Major achievements in safflower breeding and future challenges.

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Abstract
Arguably the most important safflower breeding achievements over the past half century have come from India and California. Breeding for disease resistance globally and medicinal uses of safflower as developed in China, warrant special attention. Gila, grown on more area than perhaps any other, needs special recognition. The recent major breakthroughs towards biotechnological uses of safflower, as well as long-time efforts at successful hybrid development, can be anticipated to result in major achievements of the future.

No other country has had as much safflower research carried out as has India; nor has there been more safflower production over millennia than in India. The All India Coordinated Research Project on Oilseeds included safflower from the early 1970's, improving productivity greatly. Under the mentorship of P.F. Knowles, the University of California at Davis, has contributed more than any other single centre towards our understanding and knowledge of genetics and inheritance of *Carthamus tinctorius* L. Resistances to major safflower diseases have been developed in safflower cultivars and germplasm in various countries: resistances to alternaria, rust, phytophthora and sclerotinia head rot. Medicinal uses of safflower, while widespread in China for centuries, may spread around the world. Released in 1958, Gila provided more global production than any other cultivar. Biotechnology of safflower is being aggressively developed, both in Canada and in India. Major advances and utilizations can be expected in this field of research and development. While major breakthroughs in hybrid safflower have been largely elusive, globally speaking, the future holds promise for such techniques.

Key words: breeding achievements - disease resistance – Gila – biotechnology - hybrid safflower

Books and chapters dealing with safflower breeding

Good safflower reference books, while now over 25 and 47 years old, include the revised chapter from his earlier 1971 work, on safflower by the 'Consultant in Tropical Agriculture' E.A. Weiss, in Victoria, Australia (Weiss 1983) and by V.M. Chavan, in his chapter on "Niger and Safflower" published by the Indian Central Oilseeds Committee in Hyderabad, Andhra Pradesh, India, 23 years earlier (Chavan 1961).

A chapter on safflower breeding, co-authored by me with Jerry Bergman is currently 'in press' as part of a volume on Oil Crop Breeding in the Springer Series "Handbook of Plant Breeding" (Mündel and Bergman 2008).

As safflower researchers, we have had a truly international opportunity to be updated by way of presentations and the printed 'Proceedings' starting with 1981 for the First International Safflower Conference in Davis, California, USA; 1989 Hyderabad, India; 1993 Beijing, China; 1997 Bari, Italy; 2001 Williston, ND, USA; 2005 Istanbul, Turkey; and now, 2008 for the 7th International Safflower conference here in Wagga Wagga, Australia.

And let us not forget to be ever-thankful for the collection by FAO and the tireless editing and production by José Fernández Martínez of the IAS, Córdoba, Spain, of the "Sesame and Safflower Newsletter," which has provided us more than two decades of always-current safflower research and development information.

Genetic improvement and interspecific-crossing
Genetic improvement in safflower has commonly aimed at improving yield, oil and other agronomic characteristics, including resistance to diseases, insects and abiotic stresses. It has relied to a great extent on exploiting the existing variability in commercial cultivars and land races, and to a limited extent also on crosses with closely related species (Ashri and Knowles 1960). As cultivated safflower may have its origins from the two related species, wild and weedy safflower, *Carthamus oxyacanthus* M.Bieb. from Afghanistan, adjoining Pakistan and northern India, and from the saffron thistle, *Carthamus lanatus* from Ethiopia (Chavan 1961), these have been extensively used in generating important germplasm and thence varieties of domestic safflower.

Genetic improvement in oil content has been rather spectacular in the US. In the mid 1970's, premiums were paid by the industry in the Great Plains to producers for delivery of a crop with higher than 34% oil. Within 30 years, this had increased by 15%, to 48-50% oil ('Nutrasaff' developed from Jerry Bergman's Montana State University safflower breeding programme): i.e. a 45% increase in oil level. Increased oil levels in the US were attained both incrementally, by the reduction of hull thickness, and by single genes, such as those associated with striped-hull, reduced-hull, and partial-hull.

India – as country
In India, where typically more safflower is grown than anywhere else in the world, safflower landraces have been cultivated over millennia. Thus it is not surprising that there are and have been more safflower researchers in India than in any other country, trying *inter alia* to improve the productivity of local safflower.

For decades, safflower research in India was in the hands of individual government centres or agricultural universities, as well as private sector research such as at Maharasthra Hybrid Seeds (Mahyco) of B.R. Barwale at Jalgaon and the Nimbkar Agricultural Research Institute (NARI) at Phaltan in Maharashtra State. Using mass-selection at Niphad, in the former Bombay State, the first commercial safflower, N-630 was released in 1942. At Annigeri, in 1969, A-1 was released following a pedigree breeding method.

Country-wide coordination of safflower breeding / testing
The All India Coordinated Research Project on Oilseeds (AICORPO), initiated in the late 1960's, included safflower from 1972, starting with 8 research positions, which peaked at 29 in the late 1980's, and has remained over 20 from 1980 to the present. In 1977 the Directorate of Oilseeds Research (DOR) was established and a Project Coordinator for Safflower was posted first in Sholapur and eventually in Hyderabad. Aside from developing high yielding cultivars for the diverse regions growing safflower, with regional and national uniform variety tests, in both spiny and non-spiny versions, these programs have by now developed a wide range of plant types, such as appressed, semi-compact, spreading, with a result of at least 18 high-yielding cultivars and four hybrids of regional and multi-regional importance (Sujatha 2006).

While safflower yields in the country averaged less than 200 kg/ha in the years just preceding this coordination, by the 1990's, average countrywide yields ranged from 500 to 650 kg/ha (AICRP on Safflower, 2006). Cultivar improvement must have been a major contributing factor to these increased yield levels. Nevertheless, oil content stayed quite constant, around 28 – 30%, with only an occasional cultivar reaching to 35% oil.

Spineless yet good yielding
Realizing that safflower harvesting is done manually, to facilitate its introduction in non-traditional areas, A.R. Sawant, working at the College of Agriculture of the Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV) in Indore, Madya Pradesh, embarked on a project to breed high-yielding spineless safflower. In this endeavour, he was assisted for some years by a grant by the International Development Research Centre (IDRC) from Canada. JSI-7, the first-ever spineless cultivar in India, was released in 1990. A decade later, in 2000, the spineless NARI-6 was released by NARI, providing a dual income to farmers, as the florets can easily be collected from

non-spiny safflower after the crop matures and is thus sold for food and textile dye.

P.F. Knowles – and his research group at the University of California, Davis

Decades of studies at UC-Davis, by international graduate students as well as local technicians, under the mentorship of Paulden Knowles, originally from Canada, spread safflower knowledge worldwide to many countries! Together with his students, Knowles worked out the cytotaxonomic relationships among not only most of the safflower species but also between these species and their wild relatives (e.g. Ashri and Knowles 1960). His interests ranged from the breeding, genetics, cytogenetics, to the germplasm conservation of safflower.

UC-1 and the start of high oleic safflower:

Over the past dozen years, high oleic acid safflower has been favoured by nutritionists and for frying, hence by the commercial trade. Knowles had interest in the genetic modification of the fatty acid composition of vegetable oils. One of his safflower introductions, carried the gene *ol*, which modifies safflower oil from a high linoleic to a high oleic type (Hill and Knowles 1968). As ascertained by Knowles' research group 40 years ago, three alleles at one locus were found to govern the levels of linoleic and oleic acid, with the intermediate levels being temperature sensitive. Starting with the release of UC-1, the first commercial safflower cultivar with high oleic acid (Knowles 1968), high oleic cultivars, at first quite slowly, in the past 15 years rather quickly, were developed around the world: e.g. S-317 from SeedTech in California; Rinconada in Spain.

Germplasm collection around the world

The collections of cultivated, wild and weedy relatives of safflower from around the world were the product of plant exploration expeditions Knowles undertook in the 1950s, 1960s and 1970s. The widely-used USDA World Collection owes most of its safflower lines to Knowles' collecting efforts.

China – medicinal uses

While in most countries where safflower is used, this is for the edible oil, China has for centuries, if not millennia, used safflower for medicinal purposes. In relatively recent years, research has combined traditional Chinese medicine with modern scientific principles.

Li and Mündel (1996) provide a summary of examples of medicinal uses of safflower in China.
In China, safflower is grown almost exclusively for its flowers, which are used in the treatment of many illnesses as well as in a medicinally-useful, tonic tea. The main active ingredient in safflower medicines is safflower yellow, which is water-soluble; but alcohol extracts are used in some preparations. Safflower dilates arteries, reduces hypertension and increases blood flow and, hence, oxygenation of tissues, as well as inhibiting thrombus formation. Thus cardiovascular disease treatment is one of the main uses of safflower in China.

In many cases, safflower decoctions are used in combination with various other herbs and additional ingredients. Many clinical and laboratory studies support the use of safflower medicines for menstrual problems, cardiovascular disease and pain and swelling associated with trauma. Safflower decoctions have been used successfully in restoring fertility to almost 75% of women treated becoming pregnant, after having been infertile for up to 10 years. Also male sterility could be reduced.

Disease resistances

As reviewed by Mündel and Huang (2003) a number of the major diseases of safflower can be controlled by breeding for resistance as well as by the use of cultural practices. Breeding for resistance to alternaria, sclerotinia and rust are three examples which I will elaborate on a bit here.

Alternaria resistance

A series of cultivars was released with resistance to alternaria leaf blight (caused by the fungus *Alternaria carthami* Chowdhury). These cultivars were developed by mass-selection of resistant plants from crossing of existing cultivars in a disease nursery initiated in the early 1960's from fields naturally infested with a multitude of diseases in Sidney, Montana, USA. This produced cultivars with improved field resistance to several diseases, including leaf blight caused by *Alternaria carthami* and bacterial blight caused by *Pseudomonas syringae* van Hall. This was followed by crossing to commercial cultivars and resulted in e.g. the cultivar, Girard, which had very good resistance in the field to alternaria (Bergman *et al*. 1989).

Contributions to disease resistant cultivars and germplasm have also been made by safflower programs in countries with very modest safflower breeding programs. For example, in Australia the cultivar Sironaria, the birdseed / linoleic cultivar, was developed in a backcrossing program to Gila, selecting plants to incorporate resistance to races of *A. carthami*(Harrigan, 1987). Sironaria has become the most widely grown cultivar in Australia.

Sclerotinia field resistance
In Canada, Saffire was developed as part of my small safflower breeding program at Lethbridge, Alberta, by mass-selection from a bulk derived from selections from India, having good field resistance to head rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary (Mündel *et al.* 1985). This cultivar, though modest production at best has occurred, is almost the only safflower cultivar currently grown in Canada, is a high quality (solid white) birdseed.

Rust resistance genes
As infection with safflower rust (caused by *Puccinia carthami* Cda) at the seedling stage can cause serious yield reduction in warm soils, five improved safflower lines (PCA, PCM-1, PCM-2, PCN, and PCOy) have been developed, each carrying a different dominant gene for rust resistance (Zimmer and Urie, 1970). Rust-resistant cultivars and germplasm lines have been developed for various safflower production areas.

Germplasm screening for disease and insect resistance
Important examples of major screening of safflower germplasm against diseases and insects, as well as describing in detail thousands of lines are those from Israel and China, with important germplasm lines identified for use in breeding programs world-wide (Ashri 1971; Li, Zhou and Rao 1993; Wang Zhaomu 1993).

Gila
Working in Arizona, Dave Rubis released 'Gila', in 1958. This was an F_3-derived, thus highly heterogeneous, cultivar, which showed wide global adaptability. Gila thus became popular, indeed dominant, in a number of countries outside the USA including Mexico, Australia, Argentina, for much of the next three decades (Anon, 1959; Li and Mündel 1996). Gila was reputed to have been grown in more countries and covered more area than any other single cultivar. Susceptibility to endemic diseases, relatively low oil levels and improved yield in later-released cultivars, resulted in the gradual decline in Gila production. However, it has been incorporated in many future cultivars by crossing and backcrossing, in numerous countries (e.g. Mexico, Australia) (Harrigan 1987).

Future Challenges for Safflower Breeding:

CULTIVARS FROM PROMISING GERMPLASM: INDIA AND GLOBALLY

Screening for resistance to a number of the common safflower diseases has generally not directly resulted in resistant cultivars in India, but has provided useful resistant or moderately resistant germplasm, which thus become the building blocks of potential future cultivars (Mündel and Huang 2003).

PAUL KNOWLES and derivatives:

This 'World Collection' has resulted in valuable material and indeed provides the core of collections of safflower in many countries and even more institutions, benefiting safflower breeding and germplasm research around the world.

Medicinal uses: beyond China

While medicinal uses of safflower have dominated safflower use in China, it can be hoped that these aspects can be further studied, expanded and applied globally in the future. The use of biotechnology for pharmaceutical purposes could greatly assist such expansion.

Intergration of new biotechnologies in safflower breeding programs

Biotechnology of safflower is being aggressively developed, both in Canada and in India. Major advances and utilizations can be expected in this field of research and development.

SemBioSys
The international leader in these technologies is the Calgary, Alberta, Canada-based company, SemBioSys Genetics Inc., which has found safflower to be a very attractive host for the production of high value proteins such as pharmaceuticals and industrial enzymes by genetically transforming safflower tissue so that the proteins of interest will accumulate in the seed of the mature transgenic plant. Their system involves the genetic attachment of commercially viable target proteins to oleosin, the primary protein coating the oil-containing vesicles (oilbodies) of the seed. This attachment allows the target protein to be purified along with the oilbody fraction which floats to the surface of a ground seed/water slurry upon centrifugation.

While the protocols for regeneration have been developed satisfactorily, of the numerous cultivars and germplasm lines tested, those lending themselves the most to transformation at SemBioSys have been 'S-317', an older high-oleic cultivar developed by SeedTec in California and 'Centennial', a linoleic safflower cultivar developed by the safflower breeding program of Montana State University by Jerry Bergman and his group.

While the range of products being developed by SemBioSys are on the S-317 background, for production in Chile, newer, more disease resistant and locally adapted cultivars will be needed in the future, to facilitate this 'molecular farming' over a range of agro-ecological niches.

India
With the development of protocols for genetic transformation of safflower based on callus-mediated regeneration of seedling explants in Hyderabad (Sujatha 2007), major contributions towards the use of biotech in safflower breeding can be expected there. Marker assisted selection for numerous characters can be expanded. Specifically, items of primary importance in India, such as the development of transgenic cultivars with resistance to aphids and herbicides, as well as of global concern, cultivars with tocopherol content with improvements in both antioxidant activity and vitamin E content, can be expected.

In the future, it can be hoped, that the various corporate and government endeavours in safflower biotechnology become fully integrated with safflower breeding programs to provide significant genetic information through increased use of molecular markers; and to provide a wide range of safflower lines / cultivars for improvement in agronomic characters as well as, and specially also for the production of a multitude of specific pharmaceuticals, proteins, specialty enzymes, specialty oils. While specific specialty safflower cultivars may not require significant production areas to cover global needs, as more and more of these come 'on-stream', the total safflower area can be expected to provide significant income to farmers, processors, manufacturers and marketers of the range of derived products.

Hybrid Breeding
While major breakthroughs in hybrid safflower have been largely elusive, globally speaking, the

future holds promise for such techniques.

U.S.A.

A.Barney Hill, working for Cargill at the time, initiated a program to produce safflower hybrids in 1972. This system for the development of cytoplasmic male sterile (CMS) lines relies on the use of the wild safflower, *C. oxyacanthus* as female and the domestic safflower, *C.tinctorius* as restorers of CMS and as recurrent males. After various partnerships, now his Safftech Hybrid Safflower is linked with the Montana-based Safflower Technologies International, with Jerry Bergman and group, in the production of birdseed safflower hybrids. And with 2008 developments progressing, it is expected that this partnership will produce high oil hybrids (well above 40%) which can be globally commercialized over the next few years, though this system is not available to the public.

India

High heterosis for seed yield and oil have been reported for safflower, and both dominant and recessive genetic (GMS) as well as cytoplasmic male sterility (CMS) systems have been developed in India, with numerous hybrids released over the past decade, including all three systems (Sujatha 2007).

However problems have beset these and the development of a cytoplasmic-genetic male sterility system (CGMS): manual roguing the 50% male-fertile plants appearing in the female parent of the GMS system is very time consuming, thus costly; while roguing of non-spiny genetic male sterile lines is economically viable, in the case of the more-common spiny genetic male sterile lines, roguing of MF plants is cumbersome and tedious and hence is commercially not feasible; the CGMS system is hindered due to the unavailability of suitable male sterility maintainer genotypes.

A Maharasthra private seed company, MAHYCO, has not only developed the male sterility maintainer genotype for the sterile cytoplasm but has developed and is commercializing the first CMS-based safflower hybrid in India, MRSA-521, expressing high wilt resistance. It is expected that hybrid seed will be made available much more cheaply to producers as compared to the seed of GMS-based hybrids in safflower. This should lead to the rapid expansion of hybrid safflower area in India, beyond the 5% of total safflower grown reported in recent years.
If biotechnological tools, such as genetic engineering of incorporation of a mitochondrial male-sterility causing factor of sunflower into safflower, can be perfected, safflower hybrids can be more readily produced.

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