



Safflower germplasm: past, present, and future

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Abstract

Germplasm collections are a critical resource for development and improving safflower (*Carthamus tinctorius* L.) cultivars and germplasm. Our objective here is to describe the safflower germplasm collection at the Western Regional Plant Introduction Station (WRPIS), Pullman WA, USA, in light of past, present, and future safflower germplasm uses and needs. The WRPIS maintains 2477 *C. tinctorius* accessions of which 90% are available for distribution. As many as 300 safflower accessions are regenerated each year under screen cages to prevent genetic contamination by insect cross pollination. The active collection is maintained at 4°C and 24% r.h. to facilitate management and distribution, while long-term, security back-up of accessions is at -18°C. Over the last ten years 11485 seed packets have been distributed from the WRPIS with 77% to scientists outside the US. The GRIN database contains 61982 data entries for safflower accessions including information on growth and development, disease resistance, oil content, and fatty acid profiles. Recent innovations in safflower germplasm using WRPIS germplasm include high vitamin E types, high saturated fatty acid types, the first mapped safflower genes, and winter adapted safflower. Current acquisition needs for the WRPIS include collections in southwest and central Asia and wild *Carthamus* species. Future uses of safflower are expected to include expanded use of hybrids, biofuels, new fatty acid combinations, and transgenic safflower for producing pharmaceuticals. In all these efforts, the WRPIS and other genebanks will play a central role by providing the needed genetic resources.

Key Words: *Carthamus tinctorius* L. - fatty acids - winter safflower -germplasm, genetic resources

Introduction

Genetic resources are the essential raw materials needed for improving crops and for developing new, value added uses. Safflower (*Carthamus tinctorius* L.), with its numerous and varied uses (Li and Mündel, 1996), has benefited from the diversity of genetic resources conserved and distributed by genebanks.

A germplasm directory for safflower was compiled by Zhang and Johnson (1999) which documented 18 different collections in 14 countries. This publication can be found at the safflower web page (<http://safflower.wsu.edu/>). India reported the largest collections with nearly 10,000 total accessions held at both the National Bureau of Plant Genetic Resources in New Delhi (2393 accessions) and the Project Coordinating Unit for Safflower in Solapur (7525 accessions). Other significant collections are in China, Mexico, and the United States. The U.S. safflower collection was developed starting in the late 1940's and is located at the Western Regional Plant Introduction Station (WRPIS) at Pullman, Washington (http://www.ars.usda.gov/main/site_main.htm?modecode=53481500). It now includes more than 2400 *C. tinctorius* accessions. The WRPIS is part of a national network of germplasm repositories that collectively make up the USDA-ARS National Germplasm System (NPGS). The US collection is represented by germplasm from more than 50 countries, and accessions are available to scientists worldwide. For this paper, WRPIS activities are divided into three sections: germplasm acquisition and regeneration, maintenance and distribution, and evaluation and enhancement. The objective is to describe the WRPIS activities within each section in light of past, present, and future safflower germplasm uses and needs.



Material and Methods

Acquisition and Regeneration There are three fundamental ways in which the WRPIS collection has been assembled: field collection, exchange, and donation. Field collections are cooperative between the WRPIS and a given national program in another country. Exchanges and donations result when genetic resources are transferred from one country or organization to the WRPIS. Donations from discontinued breeding programs have provided germplasm of particular value. All plant registrations in the journal *Crop Science*, and recently in the new *Journal of Plant Registrations*, automatically become part of the NPGS.

Accessions usually require an initial seed increase or regeneration before the quantity and quality of seed is adequate for storage and distribution to users. In addition, after the initial stock of regenerated seed is depleted, or has low germination, accessions must be regrown to replenish seed stocks. The two most important considerations are plant populations and isolation. Adequate plant populations are needed because most safflower was collected as landraces with significant variation within accessions. Thus, plant populations must be adequate to ensure that genetic changes in populations are minimized. At the WRPIS the goal is to have regeneration populations of at least 100 plants, which provides a high probability of maintaining all but very rare alleles (Johnson et. al., 2004).

As many as 300 safflower accessions are regenerated at the WRPIS each year. Regeneration is completed at the WRPIS Central Ferry WA research farm in the Snake River canyon (46.6733° N and 117.7541° W). The elevation is 206 m with a Spofford silt loam soil (fine-silty mixed superactive mesic Typic Natrixerolls). Seeds are generally planted in March and harvested in September. Central Ferry has typically hot, dry summers and plants are drip irrigated as needed through anthesis. To avoid genetic contamination by insect pollination, screen cages are constructed over plants, preventing insects from transferring pollen from one accession to another. Since most safflower is self compatible, insect pollinators do not normally need to be added to the cages. However, insect pollinators are prescribed in some cases such as the thin-hull, male sterile material developed by Rubis et al. (1966). This material is functionally male sterile based on the late dehiscence of anthers. A honey bee colony placed inside the cage ensures crossing within but not among accessions. Honey bees are also needed in wild type materials that have self incompatibly systems that reduce or prevent self pollination.

Maintenance and Distribution. After regeneration, seed longevity is promoted by cold storage to preserve seed viability as long as possible and to minimize the need for frequent regenerations, which tend to reduce genetic diversity through drift or inadvertent selection (Johnson et. al., 2004). The WRPIS maintains an “active collection” under intermediate term storage at 4°C to facilitate management and distribution. Long-term, security back-up storage of accessions is provided at -18°C at the National Center for Genetic Resources Preservation (NCGRP) at Fort Collins, Colorado (<http://www.ars.usda.gov/Main/docs.htm?docid=8071>). Upon request, distributions of genetic material in amounts of approximately 60 seeds are made to scientists without charge. Information concerning accessions is freely available on the Germplasm Resources Information Network (GRIN) (<http://www.ars-grin.gov/npgs/searchgrin.html>). This includes a cataloging of the collection origin and transfer history of each accession, available evaluation data, and identification of core collection accessions.

Evaluation and Enhancement Evaluation data is based on a set of descriptors developed at the WRPIS and also by the International Board for Genetic Resources (IBPGR, now Bioversity) (<http://www.bioversityinternational.org/publications/Web%5Fversion/237/>). The descriptors, available on GRIN (<http://www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?108>), include those traditionally used by WRPIS staff and the IBPGR descriptors. In GRIN the descriptors are divided into five general categories: chemical (seed oil and meal factors), disease, growth, morphology, phenology, and production. Evaluation is often cooperative with scientists at different sites or countries. In 1993 a core collection of 210 *C. tinctorius* accessions, about 10% of the WRPIS collection was developed (Johnson et al., 1993), and has been particularly useful when the evaluation of the entire collection was impractical (Johnson et. al, 2001).



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The journal *Crop Science* has traditionally published registration articles of released cultivars, germplasms, parental lines, and genetic stocks. In 2007, a separate publication, the *Journal of Plant Registrations*, (https://www.crops.org/publications/pdfs/2007_JPR_instructions.pdf) replaced *Crop Science* as the outlet for registering plant material under the aegis of the Crop Science Society of America. Although intellectual property rights still apply, all registered germplasm automatically become part of the NPGS, and this represents an important pool of cultivars and enhanced genetic resources for research and breeding.

Results

Acquisition and Regeneration

Of the 2477 *C. tinctorius*, 2221 or 90% are available for distribution through the WRPIS. The wild species with the most records is *C. oxyacanthus* having 106 accessions with 40 available for distribution. Overall only 60 of the 281 total accessions of wild species (21%), are available. For *C. tinctorius*, 1467 or 59% of the collection was directly collected in the field, cooperatively with farmers, or in markets. Donations from other countries and organizations accounted for 854 (34%) of accessions, and 130 are designated as formally developed as cultivars (27 total), germplasms (40), genetic stocks (1) or parental lines (2). There are 26 records without information on acquisition type.

Paul Knowles, working at UC-Davies from the 1950's into the 1980's, was the person most responsible for shaping the US safflower collection. Records show Knowles contributed to the collection, donation, and development of accessions 1259 times. Other major contributors were Li Dajue, Beijing Botanical Garden (332 records), Dave Rubis, University of Arizona (139 records), and Lee Urie (USDA-ARS (71 records).

An improved regeneration system at the WRPIS was implemented in the mid 1990's using screen cages for isolation and more than 120 plants per regeneration population. This system is providing high quality seed without the risk of insect mediated cross pollination with other accessions. However, regeneration of wild *Carthamus* species has been hampered by the receipt of seeds with low or no viability and restrictions on growing noxious weeds such as *C. oxyacanthus*. Regeneration of wild *Carthamus* was completed in a cooperative project with José Fernández-Martínez at the Institute of Sustainable Agriculture, Cordoba, Spain in 2001. This resulted in the regeneration of 1 accession of *C. glaucus*, 2 of *C. lanatus* subsp. *turkestanicus*, and 10 of *C. oxyacanthus*.

Maintenance and Distribution As indicated above, storage conditions for the WRPIS active collection is at 4°C (24% relative humidity) with a sample also backed-up under long-term storage in the NCGPR at Fort Collins, Colorado at -18°C. In addition, a regeneration sample of original seeds, or those closest to original, is under long-term storage at the WRPIS. In this way the viability of regeneration samples is preserved longer than in the active collection so seeds with the highest quality in terms of genetic diversity and viability are available for regeneration.

Distribution records show that over the last ten years, the WRPIS has sent 11485 packets of seed from 241 seed orders to researchers worldwide. Of the total packets, 8883 or 77% were sent to scientists outside the US. The most active distribution year was in 2005 with 3381 packages distributed from 47 seeds orders. Of those, 3104 were to users outside the U.S. The large total resulted from an order for all available *C. tinctorius* accessions, 2094, from Z. Mekonnen at the Awassa Agricultural Research Center in Ethiopia. For all of 2005, distributions were made to 18 countries including 263 to Egypt (for all accessions of Egyptian origin), the core collection of 210 accessions to the International Center for Agriculture in Dry Areas (ICARDA), and 199 to the Morocco Ministry of Agriculture for their breeding program. Other seeds orders included requests for high oil content accessions, those with diverse fatty acid profiles, wild types, and accessions for genomics and winter survival research. These



records illustrate not only the volume but diversity typical of safflower seed orders to the WRPIS.

Evaluation and Enhancement The safflower research community and the WRPIS have collected a substantial data set for the NPGS collection. There are 61982 entries for the safflower collection in the GRIN database (<http://www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?108>) based on 59 descriptors. This includes evaluation data collected at the WRPIS over different years and studies, and the IPGRI descriptor list developed in 1983 (http://www.biodiversityinternational.org/publications/pubfile.asp?ID_PUB=237). Descriptors traditionally used at the WRPIS include flowering date, flower color, branching pattern, growth habit, head diameter, height, oil content, iodine number, lysine content, and spininess (see GRIN and Johnson et. al, 1993). In 1994, data on oil and meal characteristics, with data on fatty acid profiles, was added for 1,000 accessions including the safflower core collection of 210 accessions. This resulted in 9265 data points (Johnson et. al, 1999). In 1996 the safflower core collection was evaluated for plant development and morphology based on IPGRI descriptors (Johnson et. al., 2001), adding 6018 data points.

Among the Crop Science registrations are materials representing some of the most significant advances in safflower germplasm (<http://www.ars-grin.gov/cgi-bin/npgs/html/csr.pl?SAFFLOWER>). The first registration was for Nebraska 10 (PI 572428) by J. Williams in 1964. It was described as an “early maturing, high yielding variety,” developed as a single selection from 852-95 by C.E. Classen at Alliance, Nebraska, USA in 1946. Knowles (1968) registered UC-1 (PI 572434), the first safflower with a fatty acid profile similar to olive oil; that is, 78% oleic and 15% linoleic. This was essentially the reverse of traditional, high linoleic safflower. Other notable contributors include germplasm registrations for rust, verticillium, fusarium, rhizoctonia, and phytophthora root rot resistance by C.Thomas, D. Zimmer, and L. Urie. H. H. Mündel and cooperators released three early developing cultivars and 4 germplasms for the Canadian Prairie. J. Bergman and cooperators have registered 13 cultivars, the most of any contributor. These include those developed for disease resistance, high oleic acid content, high linoleic content, and for bird and livestock feed. Oil and meal evaluations by Johnson et. al (1999) lead to work by Velasco and Fernández-Martínez (2004) to register CR34 and CR-81, high alpha-tocopherol germplasm (Vitamin E). The release CR34 was derived from PI 304597 and CR81 from PI 406001.

A cooperative germplasm exchange with Li Dajue at the Beijing Botanical Garden in China in the late 1980's and early 1990's lead to the first registrations of three winter hardy safflower lines, PI 651878, 651879, and 651880 (Johnson and Li, 2008a). These were developed by overwintering PI's 543995, 544006, and 544017 identified with overwintering capability, and selecting surviving plants at Pullman, WA over two cycles of selection.

Although unsaturated vegetable oils are considered most healthful, *trans* fats resulting from partial hydrogenation of vegetable oils are widely considered detrimental to human health (Mozaffarian et. al, 2006). The partial hydrogenation makes liquid vegetable oil solid at room temperature to increase shelf life and make vegetable fats for spreads and baking. Increased saturated fatty acid content, resulting in more viscosity, could reduce or eliminate the need for hydrogenation of vegetable oils for solidification. Hamden et. al (In press, Crop Science) selected accessions based on their fatty acid profiles available in GRIN (Johnson et. al, 1999) and developed safflower oil with high saturated fatty acid for potential applications in the food industry. Line CR-50 with high palmitic acid was developed from PI 306686 and CR-13 with high stearic acid was developed from PI 198990.

Recently, molecular techniques have been used to evaluate the safflower core collection with AFLP makers (Amplified fragment length polymorphisms) (Johnson et al., 2007). This has lead to a clearer understanding of the genetic variation within and among accessions and large geographic regions. Accessions were divided into seven regions—five which roughly correspond to Vavilov's main “centers of origin” of cultivated plants (S.C. Asia, China, S.W. Asia, E. Africa, and the Mediterranean)(Valilov,1997). There were also groups from E. Europe and the Americas. Even with many shared alleles, the regions were found to represent



different safflower gene pools. Thus, untapped genetic variation for breeding programs is available both within and among accessions and regions. However, AFLP marker diversity was only weakly correlated with variation in phenotypic characteristics. This is not surprising given that random molecular marker variation is mostly associated with genetic drift and migration, whereas adaptive traits are associated more with selection.

Hamdan et. al (2008) have developed the first molecular markers closely linked to a trait in safflower. They developed SCAR (sequence characterized amplified region) markers linked to the *Li* gene controlling very high linoleic acid content and the *Ms* gene controlling nuclear male sterility in safflower. Additional work using these and other approaches, such as association mapping, are needed to enhance the utility of molecular markers to tag adaptive traits in safflower.

Discussion

Acquisition and Regeneration The WRPIS has an extensive collection of safflower (*C. tinctorius*) genetic resources and a sound regeneration program that is providing high quality seed for distribution. Many valuable accessions have been collected or donated by scientists and organizations. However, valuable germplasm from safflower breeding programs can be easily lost when programs are discontinued or breeders retire. Fortunately the WRPIS recovered key components of programs lead by Dave Rubis, Paul Knowles, H-H. Mündel. But the potential for loss of useful, improved or unique material will continue as other programs are lost or redirected.

There are still areas where new collections of safflower should be made and evaluated. Many parts of southwest and central Asia seem to be poorly represented at the WRPIS even though they are likely centers of diversity and origin (Knowles, 1989). Although Iran and Turkey appear relatively well collected with 200 and 125 accessions, respectively, there are only a few accessions from Iraq, Syria, Kazakhstan, Uzbekistan, and Tajikistan and none for Kyrgyzstan and Turkmenistan. A major need for the WRPIS collection is for expanded collection and availability of key wild *Carthamus* species, especially those that cross readily with *C. tinctorius*, but also the wider gene pool for potential use in transgenic and basic research. Wild *Carthamus* species are potentially useful for disease and insect resistance, new fatty acids combinations, molecular genetics, and taxonomic studies.

There are about 25 species of wild safflower divided by Ashri and Knowles (1960) into different sections based on chromosome number. Many of these are weedy, such as *C. oxyacanthus*, a noxious weed in the US, complicating its regeneration at the WRPIS. Species with 12 chromosome pairs tend to cross readily. These include safflower (*C. tinctorius*), *C. flavescens* = *persicus*, *C. oxyacanthus*, and *C. palaestinus*. The WRPIS has no *C. flavescens* = *persicus* available, only 40 *C. oxyacanthus*, and just one *C. palaestinus*. *C. flavescens*, from areas of Turkey, Syria, and Lebanon, is entirely self-incompatible. *C. oxyacanthus*, indigenous from northwestern India to central Iraq, is a mixture of self-incompatible and self-compatible types. *C. palaestinus*, found in the desert areas of western Iraq, Jordan, and southern Israel, is a self-compatible species. Additional details concerning crossing safflower with wild *Carthamus* species can be found in Knowles (1989).

Partnerships for collecting, regenerating, and exchanging wild species is one way that the WRPIS and other gene banks could provide wild *Carthamus* genetic resources. This approach was used to increase *C. oxyacanthus* in Spain. Self-compatibility varies, and recognizing differences in reproductive biology is critical to a regeneration protocol that produces high quality, genetically pure seed. Isolation is needed to prevent potential outcrossing and insect pollinators will be required for self incompatible types.

Maintenance and Distribution The harvest, cleaning, cold storage, and security back-up procedures at the WRPIS are a strength of our operation. As discussed above, an average of 1148 seed packets of *Carthamus* have been distributed from the WRPIS in the last 10 years, and more than three-quarters of those have been sent to scientists outside the US.



Evaluation and Enhancement For a relatively minor crop, safflower has a strong base of evaluation and enhancement data available on GRIN. The future of safflower will depend on continued germplasm supply and research. The discovery and development of high oleic safflower (low iodine values) from India and Pakistan is a remarkable example of a genetic trait that at first was of only academic interest, but later leading to widespread commercial value. Safflower germplasm with low iodine values was first discovered by Horowitz and Winter (1957) working in Australia, and independently by Qudrat-I-Khuda et al (1959) in Pakistan, and Knowles (1960) in the US. Knowles and Hill (1964) described the inheritance of fatty acids in safflower and commented that the aberrant high oleic types were “of no commercial interest.” In time this changed dramatically. Knowles (1968) registered the first high oleic cultivars, UC-1, and now high oleic safflower dominates the US market.

Winter safflower; that is, fall planted, represents a new approach that could significantly increase safflower utilization through higher potential yield, and as a rotation crop with wheat in dry areas. Johnson and Li (2008a) recently released three winter hardy safflower lines that should allow winter safflower cultivars to be developed. Johnson and Li (2008b in press, *Crop Science*) found low, prostrate habit in the fall was essential for winter survival. But they also found a large amount of variation in winter survival within the prostrate types studied. This suggested that additional prostrate accessions with even better winter survival might well be found among the 2477 safflower accessions in the WRPIS collection.

These recent innovations in safflower, including high vitamin E safflower (Velasco and Fernández-Martínez. 2004), development of new fatty acid types (Hamdan et. al, in press *Crop Sci.*), and the first mapped safflower genes (Hamdan et. al, 2008), together with germplasm historically important to the crop, show the value and utility of genebanks. Future uses of safflower may include expanded use of hybrid safflower, biofuels, and transgenics. Work at SymbioSes Genetics is using transgenic safflower to develop lower cost pharmaceuticals such as insulin. For all these and future efforts in safflower research and breeding, the WRPIS and other genebanks will play a central role by providing needed genetic resources.

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