Indian J. Genet., **54** (3): 235–241 (1994)

AN ATTEMPT OF HYBRIDIZATION BETWEEN *CARTHAMUS TINCTORIUS* AND *HELIANTHUS ANNUUS* AIDED BY GRAFTING

N. D. JAMBHALE

Unit for Advanced Propagation Systems, University of London (Wye College) Wye, Ashford, Kent, TN25 5AH, U.K.

(Received: November 22, 1993; accepted: January 21, 1994)

ABSTRACT

Safflower (*Carthamus tinctorius*) grafted on sunflower (*Helianthus annuus*) on pollination with sunflower pollen gave three seeds out of which one developed into a plant. Like safflower, the pollen mother cells of hybrid plant formed 12II separating regularly. It has normal pollen and seed fertility. The putative hybrid plant resembled mother plant (safflower) for most of the morphological traits except that it has a few bifurcated and less spiny leaves, slight pubescence and reddish tinge on white stem and relatively taller than either of the parent. However, none of these new traits could be transmitted in the F_2. The normal diploid safflower plant appeared in the safflower x sunflower hybrid may be due to diploidization of egg nucleus giving rise to parthenogenetic diploid embryo.

Key words: *Carthamus tinctorius, Helianthus annuus,* wide hybridization, grafting, , parthenogenetic diploidy.

Hybrids between distantly related crop species or genera have been attracting the attention of breeders to solve some breeding problems [1]. Sexual crosses between distantly related genera and species, is however, a remote possibility. There are some reports which claim successes in making conventional crosses between distantly related crop species like rice with sorghum [2–4] rice with reed, wheat and maize [5] *Tripsacum dactyoides* (2n = 36) with *Zea mays* (2n = 10) [6] and *Gossypium hirsutum* and *G. arboreum* and *G. herbaceum* [7]. In all these cases, the hybrids resembled to mother plants with some paternal characters introgressed. Origin of such counterfeit hybrids have been attributed to the exchange or transfer of certain genes between the sperm and egg genomes without involvement of actual nuclear fusion [6].

Some reports [8–12] claim that incompatible distant crosses could be made compatible by grafting the parents prior to crossing. Grafting safflower onto sunflower stock and pollinating scion safflower flowers with sunflower, normal seeds were obtained which

*Present address: Department of Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri Dist. Ahmednagar 413722.

produced 23 vigorous plants with smaller spines, less husk than normal safflower [8]. Hall [9] found that mature wheat embryos grafted to rye endosperms and pollinated with rye pollen produced five times the number of hybrids as ungrafted controls. Evans and Denward [10] grafted seven *Trifolium* species in all possible combinations and interspecific hybridizations were attempted. There existed correlation between the success of grafting and the success of fertilization. Grafting *Corchorus oilitorius* and *C. capsularis* reciprocally and using the grafted plants for crossing led to the striking increase in fruit set. However, mature seeds were shrivelled and empty [11]. Grafting young seedlings of *Cucumis metuliferus* on *C. melo* root stocks and vice versa followed by reciprocal crossing gave three crossed fruits possibly of hybrid origin [12].

The so-called wide hybrids reported were mostly identical to the mother plant with few paternal characters introduced. Such a transfer of characters between plants without adopting repeated backcrossing has great significance in plant breeding. It is necessary that this method be examined in different plants. An attempt was made to obtain graft aided hybrid between *Helianthus annuus* and *Carthamus tinctorius*, the results of which are presented in this paper.

MATERIALS AND METHODS

Safflower genetic male sterile line MS 105 (which segregate into 1 fertile : 1 sterile) and sunflower CMS 86 A, and its maintainer CMS 86 B were sown in pots filled with compost and maintained in the glass house of Wye College, University of London, U.K. during 1990. Well developed 10–15 days old seedlings were used for grafting. The stock plants were cut at 4–5 cm height and a split was formed in its centre to a depth of about a 1 cm. The scion shoot was prepared by removing cotyledonary leaves and slicing the base into a wedge shape. The scion was then inserted into the split made on the stock and bound in a position with a narrow band of nesca film. The graft was then kept in the fogging unit with relative humidity of more than 90% for 2–3 days, followed by transferring it in relative humidity of 65%, for 5–6 days, and then in the normal area of glass house. The nesca film was removed after 20–25 days of grafting. Male sterile floret of the head born on the safflower scion grafting on safflower stock were pollinated by safflower pollen collected from the pollen parent raised separately. Similarly, male sterile florets borne in a head of sunflower scion grafted on safflower stock were pollinated by safflower pattern collected from the pollen parent raised separately. The crossed seeds were harvested, dried and stored. Crossed seeds and parental seeds were sown in pots filled with compost in the greenhouse. Observations were recorded on the morphological attributes and meiotic chromosome behaviour following standard techniques.

RESULTS AND DISCUSSION

The success in the reciprocal grafting between sunflower and safflower was fairly good, 63.33% in safflower (MS 105) (scion) grafted on sunflower cms 86 B (stock) and 40% in

sunflower CMS 86 A (scion) grafted on safflower (MS 105) (Stock). Eight safflower scions grafted on sunflower stock and four grafts of sunflower on safflower survived and reached maturity (Table 1). The grafted plants were less vigorous and produced small single terminal

Table 1. Graft compatibility in between sunflower and safflower

Stock	Scion	Total grafts made	Successful grafts (%)	No. of grafts	
				survived up to flowering	with male sterile scion
Sunflower CMS 86 B	Safflower MS 105	30	63.33	8	3
Safflower MS 105	Sunflower CMS 86 A	20	40.00	4	2

heads. Ryzeeva [8] obtained a graft by cleft grafting 10-day-old safflower and a second by grafting dormant buds of 20-day-old safflower on 30-day-old sunflower plants. Moldenhawer [13] also reported successful grafts between sunflower and safflower.

The safflower scion grafted on sunflower stock exhibited poor growth and formed a small head terminally. Flowering of the grafted safflower plant was delayed by 15 days. Out of the eight grafts which reached maturity, three were male sterile and four normal male fertile. Sunflower grafted on safflower also exhibited poor growth and formed smaller heads. Out of the four successful grafts, two survived and reached maturity. Like the mother strain both the grafted plants were male sterile.

Pollination of ungrafted male sterile safflower plants with the pollen of sunflower or sunflower pollinated with safflower pollen did not form any seed. The male sterile heads of the graft on safflower stock gave two seeds on pollination with pollen of safflower. However, none germinated. The male sterile florets borne in the head of safflower graft on sunflower pollinated with sunflower pollen gave three seeds, one of which germinated

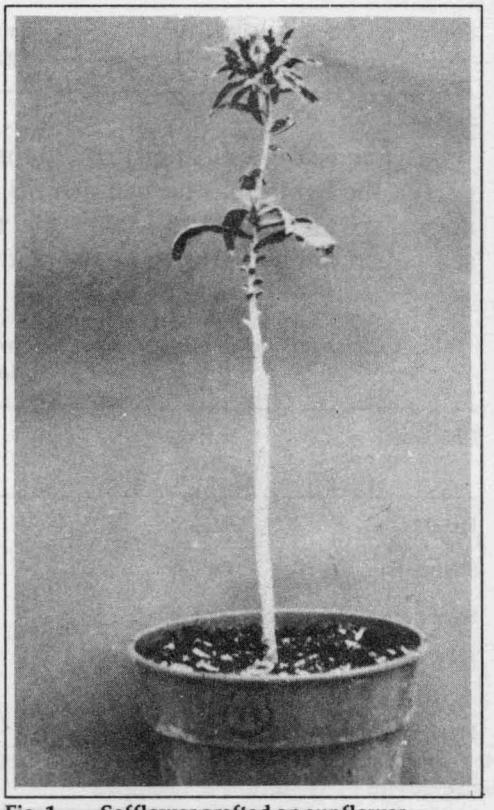

Fig. 1. Safflower grafted on sunflower.

(Table 2). The putative hybrid plant generally resembled safflower but was more vigorous than the female parent. The "hybrid' plant resembled the mother plant (safflower) for most of the morphological traits except that it has few bifurcated and less spinous leaves. Its stem was white with reddish tinge and much less pubescent than in parent genotype. It was fertile, taller than the safflower parent and almost comparable to sunflower.

Table 2. Reciprocal cross compatibility between sunflower and safflower with and without grafting

Female parent	Male parent	No. of heads pollinated	No. of seeds obtained	No. of seeds germinated
Safflower MS 105 male sterile, control	Sunflower CMS 86 B	10	0	—
Safflower MS 105 male sterile grafted on sunflower	Sunflower CMS 86 B	3	3	1
Sunflower CMS 86 A male sterile, control	Safflower MS 105	10	0	—
Sunflower CMS 86 A male sterile, grafted on safflower	Safflower MS 105	4	2	0

Cytological examination of the anthers on the hybrid plant revealed that like safflower parent, the hybrid has normal meiosis forming 12 II, which separated regularly. It had normal seed fertility. A possibility that the hybrid plant arising from pollination by stray self pollen can not be valid as the plant was distinct from the female plant and that no

Table 3. Characteristics of probable hybrid between safflower and sunflower obtained by means of grafting prior to pollination

Character	Safflower MS 105	Graft aided safflower x sunflower hybrid	Sunflower CMS 86
Height (cm)	80	95	85
Stem colour	White	White with reddish tinge	Pale green
Stem pubescence	Glabrous	Mildly pubescent	Hairy
Leaf	Entire spinulose	Entire, spinulose and serrate, bifurcated leaves	Entire, coarsely and irregularly toothed, pointed at apex
Flower colour	Creamy, white	Creamy, white	Yellow
Seed colour	White	White	Black

safflower pollen source was available in the greenhouse and that female plant was completely male sterile.

The F_2 generation comprising 23 plants raised from the selfed seeds of the hybrid plant were all alike and resembled safflower mother plant for all the characters. The unusual phenotypes observed in the putative hybrid are thus not heritable. The hybrid plant thus, neighter was a true hybrid nor it has introgressed characters of male sunflower parent Ryzeeva [8] observed small spines and less seed husk in graft aided safflower x sunflower hybrid. However, there is no report on the expression of these characters in subsequent generations.

In rice x sorghum hybrids, Chinese workers have observed bigger spikes, bolder grain, higher yield and better drought tolerance, than in the maternal rice parent [2, 3]. It was shown that the sorghum pollen tubes do penetrate the rice ovaries. However, due to

Fig. 2. a) Sunflower; b) putative hybrid of safflower x sunflower; c) safflower.

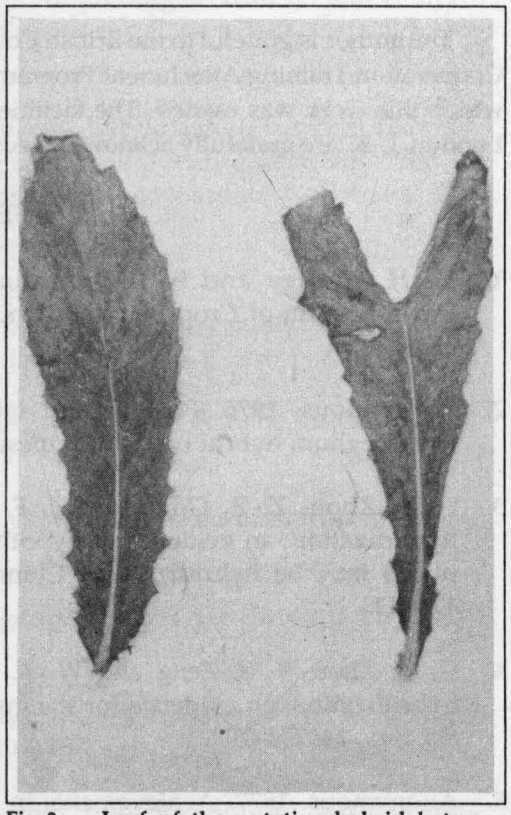

Fig. 3. Leaf of the putative hybrid between safflower x sunflower (left) compared with the normal safflower leaf (right).

incompatibility, the chromosomes of pollen are destroyed and DNA segments of sorghum integrate with the resident DNA of rice in some way and cause phenotypic alterations [3, 4]. Gene transfer from one plant to another without nuclear fusion has been reported using heavily irradiated pollen. This unorthodox genetic phenomenon was termed as egg transformation [14–18]. The irradiated pollen stimulated diploidization of egg nucleus. Subsequently the normal cell cycle resume thereby giving rise to parthenogenetic diploid embryo [16]. In the present investigation germination of sunflower pollen on safflower stigma, the pollen tube reaching the ovary and parthogenetic diploid development of embryo might have been facilitated by the safflower scion growing on sunflower root stock.

The forced hybridization between safflower x sunflower effected with the aid of grafting did not achieve genetic transfer.

ACKNOWLEDGEMENTS

The author is grateful to the British Council, London for the award of British Technical Cooperation Training Attachment Programme in Plant Biotechnology during the tenure of which this work was carried. The facilities provided by the Wye College, University of London, U.K. are gratefully acknowledged.

REFERENCES

1. H. H. Hadley and S. J. Openshaw. 1980. Interspecific hybridization. *In*: Hybridization of Crop Plants. Amer. Soc. Agron. Crop Sci. Soc. Amer., Madison: 133–159.

2. Anonymous. 1976. Practice and understanding of selection and breeding on rice–sorghum hybrid type (in Chinese). Acta Bot. Sin., **18**: 334–339.

3. G. Y. Zhou, Z. Z. Gong and Z. F. Wang. 1979. A molecular basis of remote hybridization: an evidence of hypothesis that DNA segments of distantly related plants may be hybridized (in Chinese, English summary). Acta Genet. Sin., **6**: 414–420.

4. G. Y. Zhou, Y. S. Zeng and W. X. Yang. 1981. The molecular basis of remote hybridization: an evidence for the integration of sorghum DNA into rice genome. Sci. Sin., **24**: 701–709.

5. Anonymous. 1977. Achievements in the hybridization between rice and reed (in Chinese). Acta Bot Sin., **19**: 88–89.

6. J. M. I. DeWet, Newell and D. F. Brink. 1984. Counterfeit hybrids between *Tripsacum* and *Zea*. Amer. J. Bot., **71**: 245–251.

7. D. V. Ter-Avanesian. 1977. Artificial alien pollination as a model of natural introgressive hybridization on the example of cotton. Indian J. Genet., **37**: 425–432.

8. O. I. Ryzeeva. 1952. Vegetative hybridization between safflower and sunflower (in Russian). Breed. Seed Grow., **5**: 77–8.

9. O. L. Hall. 1954. Hybridization of wheat and rye after embryo transplantation. Hereditas, **40**: 453–458.

10. A. M. Evans and T. Denward. 1955. Grafting and hybridization experiments in the genera *Trifolium*. Nature, **175**: 687–688.

11. K. Sulbha and M. S. Swaminathan. 1959. Effect of grafting on fruit set and embryo development in crosses between *Corchorus olitorius* and *C. capsularis*. Curr. Sci., **28**: 460–461.

12. N. E. Fanourakis. 1988. Possibility of interspecific hybridization between *Cucumis metuliferus* and *Cucumis melo* by reciprocal grafting. *In*: Cucurbitaceae 88th Proc. of Eucarpia meeting on Cucurbit Genetics and Breeding, Institute National de La Research Agronomique: 181–186.

13. K. L. Moldenhawer, Martynowska, H. Pawlowska and Z. Ruskowna. 1952. Studies on seed progenies of vegetative hybrids between sunflower and safflower or *Xanthium strumarium*. Roczn Nauk. Rol., **60**: 119–30.

14. K. K. Pandey. 1975. Sexual transfer of specific gene without gametic fusion. Nature, **256**: 310–313.

15. K. K. Pandey. 1978. Gametic gene transfer in *Nicotiana* by means of irradiated pollen. Genetics, **49**: 53–69.

16. K. K. Pandey. 1980a. Further evidence for egg transformation in *Nicotiana*. Heredity, **45**: 15–29.

17. K. K. Pandey. 1980b. Parthenogenetic diploidy and egg transformation induced by irradiated pollen in *Nicotiana*, N. Z. J. Bot., 203–207.

18. K. K. Pandey and M. Phung. 1982. Herting effect in plants: induced parthogenesis through the use of irradiated pollen. Theor. Appl. Genet., **62**: 295–300.