HELIA, 17, Nr. 21, p.p. 87-92 (1994) UDC 633.854.78:582.998.2

PROGRESS REPORT OF THE WORKING GROUP OF THE EVALUATION OF WILD *Helianthus* SPECIES FOR THE PERIOD 1991-1993

G. J. Seiler

USDA-ARS Northern Crop Science Lab., P.O. Box 5677, Fargo, North Dakota, USA

SUMMARY

This report presents research accomplishments of the working group for the evaluation of wild *Helianthus* species since the last progress report at the FAO meeting in 1991 at Pisa, Italy. The working group conducted broad and extensive investigations on the wild species with a common goal to increase the genetic diversity of cultivated sunflower using wild species to make it better global crop.

The accomplishments of the working group have added new data on exploration and maintenance and characterization of wild sunflower species, germination and dormancy, phylogeny and classification of *Helianthus*, interspecific hybridization, cytogenetics of species and interspecific hybrids, cytoplasmic male sterility and fertility restoration, oil quality and protein content, drought tolerance, disease resistance and regeneration of wild *Helianthus* species, and this way they added considerable knowledge to informational database about the wild sunflower species and their potential use in improving cultivated sunflower.

INTRODUCTION

At the beginning of the current research cycle 1991-1993, the name of the working group was changed from Evaluation of Morphological, Physiological, Biochemical Characters and Taxonomic Aspects of Wild *Helianthus* Species to Evaluation of Wild *Helianthus* Species. This was a name change only and did not change the broad scope of the working group. The working group has become global with participants joining from Mexico, India, and Russia. The working group conducted broad and extensive investigations on the wild species as a means to increase the genetic diversity in cultivated sunflower. Much of the research progress has been presented at national and international meetings and published in scientific journals, International Sunflower Conference Proceedings, and in HELIA.

The achievements of the working group have been very good in spite of a constantly changing global structure and difficult economic times. This shows the dedication of the participants to improve the sunflower crop in spite of the adversities that many have to work around. Participants of the working group all share a common goal and that is to increase the genetic diversity of cultivated sunflower using wild species to make it a better global crop. The working group has made significant progress in expanding our knowledge of the wild species, but the job is not finished by any means. The enthusiasm and need for wild sunflower germplasm has not changed.

This report presents research accomplishments of each participant since the last progress report at the FAO meeting in 1991 at Pisa, Italy. Reports for the 1991-1993

period are presented by participant. More detailed information can be obtained from participants.

ACCOMPLISHMENTS

The accomplishments of the working group have added considerable knowledge to informational database about the wild sunflower species and their potential use in improving cultivated sunflower.

Plant Exploration

The acquisition phase of the wild species collection continued during the current period. One hundred-forty populations (107 annual and 33 perennial) representing nine different species were collected from the Central Great Plains of the USA during an exploration in 1991.

Exploration for wild *Helianthus* was undertaken for the first time in Montenegro in 1990. Sixty-three populations of the perennial *H. tuberosus* (Jerusalem artichoke) were collected. They are presently located at the Institute of Field and Vegetable Crops (IFVC), Novi Sad. They will be evaluated and characterized.

Explorations for *Helianthus* and closely related genera have been initiated in Mexico by Dr. Gomez-Sanchez, INIFA, Durango. Fifty-two populations representing nine species of *Helianthus* have been collected from Baja, western, and central Mexico. Nineteen populations representing 10 species of *Viguiera* and three populations representing two species of *Tithonia*, closely related genera of *Helianthus*, have also been collected. The collection is maintained at INIFA, Durango.

Maintenance and Characterization of Wild Sunflower Species

The Institute of Field and Vegetable Crops at Novi Sad reported on the use of domesticated bee to increase seed set of wild sunflower accessions. They reported seed set was significantly lower for sibbing or selfing vs. bee pollination or open pollination. However, *H. petiolaris* produced less seed by bee pollination than open pollination.

In related maintenance problems of self-compatibility, it was reported from St. Petersburg, Russia that perennial *H. tuberosus* had the lowest seed set, *H. maximiliani* the highest, while many perennials set no seed. In France, it was reported that 96% of the annual accessions in the collection produced more than 200 seeds with 30 sibcrosses/accession, while in the perennial species seed set was low, especially in *H. tuberosus* and *H. pauciflorus* (=*rigidus*).

France, Mexico, Russia, and Bulgaria have made significant progress in the characterization of the wild accessions. France now has a catalogue of 400 accessions with agronomic and morphological data. Mexico has begun characterizing the wild species accessions of Mexico for basic descriptors and agronomic information.

Germination and Dormancy

Methods to overcome the high degree of dormancy in wild and interspecific crosses were studied. In Italy, the most effective treatment found was scarification plus GA_3 (100 ppm) at cold temperature (5°C). Perennial *Helianthus grosseserratus* and *H. mollis* had the best response to the treatments, while *H. nuttalli* and *H. maximiliani* responded the

least. The USA reported that the age of achenes at harvest of *H. annuus* and *H. petiolaris* had a significant influence on germination. A majority of germinations took place by 21 days. Achene dormancy does not appear to be overcome using a combinations of storage temperatures of times. Despite large differences in germination percentage, the coefficient of velocity is similar for all species.

Phylogeny and Classification of *Helianthus*

The group in France presented the use of RAPD markers to discriminate the *Divaricati, Ciliares*, and *Helianthus* sections of the genus *Helianthus*. The wild species and cultivated *H. annuus* were clearly separated into three main groups corresponding to the three sections. The cultivated forms are in the first plane, indicating that their diversity comes from the other species. RAPDs are good phylogenetic markers, and lead to a molecular taxonomy which appears different from that based upon rDNA and those based on anonymous RFLP.

Genetic diversity of 63 *Helianthus tuberosus* (Jerusalem artichoke) populations collected in Montenegro were analyzed for 31 morphological characteristics. The populations were grouped into two clusters which indicated obvious differences in origin. One cluster was further subdivided into groups. It was concluded that Jerusalem artichoke populations displayed a high interspecific variability which makes them valuable for breeding programs.

Interspecific Hybridization

Participants were very successful in the area of interspecific hybridization. A substantial number of interspecific hybrids between wild species and cultivated genotypes were developed by the participants. These lines have been backcrossed with cultivated genotypes to develop inbred lines. The inbred lines are being evaluated for various agronomic characteristics such as pest resistance, drought tolerance, oil and protein quality, and cytological analyses.

Participants of the working group at General Toshevo, Bulgaria, have began a program of particular interest and that is intergeneric hybridization with closely related genera *Tithonia* and *Verbesina*. A successful cross with *Tithonia rotundifolia* has been made and was found to possess Rf genes for sunflower cytoplasm and also to have mildew resistance.

Some practical information was presented from Italy on interspecific embryogenesis. Interspecific crosses involving *H. mollis* aborted in three days, *H. grossesserratus* aborted in four days, *H. strumosus* aborted in 11 days, while *H. giganteus* lasted 30 days.

Cytogenetics of Species and Interspecific Hybrids

Significant information has been obtained in this area since the last report. Dr. Jan of Fargo has developed amphiploids ($2n = 68$) of *H. gracilentus* x "Peredovik" P21, *H. mollis* x P21, *H. maximiliani* x P21, and *H. nuttallii* x P21. These amphiploids possess restored fertility. A hexaploid amphiploid was also produced from *H. hirsutus* x P21. This may add to our understanding about the origin of *H. hirsutus* as an autotetraploid or allotetraploid. The near normal pollen stainability (83%) of $2n = 68$ BC_1F_1 plants seems to suggest an autotetraploid origin.

In Italy, cytological analysis of hybrids between *H. debilis* spp. *debilis* and *H. annuus* has been undertaken. They concluded that *H. debilis* seems to have taken part in an allopolyploid differentiation of the genus *Helianthus*. Cytological analysis of *H. praecox* spp. *praecox* and interspecific crosses with cultivated sunflower was reported at Sofia, Bulgaria. They concluded that cultivated sunflower is closely related to *H. praecox*. After two backcrosses of F_1 hybrids, the chromosome irregularities were very low and complete pollen and seed fertility was restored.

Cytoplasmic Male Sterility and Fertility Restoration

Several new cytoplasms from the wild species have been discovered. At Fargo, six *H. annuus* and one *H. maximiliani* cytoplasms and their respective fertility restoration genes were discovered. Inheritance study of the fertility restoration gene(s) for cms-RIG was completed which indicated that two complementary genes were needed for fertility restoration. The participants in St. Petersburg reported that no fertility restoration genes were found for the cms-RIG at their research center. Several new cms's have been reported including *H. debilis* spp. *vestitus, H. praecox* spp. *hirtus, H. praecox* spp. *runyonii, H. hirsutus, H. rigidus*, and *H. strumosus* at General Toshevo, Bulgaria. In France, new cms BOL1, ANO1, and ANL2 appeared to be superior to PET1 in hybrid seed yield trials. Preliminary data also indicated a lower incidence of *Phomopsis* in ANL2, EXI1, and CMG1 than in PET1.

Oil, Oil Quality, and Protein

Wild populations of *H. annuus, H. debilis, H. petiolaris* and *H. mollis* evaluated in Bulgaria had oil concentrations between 30 and 41%. Populations of wild sunflower from the Great Plains had oil concentrations up to 30% in annual and perennial species. In Spain, the highest kernel oil was observed in *H. niveus* and *H. anomalus*. High and stable linoleic acid ranging from 66 to 76% was observed in *H. exilis, H. mollis, H. nuttallii, H. rigidus, H. maximiliani, H. gracilentus, H. glaucophyllus, H. divaricatus, H. grosseserratus, H. hirsutus*, and *H. debilis* in Spain. They had high stability index values of 0.86 to 0.90. In the Great Plains, *H. pumilus* had a linoleic acid concentration of 74%.

Kernel protein concentration varied from 24.3% in *H. praecox* spp. *hirtus* to 53% in *H. decapetalus*. In Spain, kernel proteins averaged 37.1% in wilds vs. about 22% in cultivated sunflower. In Bulgaria, protein concentration in kernels of *H. annuus* (wild) was 25% compared with 49% in *H. glaucophyllus. Helianthus petiolaris* and *H. praecox* had high lysine concentrations with 4.1 and 4.2%, respectively.

Drought Tolerance

In Italy, *Helianthus argophyllus* has reduced transpiration, photosynthesis activities, and leaf hydration in a -0.8 to -1.2 MPA range of pre-dawn leaf water potential. In the presence of water stress, it also has a higher water use efficiency due to reduced transpiration and efficient stomatal control. It can also maintain a higher relative water content than cultivated sunflower. In Spain, interspecific *H. argophyllus* hybrids are being developed with increased pubescence for drought tolerance. The evaluation of seven annual xerophytic *Helianthus* species in France under various irrigation levels has shown variability in morphological traits (leaf size and number) and some physiological characteristics of the leaf (transpiration water use efficiency). Compared with *H. annuus*

species, most wild species have a substantially increased leaf water efficiency, particularly in limited water supply situations. The French have concluded that *H. argophyllus* is interesting for drought breeding because it possesses several usful characteristics of other species such as low water permeability of the leaf, high water efficiency, and high dry matter production.

Disease Resistance

New sources of resistance to downy mildew (DM) Race 4 have been discovered from four wild *H. annuuss* populations at Fargo. The resistance is a single dominant gene controlled and each population possesses a separate gene which is different than the PL6 gene. In Russia, an interspecific cross of *H. annuus* x *trachelifolius* (=*decapetalus*) has shown resistance to downy mildew. In Bulgaria, the wild species *H. tuberosus, H. eggertii, H. rigidus, H. laevigatus, H. divaricatus, H. mollis, H. salicifolius, H. giganteus*, and *H. nuttallii* showed tolerance to DM. In the US, interspecific crosses with *H. praecox* spp. *runyonii, H. paradoxus, H. deserticola* and *H. argophyllus* have resistance to Race 2. One selection of *H. argophyllus* 1575 was resistant to 5 races of DM. Eleven perennial species appear to be resistant to multiple races of DM using the leaf disk immersion technique.

Wild *Helianthus annuus* and *H. petiolaris* possessed single dominant genes in the homozygous condition for resistance of each of four races of rust (1, 2, 3, 4). Each population had rust-resistance genes unique to the specific races. Germplasm lines with resistance to four races of rust derived from seven *H. annuus* populations and designated PH1 to PH7 were released in September of 1993 by Dr. Jan, Fargo.

In Spain, 23 species of perennial sunflower were immune to broomrape. Most annual species except *H. anomalus* and *H. exilis* were susceptible. Interspecific lines derived from *H. anomalus, H. argophyllus*, and *H. deserticola* showed some level of resistance against broomrape races SE-192, while only *H. anomalus* was partially resistant to race CU-192.

Resistance to *Erysiphe cichoracearum* (powdery mildew) was observed in Bulgaria for *H. decapetalus, H. glaucophyllus*, and *H. resinosus*. High degrees of resistance to *Phoma* were observed in interspecific lines derived from *H. laevigatus, H. ciliaris*, and *H. strumosus*.

Some accessions of wild *H. tuberosus, H. resinosus, H. giganteus, H. decapetalus, H. annuus, H. argophyllus*, and *H. debilis* showed resistance to *Phomopsis* in Bulgaria. Interspecific crosses with *H. divaricatus, H. mollis*, and *H. smithii* also showed resistance. In France, interspecific hybrids based on *H. argophyllus, H. debilis, H. exilis*, and *H. petiolaris* spp. *fallax* were as tolerant to *Phomposis* as the tolerant check "Agrisol". In order to gain a better understanding of resistance of sunflower to *Phomopsis*, the participants of the IFVC, Yugoslavia evaluated wild species for flavonoid content and etheric oils (terpenes) and concluded that there was not a significant relationship between these products and *Phomopsis* resistance. They also evaluated culture filtrates as a screening mechanism. There did not appear to be a consistent pattern for resistant and susceptible genotypes.

Three wild species, *H. argophyllus, H. debilis*, and *H. bolanderi* showed some promise for tolerance to *Sclerotinia sclerotiorum* in Bulgaria. In the US, field testing of interspecific lines derived from *H. praecox* spp. *runyonii, H. resinosus, H. paradoxus, H. anomalus,*

H. petiolaris, H. tuberosus, H. exilis, and *H. deserticola* had varying levels of tolerance to *Sclerotinia* compared to the inbred line HA89.

Regeneration of Wild *Helianthus* Species

The wild perennial *H. salcifolius* had the highest percentage of callus formation from anthers with 56% followed by *H. mollis* with 38%, and *H. praecox* with 27% as reported from Dobroudja, Bulgaria. The highest regeneration was observed in *H. salicifolius* (27%). For pollen culture, a liquid and solid medium is used with gamma rays in doses of 5 GY and 10 GY. A callus was induced for *H. salicifolius, H. mollis, H. smithii*, and *H. eggertii*. Interspecific *H. annuus* x *H. eggertii* had 69% callus formation, while *H. annuus* x *H. smithii* had 41%. Plants were produced only from the *wild species H. mollis* and *H. salicifolius*.

INFORME DEL PROGRESO DEL GRUPO DE TRABAJO SOBRE EVALUACION DE ESPECIES SILVESTRES DE *Helianthus* PARA EL PERIODO 1991-1993

RESUMEN

Este informe presenta los avances de la investigación del grupo de trabajo para la evaluación de especies de *Helianthus* desde el último informe de reunión FAO en 1991 en Pisa, Italia. El grupo de trabajo llevó a oabo amplias y extensivas investigaciones sobre especies silvestres con el objetivo común de incrementar la diversidad genética del girasol cultivado utilizando las especies silvestres para obtener un cultivo mejor.

Los avances del grupo de trabajo han añadido nuevos datos en la exploración, mantenimiento y caracterizacion de especies silvestres de girasol, germinación, dormancia, filogenia y clasificación de *Helianthus*, hibridación interespecífica, citogenética de especies e híbridos interespecíficos, androesterilidad citoplàsmica y rostauración de la fertilidad, calidad de aceite y contenido de proteina, tolerancia a sequía, resistencia a enfermedades y regeneració de especies silvestres. Esto añadio un considerable conocimiento a la base de datos informacional aceroa de las especies silvestres y ou potencial de uso para mejorar el girasol cultivado.

RAORT DES TRAVAUX REALISES AU SEIN DU GROUPE DE TRAVAIL SUR L'EVALUATION DES ESPECES SAUVAGES D'*Helianthus* DURANT LA PERIODE 1991-1993.

RÉSUMÉ

Le rapport présente les résultats obtenus dans le groupe de travail pour l'évaluation des espèces sauvages d'*Helianthus* sauvages, depuis la consultation F.A.O. de Pise (Italie) en 1991. Le groupe de travail a conduit des recherches approfondies sur les espèces sauvages avec pour objectif commun d'accroître la diversité génétique du tournesol cultivé et contribuer à son amélioration par l'utilisation des espèces sauvages.

Les résultats obrenus dans le groupe de travail ont apporté de nouvelles données sur l'exploration, le maintient et la caractérisation des tournesols sauvages, la phylogénie et la classification des *Helianthus*, l'hybridation interspécifique, la cytogénétique des espéces et des hybrides interspécifques, la sterilité mâle cytoplasmique et la restauration de la fertilité, la qualité de l'huile et la teneur en protéines, la tolérance à la sécheresse, la résistance aux maladies et la régénération des espèces sauvages d'*Helianthus*. Ainsi, ces résultats ont considérablement enrichi les informations de la base de données relative aux espèces suavages de tournesol et précisé leur intérêt pour une utilisation potentielle dans l'amélioration du tournesol.