Summary of Safflower Research carried out at the Nimbkar Agricultural Research Institute (NARI), Phaltan

Indian Council of Agricultural Research (ICAR), New Delhi sponsored safflower research at the Nimbkar Agricultural Research Institute (NARI), Phaltan since 1974 first in the form of an integrated scheme for safflower improvement (1974-1979) and subsequently as an All India Coordinated Research Project on Oilseeds (AICRPO) (1980-till date). ICAR also sanctioned different AP-Cess funded projects on varied aspects of safflower. The safflower research carried out at NARI is summarized below under the following heads:

1. Varietal development
2. Hybrid development
3. Studies on existence of apomixis in safflower
4. Development of promising ideotypes in safflower
5. Development of high oleic safflower
6. Rapid ploidy determination using leaf tissues in safflower
7. Studies with safflower flowers
8. Uses of safflower other than as an oilseed

1. **Varietal development**:

AICRP (Safflower) at NARI, Phaltan has made significant contribution in development of high yielding, high oil-containing and wilt-resistant varieties of spiny and non-spiny nature in safflower. The details of the varieties developed are listed below:

Variety	Year of release	Spiny Non-spiny	Breeding method	Average		Recommended area and production conditions
				Seed yield (Kg/ha)	Oil content (%)	
Nira	1986	Spiny	Pedigree	1554	32.5	Maharashtra, irrigated
NARI-6	2001	Non-spiny	Pedigree	1024	35	All India, rainfed
NARI-38	2007	Spiny	Pedigree	2038	31	All India, irrigated
NARI-57	2015	Spiny	Pedigree	1519	37	All India, irrigated
NARI-96	2018	Spiny	Pedigree	2023	33.2	All India, irrigated

High oil safflower variety NARI-57

Safflower variety NARI-96

2. **Hybrid development**:

Hybrid development in safflower was pioneered at NARI in 1979 by producing hybrid seed using gamma ray-irradiated seed as female parent combined with pollinating activity of bees. Multilocation testing revealed superiority of the hybrid over elite lines under adverse conditions. This study became the basis for hybrid development in safflower in India. Subsequently, in 2001 NARI released the first non-spiny hybrid NARI-NH-1 based on genetic male sterility for commercial production in India. This hybrid in true sense established the feasibility of growing non-spiny safflower in India. NARI also released a spiny hybrid NARI-H-15 in 2006. It was also based on a non-spiny genetic male sterile line to enable commercial production of hybrid seed since roguing of fully grown spiny male

fertile plants from a spiny genetic male sterile line during flowering would have been difficult. NARI has also the credit of releasing the first thermosensitive genetic male sterility-based hybrid NARI-H-23 in 2014. The details of the hybrids released are as below:

Hybrid	Year of release/ notification	Spiny/ Non-spiny	Male sterility system	Average		Recommended area and production conditions
				Seed yield (Kg/ha)	Seed oil content (%)	
NARI-NH-1	2002	Non-spiny	GMS	1936	35	All India, irrigated
NARI-H-15	2006	Spiny	GMS	2201	31	All India, irrigated
NARI-H-23	2014	Spiny	TGMS	1711	35	All India, irrigated

Non-spiny hybrid NARI-NH-1

TGMS-based safflower hybrid NARI- H-23

(i) **Development of genetic male sterility systems in safflower**: Gamma-irradiated seed could not be used for commercial scale hybrid seed production due to many difficulties associated with it. Similarly exotic genetic male sterile lines UC-148 and UC-149 procured from USA in 1984-85 could not be successfully used for hybrid seed production due to inherent shortcomings which existed in them. Therefore, it was decided to search for new sources of male sterility in safflower. This resulted in identification of two sources of genetic male sterility designated as MSN and MSV respectively. Male sterile lines of both spiny and

non-spiny nature giving high seed yield and having desirable traits were developed from these sources. Male sterility in both these sources was controlled by single recessive genes. The non-spiny hybrid NARI-NH-1 and spiny hybrid NARI-H-15 were developed from the non-spiny genetic male sterile lines developed from each of these sources of male sterility.

(ii) **Development of dwarf male sterility associated with male sterility marker trait in safflower**: Six dwarf plants of 30 cm height were identified in the 100 cm tall genotype BLY 1035 during 1992-93. The dwarf plants were observed to be male sterile during flowering of the crop. Their pollination with tall plants of different genotypes gave tall and fertile plants in F_1. The inheritance of male sterility and dwarfness was observed to be monogenic recessive. The genes expressing both male sterility and dwarfness were found to be tightly linked in coupling phase. This makes it easy to identify male sterile and fertile plants at 40-45 days after sowing (DAS) as the male sterile plants remain dwarfs of 5-10 cm, but fertile plants attain a height of 15-20 cm. Thus the male fertile plants can be rogued out at 40-45 DAS leaving a pure stand of male sterile plants in the seed production plot. The dwarf male sterility-based hybrid showed a standard heterosis of 15-20%.

(iii) **Development of cytoplasmic male sterility in safflower**: Cytoplasmic male sterility was developed at NARI following: (1) interspecific crossing and (2) induced mutagenesis with streptomycin.

The work on development of cytoplasmic male sterility using interspecific crossing was initiated in 1996-97 and cytoplasmic male sterile plants were identified in F_3 generation of a cross between *C. palaestinus* and *C. glaucus* during 1997-98. Male sterility maintainer and male fertility restorer genotypes were also identified for the male sterile cytoplasm so identified. However cytoplasmic male sterility appears to be temperature-sensitive as the expression of male sterility was excellent at the locations like Indore in Madhya Pradesh and Mauranipur, Jhansi in Uttar Pradesh which have cooler winters than at Phaltan in western Maharashtra.

In order to have diverse sources of cytoplasmic male sterility in safflower a programme to induce cytoplasmic male sterility through mutagenesis with streptomycin was initiated during 2001-02. The seeds of safflower genotype NARI-2 were subjected to streptomycin dosages of 50, 500, 1000 and 2000

mg/l of water for 40 hours. The treatment of 50 mg/l of water to safflower seed gave cytoplasmic male sterile plants. The male fertility restorer genotypes for the male sterile cytoplasm were also identified however male sterility maintainer genotypes identified showed variable expression in different years. Efforts are underway to identify a genotype with stable expression of male sterility across the environments.

(iv) **Development of thermosensitive genetic male sterility (TGMS) in safflower**: Development of TGMS in safflower was an outcome of efforts initiated to explore the possibility of development of cytoplasmic male sterility from the derivatives of a CMS-based hybrid of exotic origin. This exploration of hybrid derivatives during 1998-99 resulted in identification of TGMS during 2005. These express 100% male sterility during winter when average daily minimum and maximum temperatures are $<16^{o}$C and $<32^{0}$C respectively in the period from capitula formation to completion of flowering. The fertility is restored 100% when they are grown during summer with average daily minimum and maximum temperature $>21^{o}$C and $>39^{0}$C respectively in the period from capitula formation to completion of flowering. The TGMS in safflower is controlled by inhibitory genes. .NARI released the first TGMS-based hybrid NARI-H-23 for commercial production in India during 2014.

TGMS capitulum in flowering

3. **Studies on existence of apomixis in safflower**:

Embryological studies of fasciated derivatives of an interspecific cross between *C. palaestinus* and *C. tinctorius* producing twin embryos and fused multiple seeds to determine the origin of such seeds were carried out to determine the origin of such seeds. It was found that there was fusion of two to three ovaries forming uni- or bilocular structures with one to five ovules in each locule. The ovules had both

sexual and aposporic embryo sacs. The multiple embryo sacs were found to have originated from nucellar epidermal cells located inside the integumentary tapetum. The presence of both aposporic and sexual embryo sacs in the same ovule suggests the existence of facultative apomixis in safflower.

Another genotype 238-14-2 and its derivatives also expressed similar histological characters as those described above for fasciated safflower. Pre-fertilization study of the genotypes showed that mitotic division of somatic aposporous cell led to formation of multiple unreduced embryo sacs. Presence of sexual plants in the genotype confirmed a facultative type of apomixis in it. The frequency of apomixis in genotype 238-14-2 was found to be 13%.

Inheritance of twin-embryo seeds and stem fasciation in interspecific fasciated derivatives was found to be digenic recessive with inhibitory gene action for the control of both the traits. The genes controlling the two traits were found to be closely linked in coupling phase.

Capitulum of fasciated safflower

4. **Development of promising ideotypes in safflower**:

(i) **Development of short duration (SD) safflower**: Development of SD safflower was carried out for rainfed and late-sown irrigated conditions or for shallow soils with length of growing period (LGP) of 70-80 days. The SD safflower flowers in 40-50 DAS and matures in 80-90 DAS as compared to normal duration (ND) safflower which flowers in 75-90 and matures in 125-145 DAS. The assessment of SD safflower in comparison to ND safflower under different spacings and fertilizer levels in soybean-safflower

cropping system under delayed sown conditions was carried out. SD safflower out-yielded the ND one under both the spacings of 30 X 20 cm and 45 X 20 cm by 15%. Different levels of fertilizer did not have any effect on the yield of SD safflower indicating the possibility of reducing the fertilizer application to SD safflower thus further increasing the remuneration from the crop to the grower. Therefore SD safflower was highly productive under delayed sown conditions in soybean-safflower double cropping system. Similarly, SD varieties are also likely to be highly productive under soils with LGP of 70-80 days.

Short duration safflower in flowering (Right) and normal duration safflower (Left)

(ii) **Development of safflower producing only primary branches**: We have identified a spontaneous mutation resulting in production of only primary branches in safflower. Screening of these genotypes along with the regular checks having secondary and tertiary branches showed that the promising genotypes with only primary branches out-yielded the regular safflower check A-1 by as much as 60% under irrigated conditions. These genotypes in general showed greater capitulum diameter and higher number of seeds/capitulum than the check cultivars. The present findings suggest that genotypes producing only primary branches would be more productive under

rainfed conditions than the regular genotypes due to their shorter duration and greater harvest index.

Safflower producing only primary branches

(iii) **Development of 60-day duration single-headed safflower**: Single-headed safflower identified and developed in the programme is being transferred to SD background in order to develop single-headed safflower maturing in 60 days. The safflower so developed will be highly productive on poor soils and soils with LGP of 50-60 days. It is likely to fit into many cropping systems and will be amenable to intercropping with different crops in conventional and non-conventional areas of safflower cultivation.

Single-headed safflower

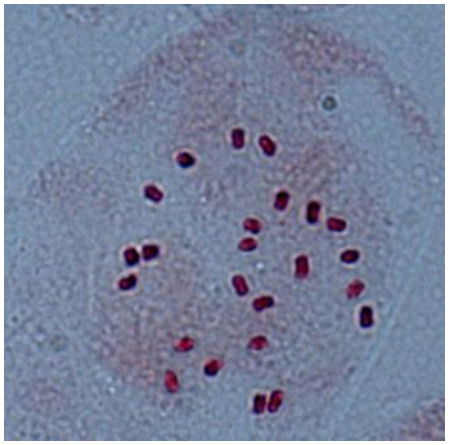
5. **Development of high oleic safflower**:

Breeding for high oleic safflower has resulted in the development of eight high yielding F_4 populations giving > 75% oleic acid in the oil. These lines are being

advanced and screened for development of high oleic cultivars giving high seed yield and oil content.

6. **Rapid ploidy determination using leaf tissue in safflower**:

Conventional method of ploidy determination using root tissues in safflower take an approximate period of 96 to 100 hours and that too without any guarantee of getting properly spread chromosomes due to their sticky nature. Our experimentation with processing leaf tissues for chromosome analysis resulted in development of a suitable technique to study chromosomes from a young leaf. This involves fixing of freshly excised pieces from a young leaf of about 1.5 cm length in a regular fixative for a period of four hours followed by 4-5 washings with water and then staining with acetoorcein for a period of 20-30 minutes. In short, the leaf processing enables chromosome analysis to be carried out in 6-7 hours. This technique with slight modifications may also be suitably used for regular karyotype investigations in safflower which in the root chromosome preparations are very lengthy and difficult due to overlapping of chromosomes at metaphase.



Safflower somatic chromosomes from leaf tissues

7. Studies with safflower flowers:

Safflower since time immemorial is known around the world for the beauty of its flowers and production of natural colours for food and fabrics from them. In addition flowers also have pharmaceutical properties to cure many chronic diseases such as hypertension, arthritis, spondylosis, coronary heart ailments etc. to name a few. With the rising global demand for safflower flowers, NARI made efforts not only to develop high-yielding non-spiny cultivars, but also to explore uses of

safflower flowers such as manufacturing food colourants and development of herbal health tea. This should help to commercialize them so that income of safflower growers can be enhanced to make the crop more profitable than the competing crops grown in the winter season. The details of the activities undertaken regarding safflower flowers are summarized below:

(i) **Study of variability of flower yield and its components in spiny and non-spiny genotypes in safflower**: At NARI 38 genotypes of spiny and non-spiny nature were screened for flower yield and its components for two years. This showed high variability for flower yield and its components. Entry 694 recorded the maximum average flower yield of 282 kg/ha. High GCV, PCV, and heritability coupled with high genetic advance (as percent of mean) were recorded for the traits such as flower yield/plant, seed yield/plant, number of primary branches/plant, number of capitula/plant, number of seeds/capitulum and 100-seed weight. These traits may be considered for selection to obtain required genetic improvement of the crop.

(ii) **Study of variability in floral traits and interrelationship among them in spiny and non-spiny genotypes**: Study of variability for floral traits in spiny and non-spiny genotypes revealed the existence of high variability for all the floral traits examined. Among the floral traits studied number of flowers/capitulum was found to be the most important trait for enhancing the flower yield in safflower. The correlation studies between flower yield and its components showed that the flower yield/plant was significantly and positively associated with number of flowers/capitulum and seed yield/plant in both years.

Non-spiny safflower capitulum

(iii) **Inheritance of flower yield and its components in safflower**: The inheritance of flower yield and its components was studied in 10-parent diallel crosses (excluding reciprocals) for two years in F_1 and one year in F_2 generations. It showed the importance of additive and non-additive gene actions in the expression of different floral traits. Parent MSN-3-8-5 was observed to be the best general combiner for days to 50% flowering, days to maturity, number of flowers/capitulum and % oil in seed in all the three generations, capitulum diameter and number of seeds/capitulum in F_1s of both the years, number of capitula/plant and flower yield/plant in F_1 of first year and in F_2 generation, petal length, anther length, stigma length, petal area/flower, seed yield/plant and oil yield/plant in F_1 of first year and number of primary branches/plant in F_2 generation. The specific cross combinations NARI-6 X GMU-4808 and MSN-3-8-5 X 126-8-2 exhibited the maximum sca effects for flower yield in all the three generations except the latter of the two crosses in F_1 of second year. Thus to exploit both additive and non-additive gene actions, hybrid vigour using genetic male sterility should be harnessed as also biparental mating in the crosses exhibiting dominant X recessive gene action should be resorted to and individual plant selections be made in the crosses showing additive gene actions.

(iv) **Heterosis and inbreeding depression for flower yield and its components in safflower**: Standard heterosis (average of two years) over newly released non-spiny safflower variety NARI-6 was worked out for all the 45 F_1s for flower yield and its components. The maximum standard heterosis of 147.46% was recorded for flower yield. The crosses which showed high heterosis for flower yield and its components also exhibited high inbreeding depression which may be attributed to non-allelic interaction of genes in the inheritance of different traits.

8. **Uses of safflower other than as an oilseed:**

(I) **Safflower flowers for colour and medicinal uses**:

(i) **Screening of safflower flowers of released cultivars for safflower yellow**: Out of the released safflower cultivars screened for safflower yellow, non-spiny safflower varieties NARI-6 and CO-1 gave the highest amounts of yellow pigment of 30.03 and 28.14% respectively. The spiny cultivars contain <10% yellow pigment in their flowers. Therefore, for commercial extraction of colour from safflower flowers, non-spiny

varieties like NARI-6 and CO-1 and hybrid NARI-NH-1 should be considered as they not only contain high amounts of pigment in their flowers but give high flower yield and their non-spiny nature makes flower collection relatively easy.

(ii) **Analysis of toxic and nutritive elements in safflower flowers:** An analysis of nutritive and toxic elements in safflower flowers of Indian cultivars was carried out at CFTRI, Mysore in order to assess the suitability of safflower flowers for human consumption. The analysis of toxic elements in the flowers of seven genotypes showed non-spiny cultivars NARI-6 and NARI-NH-1 to have Cd, As and Pb contents within the permissible limit. Flowers of non-spiny cultivars NARI-6 and NARI-NH-1 were found to be rich in protein, total sugars, calcium, iron, magnesium and potassium. Therefore, the flowers are safe for human consumption and are rich in essential components needed for good health.

(iii) **Assessment and acceptance of flower extract as food colourant**: A simple and easy to use method of colour extraction of safflower flowers was devised. The colour concentration of 4.5% was standardized and used for colouring different food items. The quantity of 4.5% concentration required for colouring different food products was: 5 ml for 250 g raw material of Jilebi, 0.5 ml for 100 g ice cream, 0.2 ml for 100 g of shrikhand, 8 ml for 100 g cake, 5 ml for 1200 g of burfi and 3 ml for 1200 g pedha.

(iv) **Development of safflower herbal tea**: After testing different combinations, safflower flower powder (0.3 g) + lemongrass (0.1 g) + cardamom (0.08 g) in 100 ml of water was found to give excellent taste, aroma and colour. From the research carried out mainly in China extract of safflower flowers can be used to cure various chronic disease like hypertension, arthritis, spondylosis, coronary artery diseases and sterility in men and women. This should help to popularize safflower flowers for human consumption and in commercializing safflower flowers to give additional income to the farmers.

Safflower petal packets

(v) **Studies on eco-friendliness of safflower colour**: The eco-friendliness test of safflower colour for toxic elements and pesticide residues was carried out at IIT, Kanpur. It revealed that the amounts of Cu, Co and Cr each were 0.01 ppm and Zn and Cd were 0.02 ppm each. Ni amount in dye was 0.9 ppm however no traces of Pb, As and Hg were found in the dye. The colour was also found to be free from the pesticide residues of BHC, DDT, Methyl parathion, Endosulfan, Malathion, DDE, DDD, 2,4-D, 2,4,5-T, Aldrin, Dieldrin, 12 Ethion, Dimethoate, Formaldehyde and all the 22 banned Amines. Thus the colour was found to be safe and eco-friendly in nature.

(vi) **Efficacy and safety of safflower herbal tea when given as an add-on therapy in patients suffering from mild hypertension**: A clinical trial to study efficacy and safety of safflower tea in controlling hypertension was carried out in association with T. N. Medical College and BYL Nair Charitable Hospital, Mumbai. The results of the clinical study revealed that the addition of safflower herbal tea to the ongoing anti-hypertension monotherapy, successfully reduced the blood pressure in patients of mild hypertension. It was noted that in safflower-treated group, the percentage decrease in blood pressure was more between day 0 and day 15 as compared to that between day 15 and day 30. However, safflower tea did not show significant effect on lipid profile parameters.

(vii) **Development of safflower petal collector**: A knapsack-type simple, suction mechanism-based battery-operated petal collector was developed for flower collection from a spiny crop of safflower. The testing of battery-operated petal collector showed that on an average a person could collect 400-500 g of flowers/day (6 hours/day) which was nearly twice as much as that collected by hand from a spiny crop of safflower.

Battery-operated safflower petal collector

(II) Young **safflower as a leafy vegetable**:

Safflower plants at an early stage of growth are used as potherb and salad locally in and around the area of its production in India. Safflower leaves are a rich source of fiber, minerals, vitamins and antioxidants. Therefore, in order to popularize safflower as a leafy vegetable it is crucial to create awareness on diet-related health benefits from this crop. Promotion of safflower as a leafy vegetable will benefit the consumers in securing nutrition and will also help safflower growers to enhance their income significantly. The aim of this study was to assess the potential of safflower cultivars/genotypes for fresh vegetable yield, nutritional qualities and monetary returns in different seasons.

In order to generate the desired information 15 safflower cultivars/genotypes were assessed for their fresh vegetable yield and quality parameters of safflower leaves under winter, summer and monsoon

conditions during 2014-15. The results showed that the average fresh vegetable yield of safflower at 30-35 days after sowing (DAS) was the maximum (7008 kg/ha) in monsoon 2015- grown crop followed by the crops grown in winter 2014-15 (3960 kg/ha) and summer 2015 (3313 kg/ha). This indicated that apart from regular winter, safflower as a vegetable crop can also be produced in both summer and monsoon seasons, thus making it possible to have a round the year supply to the consumers. The nutritive analysis of safflower leaves in comparison to fenugreek and spinach (the two popular leafy vegetables in the market) showed that safflower leaves are as nutritious as fenugreek and spinach.

The high returns in a short period of 30 days coupled with its high nutritional quality as a leafy vegetable should be publicized so that farmers can get high returns in a short time period. This will enhance the requirement for safflower as a vegetable which can be easily fulfilled from the regular safflower grown as an oilseed during winter season as the excess plants are removed at 30-35 days after sowing. The removed plants can be marketed as a leafy vegetable to obtain additional income instead of using as a fodder for animals. The lower 3-4 leaves of each plant may also be detached during the rosette stage (30-40 DAS) without any adverse effect on the productivity of the crop as an oilseed. Thus the income obtained from the sale of thinned plants and removal of the lower 3-4 leaves/plant at 30-40 DAS can meet the entire cost of cultivation of the crop in advance. This can also support all future input needs of the crop. The income likely to be generated from the seeds and flowers would be net earning in the hands of the farmer. However, in order to realize this it is important to promote safflower as a nutritious leafy vegetable among the public.

Safflower crop ready for harvest as a leafy vegetable

HOME

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