crosses the number of bivalents plus the multivalents made 2n = 4.

Configurations are tentative in some crosses due to small number of cells observed, formation of complicated multivalent configurations, or pairing failure in the majority of cells.

The chromosome number was determined for 25 of the 68 BC₁ F₁ plants. Of these plants fourteen had the euploid chromosome number 2n = 34. Nine plants had one extra chromosome, 2n = 35, while two plants had two extra chromosomes, 2n = 36. In some cases the extra chromosomes were telocentric and probably came from misdivision of lagging chromosomes. Extra whole chromosomes were only found in plants showing multivalents in meiosis so were probably derived from unequal segregation of multivalents in the F₁. The plants with extra chromosomes did not have decreased fertility or vigor. This indicates that sunflower is relatively tolerant of aneuploidy involving additional chromosomes.

The chromosomal relationships of the species are being investigated by meiotic analysis of the hybrids. Although the investigation is not yet completed, the main trends are clear. Six of the annual species, *H. niveus*, *H. debilis*, *H. bolanderi*, *H. anomalus*, *H. deserticola*, and *H. paradoxus* have similar chromosome end arrangements. None of these species differs by more than one translocation from any of the others. Structurally the chromosomes of these species are also very similar to the chromosomes of the perennial species (Heiser and Smith, 1964, Chandler, unpublished data). The chromosomes of the remaining five species have diverged from those of the core group by additional translocations. Clearly, the most divergent is the cultivated species *H. annuus*, a fact which has unfortunate implications for the efforts to use wild species as breeding material. *H. annuus* differs by at least four translocations from the vast majority of the annual and perennial species, and these translocation differences are one of the primary causes of sterility in the interspecific hybrids.

The multivalent configurations listed in Table 2 are within one translocation of the true maximum configuration. Some uncertainty arises concerning some rarely expressed con-figurations which in some crosses are difficult to distinguish from artifact chromosome associations. This problem is most serious in those hybrids exhibiting complicated configurations. For example in the hybrid *H. debilis* x *H. annuus* a chain or ring of twelve chromosomes is sometimes seen but is difficult to distinguish from a close association between the more commonly observed pair of hexavalents.

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CLUSTER ANALYSIS OF WILD *HELIANTHUS ANNUUS* ACCESSIONS.

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ABSTRACT

Wild *Helianthus annuus* L. plants are widely distributed over North America, and extensive collecting has resulted in about 400 accessions in the Davis collection. We have recorded data for 7 morphologic and 6 agronomic characters from 7 to 10 plants in each of 177 accessions. Even with these data it is an enormous task to evaluate the potential of each accession for improvement of the domestic cultivars. Consequently, the data set was analysed using cluster analysis based on the standardized Euclidean distance measure (BMDP2M). We then separated the accessions into 10 groups or 20 groups based on either morphological or agronomic data. Groups can be selected for specific characters. For instance in the morphologic data separation a cluster of 3 accessions with stem length of 72 cm can be compared to another cluster of 4 accessions with stem length of 260 cm. The cluster with the largest number of accessions (18) has a stem length of 195 cm. One group with 11 accessions was early with a mean anthesis date on May 23. There were two groups, one with 3 accessions and one of 7 accessions, with a mean of June 15 for anthesis date. The C18:2 fatty acid component of the oil for these two late groups was 62.4 and 70.5% respectively, yet the seeds apparently developed during the same period and were subjected to similar environmental conditions. These cluster results and others can be used to select likely candidates for use in a breeding program.

INTRODUCTION

Heiser *et al.*, (1969) describes six forms of uncultivated *Helianthus annuus* L., and reported that these are commonly found in every state of the United States plus areas in Canada and Mexico. Each local swarm of plants has the potential of contributing germplasm that is different from any other source. We have over 400 accessions in the USDA collection at Davis. With so many accessions, how does the plant breeder determine which might be the best for parental material?

Environmental modification of the phenotype of crop plants is well known (Goyne *et al.*, 1979). The wild *H. annuus* at different collection sites are frequently different in height, anthesis date, and other characters, but the plant breeders need to know whether these differences reflect genetic effects or phenotypic variations due to environments. The accessions can be grown in a uniform environment and morphologic and agronomic data recorded to determine the mean and variances associated with each. In most cases the agronomic data will be most important, but this will be at least partially dependent on the objectives of each breeding program.

This paper describes the use of cluster analysis to determine distinct groups containing similar types under a common set of environmental conditions. If this grouping is meaningful, one or two accessions in each group can be used as parental material to determine the possible germplasm potential which would be contributed by the collection.