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Chao-Chien Jan: Thirty-five Years of Dedicated Research Utilizing Wild Sunflower Crop Relatives for Sunflower Improvement

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Abstract: Dr Chao-Chien Jan, Research Geneticist with the USDA-Agricultural Research Service (USDA-ARS), Red River Valley Agricultural Research Center, Northern Crop Science Laboratory, Sunflower and Plant Biology Research Unit, Fargo, ND retired January, 2017 after 35 years of dedicated service. He began his research career in 1974 after receiving his Ph.D. in genetics from the University of California, Davis, CA, working with wheat. He was a postdoctoral Research Biologist at the Cancer Research Institute, University of California, San Francisco in 1975. From 1976 to 1981 he was a postdoctoral Research Agronomist, Department of Agronomy and Range Science, UC, Davis, CA. working on wheat. In 1981, Dr Jan joined the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) as a Research Geneticist at the Rice and Oilseed Unit at Davis, CA with an emphasis on cytogenetics, working on sunflower (*Helianthus annuus*) crop wild relatives (CWR) for the improvement of the sunflower crop. The sunflower program at Davis, CA was closed in 1984, and he was transferred to the USDA-ARS Sunflower Unit in Fargo, ND where he spent the rest of his career working on sunflower CWR. Dr Jan' research contributed significantly to the ability to utilize the genetic diversity of the 53 species of wild sunflowers, especially in the areas of germination, use of embryo culture and chromosome doubling to overcome embryo abortion and fertility problems. His pioneering research in interspecific hybridization, cytoplasmic male sterility and fertility restoration, cytogenetic stocks, disease resistance and mutation opened doors to genetic diversity never before available for utilization by the sunflower industry. This led to his global stature with invitations to serve as a visiting scientist and fellowships in Australia, Serbia, Spain, Romania, and China. He has hosted over 20 scientists from 15 countries, as well as countless students during his career. Due to his stature, he has been invited to present several invited plenary talks, both national and

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international, and in 2012 he was presented the prestigious Pustovoit Award, the highest award in the sunflower industry given by the International Sunflower Association for his contribution to sunflower science and technology.

Keywords: *Helianthus* species, crop wild relatives, exotic germplasm, interspecific hybridization, pre-breeding, genetic resources

The early wheat years

Dr Chao-Chien Jan began his research career after receiving his Ph.D. in genetics from the University of California, Davis working with wheat in 1974 (Figure 1).

Figure 1: Dr Chao-Chien Jan in the greenhouse at the USDA-ARS, Northern Crop Science Laboratory, Red River Valley Agricultural Research Center, Fargo, ND USA.

He was a post-doctoral Research Biologist at the Cancer Research Institute, University of California, San Francisco during 1975. From 1976 to 1981 he was a postdoctoral Research Agronomist, Department of Agronomy and Range Science, UC, Davis, CA working with Dr Calvin Qualset on the inheritance of male-sterility (MS) derived from an intervarietal cross and use of gametocides in wheat (Jan and Qualset, 1977a, 1977b). Dr Jan demonstrated that three recessive sterility genes controlled MS, and one gametocide induced high MS with minimum reduction in female fertility. He also produced intergeneric hybrids and amphiploids of hexaploid wheat (*T. aestivum*) and tetraploid wheat (*T. turgidum*) with *Dasypyrum villosum* (L.) Candargy, an annual grass native to the Mediterranean and Caucasus areas (Jan *et al.*, 1986). The male-sterility study presented the possibility for the commercial production of hybrid wheat seed if the poor pollen-shedding problem of the crop could be improved. This work demonstrated the feasibility of the use of *D. villosum* for wheat improvement and showed an analogy between the 6x amphiploid and 6x triticale. Based upon this research, De Pace's laboratory in Italy established the locations of genes controlling certain isozymes and seed storage proteins in *D. villosum* and the potential role of these genes for use as biochemical controls in transferring *D. villosum* chromosomes into the wheat genome (Montebove *et al.*, 1987; De Pace *et al.*, 1988). Short-statured, male-sterile wheat germplasm was released (Jan *et al.*, 1980). The production of fertile wheat x *D. villosum* hybrids stimulated research activities on the genetics and utilization of *D. villosum* for wheat improvement and the production of alien addition lines with *D. villosum* chromosomes in 4x and 6x wheat backgrounds.

The sunflower years

Utilization of interspecific hybridization to evaluate relationships among annual helianthus species

Working with a graduate student, John Chandler, Dr Jan used meiotic abnormalities of interspecific hybrids among 11 annual diploid *Helianthus s*pecies to construct the first phylogenetic diagram of sunflower (Chandler *et al.*, 1986). He was also successful in interspecific transfer of resistance to prevailing races of sunflower rust (*Puccinia helianthi* Schwein.), downy mildew [*Plasmopara halstedii* (Farl.) Berl. & De Toni], and powdery mildew (*Erysiphe cichoracearum* DC.) from the sunflower CWR into cultivated lines. He further determined the mode of resistance to the diseases, and produced pre-breeding germplasm

populations (Jan and Chandler, 1985, 1988). Dr Jan was the major professor for two North Dakota State University, Fargo, ND graduate students who demonstrated interspecific gene transfer of rust and downy mildew resistance (Quresh and Jan, 1993; Quresh *et al*., 1993; Rahim *et al*., 2001; Rahim *et al*., 2002). Understanding the species relationships assisted in the utilization of wild *Helianthus* species, and the introduction of disease resistance genes from wild species into cultivated lines added genetic diversity to stabilize long-term sunflower production.

Development of new methods for germinating wild sunflower seeds, embryo rescue, and chromosome doublings of interspecific hybrids

Methods for efficient germination of wild *Helianthus* species, techniques for interspecific hybridization, and procedures to improve F_1 hybrid fertility are essential for gene transfer from wild *Helianthus* species into cultivated sunflower (Chandler and Jan, 1985; Jan *et al*., 1983). Dr Jan was a leader in the development of methods that facilitated the use of the wild sunflower species to produce interspecific hybrids (Jan and Chandler, 1989). He also increased F_1 hybrid fertility by introducing a chromosome doubling technique, which successfully produced the only trisomic genetic stocks in cultivated sunflower. The germination and new embryo culture methods are now used as standard procedures throughout the world, and have led to introgression of diverse germplasm that was previously unavailable due to the difficulty in making interspecific crosses. As a result, numerous interspecific hybrids were produced for the first time by Dr Jan (Jan and Chandler, 1989). The chromosome doubling method made the production of amphiploids possible, as well as, autotetraploid P2l, which led to the development of trisomics (Jan *et al*., 1988; Jan, 1992a, 1992b). The production of trisomics from autotetraploid P21 demonstrated the feasibility of producing a set of trisomics, which enhanced linkage studies in sunflower and accelerated germplasm improvement. The successful production of amphiploids up to the hexaploid level provided a bridge for efficient gene transfer from perennial species which was instrumental in the introduction of broomrape resistance (Jan and Fernandez-Martinez, 2002). Special invitations to conferences and research institutes in Yugoslavia in 1989, China in 1993, 1997, 2002, 2005, 2006, and 2010, Spain in 1995, Australia in 1998, and Turkey in 2000 are indicative of the widespread recognition of Dr Jan's contributions.

Characterization of a unique type of cytoplasmic-nuclear interaction

Dr Jan identified and determined the inheritance of a cytoplasmic-nuclear interaction that caused a reduction in chlorophyll, photosynthesis rate, and overall plant vigor when the nucleus of inbred line HA89 was substituted into cytoplasms of perennial diploid *Helianthus* species (Jan, 1992c). Inheritance studies indicated that a single dominant gene located at the same locus fully restores the vigor, and is present in a large number of cultivated lines. A recent study indicated that *H. giganteus* has a vigor restoration gene at a different locus, suggesting variability of the gene among wild perennials (Jan and Ruso, 2000, 2002). The results also suggested that perennial *Helianthus* species may have shared a common cytoplasmic origin different from that of the annual *Helianthus* species. The observation that all 11 annual *Helianthus* species do not possess the reduced vigor (RV) cytoplasm prompted a reexamination of the evolutionary origin of diploid, tetraploid, and hexaploid perennial *Helianthus* species using classical, as well as molecular approaches. Because the vigor restoration genes can easily be selected, the utilization of these cytoplasms as sources of genetic variability for sunflower improvement is not affected by perennial species cytoplasm. The incorporation of the vigor restoration gene from *H. giganteus* into an HA89 background demonstrated the successful application of embryo rescue and chromosome doubling for difficult interspecific gene transfer. The widespread existence of vigor restoration genes in cultivated lines suggested the wide usage of *H. tuberosus* in early sunflower breeding projects in the former Soviet Union.

Development and release of pre-breeding germplasms/genetic stocks

Sunflower CWR have been undeniably beneficial in sustaining sunflower production by providing breeders with an unlimited pool of potentially useful traits (Jan and Seiler, 2007; Seiler and Jan, 2010; Seiler and Marek, 2011; Liu and Jan, 2012; Kane *et al.*, 2013; Seiler *et al.*, 2017). Parasitic broomrape (*Orobanche cumana* Wallr.) has become a threat to global sunflower production. The sunflower CWR, especially the perennial species, have been identified as reliable sources of resistance (Jan *et al.*, 2014a; Seiler and Jan, 2014). Dr Jan was instrumental in bridging the gap between the CWR and the breeding industry. He has released 100 interspecific lines over his career, including one resistant to

sunflower powdery mildew, seven to rust, four to downy mildew, one to rust, powdery mildew, and sunflower mosaic potyvirus combined, three to mosaic potyvirus, and four to *Orobanche* (broomrape) race F (Tan *et al*., 1992; Jan and Chandler, 1988; Jan *et al*., 2004a, 2004b; Jan and Gulya, 2006a; 2006b; Jan *et al*., 2002a). Cytoplasmic male-sterile (CMS) lines included six from induced mutation, two from wild *H. annuus*, and one from *H. pauciflorus* as well as a total of 14 fertility restoration lines (Jan, 1992c; Jan *et al*., 2002b; Jan, 2006; Jan and Vick, 2006; Jan *et al*., 2006a). Also included are tetraploid P21, four nuclear male-fertility lines, and one interspecific amphiploid of *H. bolanderi* x P21 (Jan, 1992a, 1992b). In addition, Dr Jan also released, as a co-author, 10 interspecific fertility restoration germplasms for the standard CMS PET1 cytoplasm, and 4 lines with reduced saturated fatty acid content (Seiler and Jan, 1994; Seiler and Jan, 1997; Vick *et al*., 2003; Vick *et al*., 2007). The vulnerability of using a single CMS source and a few restoration genes for worldwide sunflower production will be reduced using the new CMS sources and fertility restoration genes. The new disease and broomrape resistance genes will increase diversity of sunflower hybrids, reducing the cost and environmental pollution associated with chemical treatments. As a whole, these releases either provide material facilitating research or possess valuable genes for widening the crop's genetic base, reducing its genetic vulnerability, and enhance its potential for improvement. The USDA–ARS National Plant Germplasm System (NPGS) maintains and manages genetic resources for agricultural crops in the United States. The NPGS sunflower CWR collection is located at North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa, USA. The NPGS GRIN system also serves as the portal for requesting germplasms (https://npgsweb.ars-grin.gov/gringlobal/search.aspx). The germplasm lines developed by Dr Jan are freely available for research and educational purposes, although some restrictions are imposed by import regulations of receiving countries.

Development of CMS and nuclear male sterility (NMS) using mutagen and interspecific hybrids

Dr Jan was the first to use chemical mutagens in an innovative effort to create unique sources of cytoplasmic male sterility (CMS) and nuclear male sterility (NMS) in cultivated sunflower. He also identified six sources of CMS from wild *H. annuus* and one from *H. giganteus*. Dr Jan also identified fertility restoration genes for the seven CMS sources from the wild accessions and the cultivated lines. The induced CMS mutants are agronomically equal to the commercially used CMS PET1 cytoplasm, and respond to the same fertility restoration genes

as the CMS PET1 (Jan and Rutger, 1988; Havekes *et al*., 1991; Jan, 1992d; Jan, 1992e; Jan, 2000; Jan *et al*., 2006b; Jan and Vick, 2007; Jan, 1990). The NMS mutants represent four genes, and are agronomically superior to the NMS lines currently in use (Gong *et al*., 2014). These new CMS and restoration genes are available for commercial breeders to incorporate into their hybrids to reduce the crop's genetic vulnerability associated with the current use of a single CMS PET1 cytoplasm. Without the need to develop new restoration lines, the new CMS mutants are available for rapid utilization in sunflower hybrid production in the event of catastrophic failure of the current hybrid production system. These CMS mutants induced from USDA sunflower line HA89 are also unique isogenic lines for future molecular and biochemical characterization of the CMS traits.

Development of molecular tools for sunflower germplasm enhancement

Dr Jan and colleagues evaluated the genetic diversity of 23 USDA sunflower lines, created an restriction fragment length polymorphism (RFLP) linkage map for cultivated sunflower, constructed bacterial artificial chromosome (BAC) and binary bacterial artificial chromosome (BIBAC) libraries, identified RFLP linkage group-specific BAC and BIBAC clones, and developed a genomic in situ hybridization (GISH) technique distinguishing chromosome and chromosome segments of perennial and annual *Helianthus* species from those of cultivated sunflower. This produced the initial U.S. public RFLP map for cultivated sunflower, covering 1,129 centimorgans of the sunflower genome and defining 20 linkage groups, the first large and high quality BAC and BIBAC libraries, and the first useful GISH technique for sunflower (Feng *et al*., 2006; Feng *et al*., 2013; Jan *et al*., 1998; Liu and Jan, 2012; Chen *et al*., 2006; Rojas-Barros *et al*., 2008; Mulpuri *et al*., 2009). The RFLP map provided a foundation for linkage studies of major sunflower genes, as well as further saturation of the RFLP map with additional molecular markers (Jan *et al*., 1993). The utilization of this RFLP map has been expanded by its recent merging with other public and proprietary RFLP, amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) maps. The linkage group-specific BAC and BIBAC clones will speed up the development of cytogenetic trisomic lines, and the libraries will provide essential resources for comprehensive research of the sunflower genome.The new GISH technique will enables scientists to associate gene transfer with visible chromosome segments.

Development of interspecific hybrids and amphiploids resistant to broomrape

Dr Jan cooperated with Dr Jose Fernandez-Martinez of CSIC, Institute of Sustainable Agriculture, Cordoba, Spain, to provide expert support to develop broomrape-resistant sunflower germplasm. Interspecific sunflower amphiploid germplasm developed by Dr Jan was resistant to the newly evolved broomrape (*Orobanche*) race F, that attacks all commercial sunflower hybrids with resistance to the *Or5* gene (Jan and Fernandez-Martinez, 2002; Jan *et al.*, 2002a; Perez-Vich *et al.*, 2002; Velasco *et al.*, 2007). Preliminary results in Spain indicated all the cultivated lines and nearly all the wild *Helianthus* annual species are susceptible, and the perennial *Helianthus* species are almost immune to race F. An annual species, *H. debilis* subsp. *tardiflorus*, was recently found to be resistant to the even more virulent Race G (Velasco *et al.*, 2012). Dr Jan cooperated with researchers at the Institute of Sustainable Agriculture, Cordoba, Spain, by making interspecific hybrids and providing perennial *Helianthus* amphiploids from his project, along with their backcross progenies for screening, and conducted all cytological evaluations and seed increases. The Spanish cooperator conducted the broomrape screening in Cordoba. Dr Jan trained one Ph.D. student and one postdoctoral scientist from Spain (Sukno *et al.*, 1998; Sukno *et al.*, 1999; Jan *et al.*, 2014a). Many international sunflower seed companies have requested seed or pollen of the resistant germplasm, and are presently incorporating the trait into their proprietary lines. The work demonstrated the value of these interspecific amphiploids in providing genes for a crisis situation. This work also protects the sunflower crop in other continents in case of accidental introduction. New amphiploid germplasm from Dr Jan's breeding program appears to provide unique Sclerotinia resistance genes and fertility restoration genes for hard-to-restore CMS cytoplasms (Feng and Jan, 2008).

Identification of specific suppression of the fertility restoration gene(s)

Dr Jan discovered silencing genes in cultivated lines that are capable of suppressing the fertility restoration gene and reverting the plant back to male-sterile. This was the first report of silencing genes in sunflower and also the first report of its kind specific to fertility restoration. The discovery provides material for studying specific gene silencing, and will also facilitate restoration line development (Jan, 2003).

A CMS line, 514A, was recently developed from *H. tuberosus* cytoplasm, but no fertility restorer gene had been identified. Dr Jan reported the discovery, characterization, and molecular mapping of a novel *Rf* gene for CMS 514A derived from an amphiploid (Amp. *H. angustifolius*/P21, 2n = 68) (Liu *et al.*, 2013a). Progeny analysis of the male-fertile (MF) plants (2n = 5) suggested that this gene, designated Rf_6, was located on a single alien chromosome. Genomic in situ hybridization (GISH) indicated that Rf_6 was on a chromosome with a small segment translocation on the long arm in the MF progenies (2n = 34). Rf_6 was mapped to linkage group (LG) 3 of the sunflower SSR map. Eight markers were linked to this gene, covering a distance of 10.8 cM. The study discovered a new CMS/Rf gene system derived from wild species and provided significant insight into the genetic basis of this system which will diversify cultivated sunflower germplasm and facilitate the understanding of interactions between cytoplasm and nuclear genes.

Dr Jan and colleagues characterized two novel alloplasmic CMSs, designated CMS GRO1 and CMS MAX3, with defective anthers, narrow disc florets with no swollen corolla, and short, narrow ray flowers derived from two tetraploid amphiploids (AMPs) (Liu *et al.*, 2014). Among 26 tested lines, only AMP *H. cusickii*/P21 and HA 410 failed to restore male-fertility. Male-fertility restoration was controlled by at least two dominant genes. The new CMSs will facilitate the studies of the incompatibility between cytoplasmic and nuclear genes, especially for the alloplasmic CMS involving perennial species, and also provide unique ornamental flower types and CMS sources for hybrid sunflower breeding.

Identification of the genetic control of chromosome non-reduction

Helianthus consists of hexaploid (2n = 102), tetraploid (2n = 68), and diploid (2n = 34) species, which provide genetic sources for cultivated sunflower (2n = 34) improvement. Interspecific amphiploid production using colchicine has been successful in producing amphiploids that improved the hybrid fertility and facilitated backcrossing for interspecific gene transfer. Dr Jan studied an interspecific hybrid of the hexaploid *H. californicus* x HA 89 and identified the genetic control of chromosome non-reduction in *H. californicus*. The genetic control of chromosome non-reduction is assumed to have evolutionary significance on sunflower speciation from diploid to tetraploid, and to hexaploid species. Once introduced into cultivated lines, the chromosome non-reduction gene has the potential of doubling chromosomes of interspecific hybrids in the

absence of colchicine treatment, and will greatly facilitate amphiploid production and sunflower germplasm development (Jan and Feng, 2004).

Disease mapping

Long-term studies have revealed that virulence to the R_2 sunflower rust resistance gene in the line MC29 does not exist in the Australian rust (*Puccinia helianthi*) populations. Dr Jan cooperated in a study that identified three SSR markers ORS795, ORS882, and ORS938 linked in coupling to the R_2 gene, while the SSR marker ORS333 was linked in repulsion (Lawson *et al.*, 2011). The availability of reliable and heritable DNA-based markers for R_2 will enable the efficient deployment to obtain a durable and environmentally friendly host plant resistance.

The rust resistance gene designated R_{14} in a germplasm line PH3, originated from a wild *H. annuus* population resistant to 11 rust races. PH3 has seedlings with an extraordinary purple hypocotyl color. Dr Jan's group mapped both the R_{14} rust resistance gene and the purple hypocotyl gene, *PHC*, in PH3, and identified markers for marker-assisted breeding for sunflower rust resistance. R_{14} was mapped to the middle of the LG 11, with a dominant single nucleotide polymorphism (SNP) marker NSA_000064 as the closest marker at a distance of 0.7 cM, and another codominant marker ORS542 linked at 3.5 cM proximally (Zhang *et al.*, 2016). The PHC gene was also linked to R_{14} with a distance of 6.2 cM. The closely linked molecular or morphological markers could facilitate sunflower rust-resistant breeding and accelerate the development of rust-resistant hybrids.

Inbred line HA 458 is effective against all virulent races of downy mildew identified in the United States. The resistance gene Pl_{17} was controlled by a single dominant gene. Dr Jan assisted in the mapping of this gene to LG 4, using simple SSR and SNP primers to identify markers linked to Pl_{17} (Qi *et al.*, 2015). Two flanking markers, SNP SFW04052 and SSR ORS963, delineated Pl_{17} in an interval of 3.0 cM. This region can be considered amenable to molecular manipulation for further map-based cloning of Pl_{17}.

Sunflower downy mildew resistance genes have been designated as *Pl* genes. About half of the more than 20 *Pl* genes have been mapped. Dr Jan and colleagues mapped the Pl_{16} downy mildew gene in a sunflower differential line, HA-R4 (Liu *et al.*, 2012a). It was mapped on the lower end of LG 1 of the sunflower reference map, with 12 markers covering a distance of 78.9 cM. One dominant simple SSR marker, ORS1008, co-segregated with Pl_{16}, and another codominant expressed sequence tag (EST)-SSR marker, HT636, was located 0.3 cM

proximal to the Pl_{16} gene and closely linked to the Pl_{13} gene in another sunflower differential line, HA-R5. This was the first report of two tightly linked markers for both the Pl_{16} and Pl_{13} genes, which will facilitate marker-assisted selection in sunflower resistance breeding, and provide a basis for the cloning of these genes.

Recent genetic stocks and germplasm releases

Alloplasmic cytoplasms

The genetic base of the commercial sunflower crop is very narrow, based on a single female cytoplasm PETl used globally, making it extremely vulnerable to attacks by abiotic and biotic stresses. Dr Jan released 10 alloplasmic sunflower cytoplasm lines from nine annual and perennial wild *Helianthus* species. These included AP NIV (*H. niveus*), AP PRA (*H. praecox*), AP PRA 380 (*H. praecox*), AP NEG (*H. neglectus*), AP ANO (*H. anomalus*), AP MOL (*H. mollis*), AP MAX (*H. maximiliani*), AP GRO (*H. grosseserratus*), AP ANG (*H. angustifolius*), and AP DIV (*H. divaricatus*) (Jan *et al.*, 2014b). In general, most cytoplasms of wild annual species can accommodate cultivated nuclear genes without drastic adverse interactions, and are potential sources of cytoplasmic diversity for sunflower breeding. In addition, these new alloplasmic cytoplasmic sources will provide a tool for studying cytoplasmic effects, as well as cytoplasmic nuclear interactions.

Non-dormant germplasm

Seed dormancy is a physiological strategy evolved by plants to ensure survival of a species by not germinating under unfavorable conditions that may lead to death. Sunflower generally undergoes a non-deep physiological dormancy period mediated by abscisic acid, which can be overcome by the use of gibberellic acid or ethylene (Vick *et al.*, 2009). Dr Jan released a nondormant sunflower genetic stock line, NDG1, discovered in an interspecific cross with perennial *H. divaricatus*. Fresh seeds of NDG 1 will germinate on heads 45 days after pollination, and seeds harvested 28 days after pollination and artificially dried for four days will germinate within two days in Petri dishes on wet germination paper. Introduction of the non-dormant trait into a breeding program could be a useful tool for quickly advancing generations by circumventing dormancy and planting

the seed immediately after drying. This would eliminate the currently used time-consuming and costly embryo rescue technique.

White cotyledons

The appearance of white cotyledons that subsequently produce near normal true leaves and grow to maturity has not been reported previously in sunflower. Two white cotyledon genetic stocks, WCl and WC2, were discovered by Dr Jan during a study of cytoplasmic male sterility in wild annual *H. annuus* that exhibited cotyledon-specific total chlorophyll deficiency and partial true-leaf chlorophyll deficiency. These recessively gene-controlled white cotyledon sunflower genetic stocks along with accompanying molecular markers can be used as a genetic marker, and will further help in understanding the different processes of chlorophyll metabolism and photosynthesis between cotyledons and true leaves in sunflower and other crops.

Cytoplasmic male sterility

The cytoplasmic male-sterility system is important for hybrid sunflower production. Unfortunately, the system is based on a single female cytoplasmic male sterile line, PETl. Diversification of new and different cytoplasms is needed in the sunflower industry. New cytoplasms and complementary restoration genes from CWR will provide unique variation for breeders to increase genetic diversity within elite populations and parental lines, allowing sunflower to better respond to biotic and abiotic stresses. Dr Jan released two CMS lines based on *H. grosseserratus*, CMS GROl, and CMS GROl-RV, and CMS MAX3-RV based on *H. maximiliani*. Two of the releases have an unusual cytoplasmic-nuclear interaction causing plants to have reduced vigor that is controlled by a single dominant gene needed to restore normal plant growth. A considerable number of cultivated lines were found to possess the vigor restoration gene. A recent discovery of a different vigor restoration gene derived from *H. giganteus* suggested the existence of different vigor restoration genes in various perennial *Helianthus* species, compensating for specific cytoplasmic effects causing reduced vigor. The new CMS lines are characterized by defective anthers, narrow disc florets with no swollen corolla, and short, narrow ray flowers. These unique CMS lines will facilitate studies of the incompatibility between cytoplasmic and nuclear genes, especially for alloplasmic CMS involving perennial species, and also will provide unique ornamental flower types and CMS sources for hybrid sunflower

breeding. He also released CMS TUB1-HA89 (*H. tuberosus* line) and a fertility restoration line, RF TUB1-ANG for the CMS TUB1-HA89 discovered in an interspecific amphiploid, AMP *H. angustifolius* (Liu *et al.*, 2013a).

Fertility restoration genes

During interspecific gene transfer, cytoplasmic male sterility often appears after several backcrosses to cultivated sunflower. Corresponding fertility restoration genes are often identified among male-fertile progenies or from other sources derived from wild *Helianthus*. Dr Jan released four fertility restoration lines, Rf GIG2-MAX, Rf GIG2-GRO, Rf GIG2-ANG, and Rf GIG2-ATR for CMS GIG2 first observed in F_1 progeny after pollination with four interspecific amphiploids, AMP *H. maximiliani* P21, AMP *H. grosseserratus* P21, AMP *H. angustifolius* P21, and AMP *H. atrorubens* P21 (Zhang *et al.*, 2009; Feng *et al.*, 2015). Rf GIG2-MAX, Rf GIG2-GRO, Rf GIG2-ANG, and Rf GIG2-ATR are F_5 or F_6 bulks homozygous for fertility restoration genes for CMS GIG2. Rf GIG2-GRO and Rf GIG2-ANG produce normal plants, while Rf GIG2-MAX and Rf GIG2-ATR produce RV plants due to the lack of nuclear vigor restoration genes.

The inheritance of a previously identified dominant *Rf* gene in the confection sunflower line RHA 280 has been determined and designated as Rf_3. Dr Jan and colleagues reported the mapping of the Rf_3 locus using an F_2 population of 227 individuals derived from CMS HA 89–3149 × RHA 280 (Liu *et al.*, 2012b). Using 90 F_2 individuals with 42 polymorphic markers of LGs 7 and 11, the Rf_3 gene was linked with eight markers on LG 7, including five SSR markers and three expressed sequence tag (EST)-SSR markers. This was the first report of an *Rf* gene from the confection sunflower. The closely linked marker to Rf_3 will facilitate marker-assisted selection, and provide a basis for cloning of this gene.

Amphiploids

Interspecific amphiploids derived from CWR of sunflower help mine potential genes from 53 species, especially the hard-to-cross perennial species. Dr Jan released 12 interspecific amphiploid genetic stocks: AMP GRO (*H. grosseserratus*); AMP DIV/GRO (*H. divaricatus/grosseserratus*); AMP MAX (*H. maximiliani*); AMP MAX 1631 (*H. maximiliani*); AMP MAX 1113 + 1323 (*H. maximiliani*); AMP NUT (*H. nuttallii*); AMP NUT 102 + 412 (*H. nuttallii*); AMP MOL 1531 (*H. mollis*); AMP ATR) 1588 (*H. atrorubens*); AMP HIR 1126 (*H. hirsutus*); AMP HIR 1537 (*H. hirsutus*); and AMP STR (*H. strumosus*), originating from crosses of eight wild

perennial *Helianthus* species with cultivated lines NMS HA 89, P21 or HA 89, followed by chromosome doubling using colchicine. Additionally, AMP HIR 1537 (BC_1) was released to supplement the use of AMP HIR 1537, which requires embryo rescue to obtain BC_1 progeny. These amphiploids contain genetic diversity from the contributing wild perennial species, and have restored fertility to sib-pollinate for line maintenance and further backcrossing to cultivated lines for interspecific gene transfer. Otherwise, wild perennial *Helianthus* are extremely difficult to cross with the cultivated lines and the F_1 progeny are usually highly sterile, making further backcrosses difficult or impossible. These genetic stocks provide the badly needed genetic diversity from perennial *Helianthus* species, including resistance to new races of broomrape, and Sclerotinia, and will allow conventional breeding to identify and transfer genes from perennial *Helianthus* species with greater ease. The added value of these genetic stocks is that they can act as a bridge in interspecific gene transfer, allowing for easier backcrossing with the cultivated sunflower to further broaden the genetic diversity of the crop, as well as the transfer of specific target genes. The genetic stocks will also allow for the development of chromosome addition lines, with individual wild species chromosomes added to the background of cultivated sunflower lines for genetic studies of specific chromosomes.

Somatic embryogenesis from corolla tubes of interspecific amphiploids

Sunflower CWR possess an abundance of unique genes for sunflower improvement. However, transfer of wild species genes into cultivated sunflower is restricted by cross incompatibility and hybrid sterility. Alternative conservation and propagation of the interspecific F_1 plants and amphiploids through tissue culture has the potential of providing a large number of plants for breeding programs. Sunflower plant parts including shoot apices, hypocotyls, cotyledons, leaves, protoplasts, and mature embryos have been used to induce calli or somatic embryos with limited success. Somatic embryogenesis in vitro provides an efficient means of plant multiplication, facilitating sunflower improvement and germplasm innovation. Dr Jan and colleagues, using flower corolla tubes of chromosomally doubled interspecific hybrids, developed a protocol for direct embryo formation that results in high frequencies of secondary somatic embryos and regenerated plants (Fu *et al.*, 2017). These results contribute to extending the explant types and embryo induction methods in sunflower, providing a means of producing multiple hybrid plants for continuing crosses to cultivated sunflower for gene transfer, as well as for studies on somaclonal variation, plant

differentiation, and micro-proliferation. Continued validation and optimization of this system in diverse sunflower genotypes needs to be continued.

Triploid production from interspecific crosses of two diploid perennial helianthus with cultivated sunflower

Exploitation of perennial sunflower CWR for the improvement of cultivated sunflower has been limited by crossing difficulties and low fertility of F_1 hybrids. Dr Jan and colleagues discovered and characterized a unique unexpectedly high frequency of triploids while crossing a group of wild diploid perennial *H. nuttallii* and *H. maximiliani* accessions, collected near Morden, Manitoba, Canada, with cultivated sunflower (Liu *et al.*, 2017). Genomic in situ hybridization (GISH) analyses indicated that the triploid F_1 plants had two genomes from the wild pollen sources and only one from the cultivated line. Mitotic chromosome number analyses confirmed that the high frequency (overall 93 %) of triploid F_1 progenies was only obtained from the crosses of cultivated lines × N102, rather than those using other wild diploid or tetraploid perennial species as the female parents or in the reverse crosses. Since the cross-incompatibility between wild perennial species and cultivated lines is high, the higher frequency of triploids would be expected for diploid F_1 progenies and could be due to the preferred fertilization of the low frequency of 2n male gametes with the female gametes of the cultivated sunflower. Results confirmed the existence of an ultra-rare genetic alteration in these accessions that led to the abnormal whole genome transmission in crosses to female cultivated sunflower. The triploid F_1s could be the result of preferred fertilization of the low frequency of 2n male gametes with the female gametes of the cultivated sunflower, due to the dosage factors related to recognition and rejection of foreign pollen during fertilization. The triploids have been used to produce amphiploids and aneuploids. This discovery is novel and unique for sunflower and is expected to have significant implications for evolution of sunflower's higher ploidy species. Studies of the genetic control of this trait will facilitate research on sunflower polyploidy speciation and evolution, and the utilization of this trait in sunflower breeding.

Interspecific bulk populations

Fifteen diverse interspecific sunflower backcross bulk populations, SFB-CAL (*H. californicus*), SFB-DIV (*H. divaricatus*), SFB-DIV/GRO (*H. divaricatus/*

grosseserratus), SFB-GIG (*H. giganteus*), SFB-GR01 (*H. grosseserratus*), SFB-GR02 (*H. grosseserratus*), SFB-HIR (*H. hirsutus*), SFB-MAX1 (*H. maximiliani*), SFB-MAX2 (*H. maximiliani*), SFBMAX3 (*H. maximiliani*), SFB-NUTl (*H. nuttallii*), SFB-NUT2 (*H. nuttallii*), SFB-OCC (*H. occidentalis*), SFB-SAL (*H. salicifolius*), and SFB-STR (*H. strumosus*) originated from 11 wild perennial *Helianthus* species, including five interspecific amphiploids, with each bulk composed of 30 random backcross families. The new bulk population releases based on the perennial crop wild relatives offer the opportunity to utilize previously unavailable genetic resources. A significant number of the wild perennial *Helianthus* species have been identified as highly resistant to diseases and parasites of global concern including broomrape, Sclerotinia white mold, Phomopsis stem canker, downy mildew, leaf rust, Verticillium wilt, and Rhizopus head rot. Progeny populations of the perennial species crossed and backcrossed with cultivated sunflower provide unique genetic stocks that allow breeders to incorporate previously unavailable genetic diversity into elite populations.

Sclerotinia basal stalk rot and head rot germplasm

The most significant disease threat to sunflower production in humid temperate, as well as tropical and subtropical regions of the world is *Sclerotinia sclerotiorum* (Lib.) de Bary, a necrotrophic fungus that causes three distinctly different diseases on sunflower; basal stalk rot (BSR) or wilt, mid-stalk rot, and head rot (HR). Management tools for controlling this disease are insufficient; crop rotation is of marginal use due to the long-lived nature of the sclerotia, foliar fungicide application (commonly used for management of white mold in other crops) is not useful due to the unique infection process in sunflower, fungicide seed treatments provide insufficient control, and the present-day hybrids and cultivated lines lack sufficient tolerance and resistance. BSR and HR resistance is genetically complex and quantitatively conditioned by multiple genes, each having a small effect. Low levels of resistance are available in some inbred lines and hybrids, but greater levels of resistance are needed to combat this emerging pathogen, providing a more efficient, durable and environmentally friendly host plant resistance. Dr Jan released five BSR germplasms: BSR DIV 830 (*H. divaricatus*), BSR STR 1623 (*H. strumosus*), BSR CAL 2376 (*H. californicus*), BSR MAX 1018 + 1314 + 1323 (*H. maximiliani*), and BSR NUT 1008 + 1324 (*H. nuttallii*) with disease incidence (DI) of 7.0, 1.6, 1.9, 2.8, and 3 %, respectively, compared to the susceptible hybrid check Cargill/Mycogen® 270 with DI of 35 %, tolerant hybrid checks Croplan®305 with 9.6 %, and Croplan®343 with 15.2 % (Liu *et al.*, 2013b; Liu *et al.*, 2015). He also released two Sclerotinia head rot resistant germplasms,

HR MAX 1018 + 1323 (*H. maximiliani*), and HR NUT 1324 + 1008 (*H. nuttallii*) with a severity rating of 0.9 and DI 18.9 %, and 1.1 and DI of 24.5 %, respectively, compared to susceptible hybrid check Cargill/Mycogen®270 with a rating of 3.3, and DI of 70 %, and tolerant hybrid checks Croplan®305 with 2.7 severity, and DI of 59 %, and Croplan®343 with 1.7 severity rating and DI of 45 %.

Summary

Dr Chao-Chien Jan, a USDA-ARS, Research Geneticist dedicated 35 years to doing meticulous research using the sunflower and wheat CWR for crop improvement. His sunflower research in interspecific hybridization, cytoplasmic male sterility, and fertility restoration, cytogenetic stocks, disease resistance, and mutation has led to his globally recognized stature. Dr Jan's tireless efforts created interspecific hybrids of sunflower CWR that have opened the doors to genetic diversity never before available to the industry. His early pioneering research using embryo culture and chromosome doubling showed that these techniques would open a whole new world of opportunities for improving the sunflower. He has served as a mentor for many students and visiting scientists who have trained in his laboratory over the years, several of whom have become global leaders in the sunflower industry. It has been a pleasure for me to be a colleague of Dr Jan over the past 35 years interacting with him daily sharing our passion for utilizing the sunflower CWR for improving the sunflower crop.

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