Indian J. Genet. Plant Breed., (2025); 85(1): 141-147
https://doi.org/10.31742/ISGPB.85.1.16
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Exploiting heterotic behaviour for oil yield and fatty acid content in safflower (*Carthamus tinctorious* L.) in India

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Abstract

Safflower is considered an important oilseed crop in the world. The oil mainly contains unsaturated fatty acids like linoleic acid and oleic acid. Fatty acid profiling of F_2 seeds in all the crosses was found to be good along with a good amount of oil content. The highest oil content was found for the genotype EC-755664. The highest linoleic acid was found in the cross, EC 755673 X GMU 2830 followed by GMU 6854 X GMU 1217, while crosses EC 755664 X GMU 2830 and EC 755664 X GMU 1217 had a moderate average content of both linoleic acid and oleic acid. Better content of oil and fatty acid composition was observed in the crosses as compared to their parents. The percentage of linoleic and oleic acid content in F_2 seeds was good along with a high percentage of oil content. The present findings may be very useful in breeding programs on the production of hybrids with high oil and good fatty acid content and can be applied in related crop improvement work.

Keywords: Safflower, hybridization, fatty acid, oil content

Introduction

Safflower (*Carthamus tinctorious* L.) is an important oilseed crop in India and the World. It belongs to the family, Compositae, and bears a chromosome number of 2n = 24. The crop is usually self-pollinated but cross-pollination was also reported to the level of 5 to 15%. For countries like India, where the daily food product consumes lots of vegetable oil, usage and/or consumption of better oil are very much necessary. Nowadays, people are more prone to diseases like heart and blood pressure. Consuming good food and a healthy lifestyle has always been the best option to stay fit all the time. Safflower is one such crop packed with a good content of fatty acid in its oil. Linoleic acid (C18:2) is the main fatty acid (~77%) present in its oil. Besides this, the oil also has fractions of oleic acid (~11%), palmitic acid (~6%), and stearic acid (~3%) (Applewhite 1966). Continuous cultivation of the crop mainly for oil (Dajue and Mundel 1996) and flower for use as a flavoring agent and food coloring additives has been practiced for vegetable oil and textile dye production, respectively (Weiss 2000).

Safflower oil, having good fractions of linoleic acid, is used as premium edible oil, due to its reported role in reducing blood cholesterol levels, while safflower oil with a good fraction of oleic acid is used for frying purposes because of its bland flavor and stability (Smith 1993). Linoleic acid is polyunsaturated fatty acid (PUFA) while oleic acid is monounsaturated fatty acid. In spite of being good for human consumption, PUFA is prone to oxidation causing short shelf life and poor stability (Arab-Tehrany et al. 2012). The hydrogenation process of oil was done to increase the storage stability of safflower oil but during this process, trans-fat formation occurred which is not good for health. In this regard, the oil with high oleic acid content is more stable and has more extended shelf life makes the crop attractive for food industries. The leaves of the plant also contain a good amount of vitamin C, riboflavin and carotene and the young seedlings are being used as leafy

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How to cite this article: Devi Y. L., Srivastava R. and Mondal S. 2025. Exploiting heterotic behavior for oil yield and fatty acid content in safflower (*Carthamus tinctorious* L.) in India. Indian J. Genet. Plant Breed., **85**(1): 141-147.

Source of support: UGC, Ministry of Social Justice and Empowerment, India

Conflict of interest: None

Received: July 2024 **Revised:** Nov. 2024 **Accepted:** Dec. 2024

vegetables in some parts of India (Singh 2007; Asqarpanah and Kazemivash 2013). Even with good quality oil content, the exploitation of the crop is still very less due to a lack of knowledge of management of the crop and improvement in its productivity (Singh 2007). So the crop remained a neglected crop due to the presence of spines, lesser oil content and vulnerability to a number of diseases and pests (Sujatha 2008). Safflower contains a variety of fatty acids in its oil, but not all the fatty acids present are healthy. Unsaturated fatty acids are said to be healthy to consume. So, the present study is conducted by selecting genotypes having a higher content of linoleic acid and oleic acid. With the help of the crossing program, the study aimed to know the heterotic potential of crosses having a high content of oil as well as fatty acids i.e., linoleic acid and oleic acid. Also, the plant was crossed back with both the parents to know the breeding effect of oil content and fatty acid content. Therefore, the present study was aimed at the identification of the heterotic potential of parents and the crosses having a high content of oil as well as fatty acids (linoleic acid and oleic acid). Also, the hybrids were backcrossed to parents for generating BC_1 and BC_2 to find out the inbreeding effect on oil content and its fatty acid content for safflower improvement.

Materials and methods

Plant materials

A total of 6 parents were used to create 6 F_1s (hybrid combination) in the *rabi* season of 2018-2019 and a total of 6 BC_1 and BC_2 populations were also created in the next *rabi* season of 2019-2020. The F_1 plants were also grown and harvested as F_2 seeds of a single plant. Thus, hybrid cross combinations of six generations were created and grown in replicated trials in the *rabi* season of 2020-2021 using randomized block design, except for F_2 seeds maintaining a spacing of 45 × 20 cm. The list of the genotypes is given in Tables 1 and 2. The seeds were harvested and subjected to oil analysis and fatty acid profiling.

Oil and fatty acid analysis

The analysis for oil content was performed through the soxhlet apparatus using petroleum ether as solvent. The fatty acid composition of safflower oil was determined using gas-liquid chromatography of fatty acid methyl esters (FAMEs). The oil (20–25 mg) was treated with 0.5 N sodium methoxide solution (4 mL) in a glass stopper flask. The content was heated to 50℃ for 1-hour and 0.1 mL glacial acetic acid was added, the organic phase was extracted with hexane 15 to 20 mL and washed with water till neutral pH. The hexane extract was dried over anhydrous sodium sulphate and concentrated under reduced pressure to get methyl esters. After re-dissolving it in 1-mL of petroleum ether, the whole amount was added to a 1.8 mL sample vial for injection into a gas-liquid chromatography (GLC) (Model GC 2010, Shimadzu, Kyoto, Japan). GLC is equipped with an automated sampler and injector, 30 m of capillary column (RtxÒ-Wax, Restek, PA, USA). Separation of fatty acid methyl esters was carried out as per the GLC conditions as per Mondal et al. (2011). Fatty acids were identified by comparing the retention time of standard fatty acid methyl ester mixture (Supelco, Bellefonte, PA) under the same temperature condition and gas flow rate. Heterosis generally is the superiority of the hybrid over both their parents. Instead of the mean of both the parent, if the mean of the better parent is used then the term heterobeltiosis was used as suggested by Bitzer et al. (1967) to understand the better performance and improvement of heterozygote over the better parent of the cross. The test of significance of the heterosis, heterobeltiosis and standard heterosis was carried out by comparing the calculated value of 't' with the tabulated values 't' at 5 and 1% levels of significance. Inbreeding depression can be calculated by the formula.

$$ID\ (\%) = \frac{F_1 - F_2}{F_2} \times 100$$

and the significance of the inbreeding depression was tested by comparing the calculated 't' value with the table 't' value at 5 and 1% levels of significance.

Results

Safflower is an often cross-pollinated crop, so checking the purity of the hybrid is very important (Devi et al. 2022) and therefore, those genotypes that are identified true to type by molecular markers (simple sequence repeats) were used for estimation of oil and fatty acid content. The analysis of variance was performed to find out the significance of data on agronomical and quality traits Table 3. The oil content of the parental genotypes ranged from 21.43 to 34.36% in GMU-6891 and EC-755664, respectively. The highest oil content among the parents was 34.33% for the genotype EC-755664 followed by genotypes EC-755673 (33.43%), GMU-6854 (28.94%), GMU-2830 (28.29), GMU-1217 (27.12%) and GMU-1217 (27.12%) and GMU-6891 (21.30%), respectively. Yield is an important parameter for a crop. Among the parent genotypes, yield per plant was found maximum for the genotype GMU- 6854 having 72.31 g per plant followed by GMU-6891 (40.41 g), GMU-2830 (38.67 g), EC-755664 (30.76 g), EC-755673 (28.32 g) and GMU-1217, respectively.

The F_1 plants are quite robust and their yield content is quite high as compared to other cross combinations. Plants of the cross EC-755664 × GMU-2830 have the highest yield content of 225.9 g per plant followed by plants of cross GMU-6854×GMU-1217 having 150.03 g per plant. The lowest yield was observed for the cross EC-755664×GMU-1217 having 118.8 g per plant. While among the F_2 plants, highest yield

Table 1. List of genotypes used in the study and list of F_1 plants created

S. No.	Parents	Major characters	Sl. No.	F_1's (F_2 seeds)
1	GMU-1217	High oil and oleic acid content	7	EC 755673 X GMU 6891
2	GMU- 6854	High linoleic acid and lesser oil content	8	EC 755664 X GMU 2830
3	GMU-6891	High linoleic acid content and lesser oil content	9	GMU 6854 X GMU 1217
4	GMU-2830	High linoleic acid content, lesser oil content	10	EC 755673 X GMU 2830
5	EC 755673	Higher oil content , small capitulum, spreading type, more primary branch,	11	EC 755673 X GMU 1217
6	EC755664	Higher oil content Spreading branches, , small capitulum, more primary branch,	12	EC 755664 X GMU 1217

per plant was given by cross EC-755673× GMU-2830 (33.41 g/plant) followed by crosses EC-755673× GMU-6891 (31.81 g/plant), EC-755673× GMU-1217 (27.93 g/plant), EC-755664× GMU-2830 (26.47 g/plant), EC-755664× GMU-1217 (24.65 g/plant) and GMU-6854× GMU-1217 (24.39 g/plant). The highest oil content among the F_1 crosses was recorded in the cross, EC-755673×GMU-1217 having 35.28% oil content followed by cross EC-755673× GMU-2830 having 33.78% oil content. The lowest oil content was observed in the cross, GMU-6854× GMU-1217 (28.90%).

Among the F_2 seeds, cross EC-755664× GMU-2830 have highest oil content of 37.13% followed by crosses EC-755673 × GMU-2830 (33.78%), EC-755673× GMU-2830 (36.20%), EC-755673×GMU-1217 (35.52%), EC-755664× GMU-1217 (35.36%), EC-755673× GMU-6891 (34.74%) and GMU-6854×GMU-1217 (28.27%). Not much inbreeding depression was observed in the BC_1 and BC_2 plants. The yield content of different BC_1 and BC_2 is given in Table 2. The highest oil content was obtained for BC_1 [(EC-755673× GMU-1217) × EC-755673] having 35.18% while the lowest oil content was

Table 2. A list of BC_1 and BC_2 crosses used in the study

S. No.	Genotype	Generation
1.	(EC 755673 X GMU 6891) X EC 755673	(BC_1)
2.	(EC 755673 X GMU 6891) X GMU 6891	(BC_2)
3.	(EC 755664 X GMU 2830) X EC 755664	(BC_1)
4.	(EC 755664 X GMU 2830) X GMU 2830	(BC_2)
5.	(GMU 6854 X GMU 1217) X GMU 6854	(BC_1)
6.	(GMU 6854 X GMU 1217) X GMU 1217	(BC_2)
7.	(EC 755673 X GMU 2830) X EC 755673	(BC_1)
8.	(EC 755673 X GMU 2830) X GMU 2830	(BC_2)
9.	(EC 755673 X GMU 1217) X EC 755673	(BC_1)
10.	(EC 755673 X GMU 1217) X GMU 1217	(BC_2)
11.	(EC 755664 X GMU 1217) X EC 755664	(BC_1)
12.	(EC 755664 X GMU 1217) X GMU 1217	(BC_2)

Table 3. Estimates of heterosis and inbreeding depression for seed yield/plant and % of oil content

Crosses	Estimates (%)	Seed yield/plant	Oil content (%)
EC-755673 × GMU 6891	Heterobeltiosis	397.24**	39.61**
	Inbreeding Depression	76.54**	-16.84
EC-755664× GMU-2830	Heterobeltiosis	285.13**	4.67
	Inbreeding depression	76.19**	1.06
EC 755673 X GMU 2830	Heterobeltiosis	326.53**	19.24**
	Inbreeding depression	71.49**	-7.29
EC-755673×GMU-1217	Heterobeltiosis	256.08**	30.06**
	Inbreeding depression	70.33**	0.69
EC 755664×GMU-1217	Heterobeltiosis	254.88**	18.06**
	Inbreeding depression	76.36**	-10.45
GMU-6854×GMU-1217	Heterobeltiosis	418.45**	6.55
	Inbreeding depression	83.34**	2.18

** significance at 1% probability level

contributed by cross BC_2[(EC-755664 × GMU-2830) × GMU-2830] (20.69%). The list of genotypes and their oil content is illustrated in Fig. 1. There was an increase in the content of oil content in the F_2 seeds as compared to oil content in the F_1 generation. Some of the plants of F_1 have higher oil content than the parents and some have low oil content than their parents. The performance of F_1 is good in terms of yield for all the genotypes.

The fractions of fatty acid content in the genotypes were linoleic acid, oleic acid, palmitic acid, stearic acid, arachidic acid, eichosanoic acid and behenic acid. The linoleic acid content of all the genotypes ranged from 14.92 (EC-7556673) to 80.38% (F_2: EC-755673 × GMU 1217). The oleic acid content of the genotypes ranged from 12.98% (EC-755673 × GMU-2830) to 78.41 in EC-755673. Other fatty acids like palmitic acid (16:0), stearic acid (18:0), arachidic acid (20:0) and behenic acid (22:0) are all saturated fatty acids that do not contain double bonds in their structure. The palmitic acid content of the genotypes ranged from 5.13 in genotype EC-755664 to 7.12% in genotype [BC_1 (EC-755664 × GMU-2830) × EC-755664]. Understanding the fractions of fatty acid content of the oil is necessary. It was found that genotype GMU-2830 had having highest linoleic acid content of 78.66% while the highest oleic acid content was found for the genotype EC- 755673 having 78.41% among the parents. The list of the genotypes along with their oil content and fatty acid content are given in Table 4.

Among the F_1 seeds, the highest linoleic acid was found for the cross EC-755673×GMU-6891 having 71.43% followed by crosses EC-755673×GMU-2830 (68.18%), GMU-6854× GMU-1217 (51.55), EC-755664× GMU-2830 (48.70%), EC-755673×GMU-1217 (40.28%) and EC-755664 × GMU-1217 (34.11). However, the highest oleic acid was manifested by the cross EC-755664× GMU-1217 contributing 58.93% content of oleic acid. While among the F_2 seeds highest linoleic acid was obtained by the cross EC-755673× GMU-2830 (80.38%) succeeded by crosses EC-755673×GMU-1217 (78.24%), EC-755673×GMU-6891 (77.56%), GMU-6854×GMU-1217 (77.55%) and EC-755664× GMU-1217 (56.11%), respectively. The cross BC_2 (EC-755673×GMU-1217) has the highest linoleic acid content of 73.45% while cross BC_1 (EC-755673 × GMU-6891) has the highest oleic acid content of 45.19%. A comparison of different crosses with different fractions of fatty acid is given in Fig. 2.

Discussion

Linoleic acid and oleic acid are the main fatty acid composition of safflower oil. Linoleic acid also known as *Omega-6* fatty acid is considered an important component of fatty acid as it prevents the risk of heart diseases by reducing blood cholesterol levels. Oleic acid on the other hand is a monounsaturated fatty acid that contains only one double bond, also known as *Omega-9* fatty acid. Other

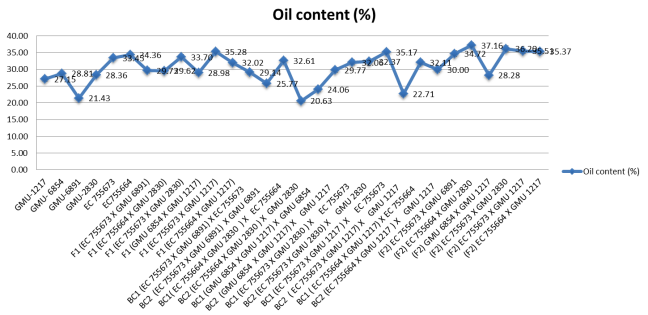

Fig. 1. Oil content in all the genotypes in Safflower

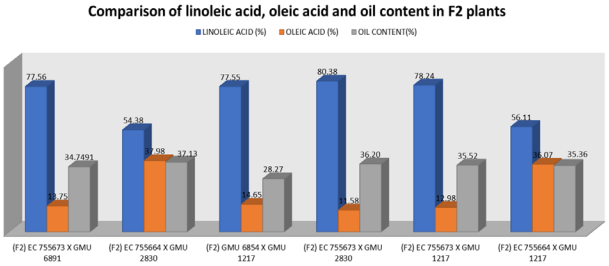

Fig. 2. Linoleic acid, Oleic acid and oil content of F_2 plants of six different crosses

than these two, other components of fatty acids like palmitic acid, stearic acid, eicosanoic acid, arachidic and behenic are also present in small quantities. Linoleic acid is 18 carbon-containing polyunsaturated fatty acids which indicates the presence of more than one double bond and is also known as *Omega-6* fatty acid. Safflower oil is considered a good source of oil and has many beneficial health effects as reported recently (Khalid et al. 2017). Through, clinical trials, a decrease in adipose tissue and body weight was observed by consumption of safflower oil (Norris et al. 2009). The oil can be used either as an oilseed crop, for industrial purposes and as biofuel by mixing it with other oils like castor oil (Thomas et al. 2012) and therefore the study on the oil and fatty acid content of safflower is very important for further research.

The comparison of linoleic acid, oleic acid, and oil content of six crosses is illustrated in Fig. 2 and it was found that the crosses EC-755673 × GMU-2830 and EC-755673 × GMU-1217 were found to have the highest amount of linoleic acid with the good content of oil percentage. Han et al. (2009) on his study found a high proportion of unsaturated fatty acids like linoleic acid used for medical purposes. In a study performed by Mihaela et al. (2013) on safflower seed, they found linoleic acid and oleic acid as the main fatty acid content of plant comprising 77.9 to 79.5% of linoleic acid and 9.5 to 11.3% of oleic acid out of total fatty acid composition of the seed. Major saturated fatty acid in safflower consists of palmitic and stearic acids of 7.2 to 8.6% and 2.0 to 2.4% respectively as reported by Ben-Moumen et al. (2015).

Table 4. Analysis of Variance of different agronomical and quality traits

Generations	Source	d.f	Characters										
			Rosette period (days)	Days to 50% flowering	Plant height (cm)	No. of primary branch/pt.	Number of capitulum/pt.	Length Of bracts on capitulum (cm)	Days to maturity	No. of seeds /capitulum	100 seed weight (g)	Seed yield /plant (g)	Oil content (%)
EC-755673 × GMU 6891	Replication	2	0.329	0.038	14.62	0.82	9.34	0.036	0.985	9.81	0.007	105.18	1.31
	Treatment	5	63.348**	142.88**	880.69**	65.72**	380.95**	0.54	33.98**	380.95**	2.682	4813.34**	73.64**
	Error	10	0.1	0.02	7.6	0.42	3.09	0.037	0.25	3.09	0.005	20.56	0.877
EC-755664 × GMU-2830	Replication	2	0.7	1.04	2.68	1.234	6.99	0.128	1.318	1.508	0.001	34.77	13.849
	Treatment	5	83.97**	98.72**	591.42**	89.52**	507.93**	1.665	78.24**	100.73**	2.567	4327.16**	99.313**
	Error	10	0.306	0.554	1.737	0.735	3.58	0.034	0.96	1.617	0.004	20.527	1.108
EC 755673 X GMU 2830	Replication	2	3.239	1.026	0.77	1.76	5.24	0.21	7.603	6.443	0.007	9.657	4.955
	Treatment	5	23.74**	93.54**	748.79**	61.88**	320.83**	1.709	60.189**	65.872**	1.191	2128.84**	86.427**
	Error	10	0.908	0.46	8.69	0.514	2.91	0.071	0.975	4.899	0.01	3.765	4.905
EC-755673 × GMU-1217	Replication	2	3.239	1.026	0.77	1.76	5.24	0.21	7.603	6.443	0.007	9.657	4.955
	Treatment	5	23.747**	93.54**	748.79**	61.88**	320.83**	1.709	60.189**	65.871**	1.191	2128.84**	86.427**
	Error	10	0.908	0.46	8.69	0.514	2.91	0.071	0.975	4.899	0.01	3.765	4.905
EC 755664 × GMU-1217	Replication	2	3.239	1.026	0.773	1.767	5.24	0.21	7.603	6.44	0.007	9.657	4.955
	Treatment	5	23.74**	93.54**	748.79**	61.885**	320.83**	1.709	60.189**	65.87**	1.191	2128.84**	86.42**
	Error	10	0.908	0.463	8.699	0.514	2.916	0.071	0.975	4.899	0.01	3.765	4.905
GMU-6854 × GMU-1217	Replication	2	2.783	0.905	16.022	1.599	3.658	0.021	1.116	10.632	0.005	1.98	4.506
	Treatment	5	105.41**	83.08**	601.21**	27.37**	323.78**	1.13	73.98**	92.704**	1.373	6673.91**	12.653**
	Error	10	0.527	0.657	4.44	0.551	1.851	0.043	0.569	3.518	0.011	4.535	3.48

** significance at 1% level, * significance at 5% level

Sabzalian et al. (2008) also found linoleic, oleic, stearic and palmitic acid as major fatty acids contributing 96-99% of total fatty acid. A small quantity of behenic, ecosenoic were also observed in different cultivars as observed by Mailer et al. (2008). Yeilaghi et al. (2012) also found safflower oil has major fatty acids of linoleic acid of 72.66 to 78.68% and oleic acid of 11.59–18.9%. Arslan and Culpan (2018) reported oleic acid content of 44.4% (from 13.97–74.74%) and 41.0% linoleic acid (from 12.21–69.83%). Camas et al. (2007) found some genotypes with high linoleic (75–80%) and low oleic acids (10–15%) while the other genotypes have low linoleic acid (12–30%) and high oleic acid (64–83%). The content of linoleic acid as the main fatty acid composition was found negatively correlated with other components of the oil with the highest being with oleic acid followed by palmitic acid as reported by Guan et al. (2008). Having a high content of oleic or linoleic acid in safflower cultivars increases the value and quality of the oil. Some reports found that there is an indirect relationship between the content of oleic acid and linoleic acid as fractions of fatty acid content in the oil as reported by Liu et al. (2016). But when they go for a correlation study, a non-significant result was found which indicates the possibility of breeding new safflower varieties having both high oil content of oleic acid and linoleic acid as the main fatty acid composition in the future. In the present study, one cross, EC-755664 × GMU-2830, showed a moderate amount of both linoleic acid and oleic acid content which opens up new research areas for improvement in safflower breeding.

The estimates of heterobeltiosis of the six different crosses for seed yield/plant and oil content (%) were found high as compared to their parents. The highest heterobeltiosis for the character oil content (%) was observed for the cross EC-755673×GMU-6891 suggesting good exploitation of heterosis for the character. Fatty acid profiling of the cross also found higher fatty acid content of linoleic acid as compared to their parents. The % of oil content for the cross EC 755664 × GMU-2830 was 4.67% which is the lowest among the crosses and the cross exhibits fatty acid profiling of medium content of linoleic acid and oleic acid. Less inbreeding depression was observed for all the crosses in terms of seed yield/plant but inbreeding depression in % of oil content was found for some of the crosses like EC-755673 × GMU-6891, EC-755673×GMU-2830 and EC 755664 × GMU-1217. Ratnaparkhi et al. (2015) found top ranking crossers showed negative heterosis for oil content while Bhima x JSI 99 showed significant positive heterosis for oleic acid PBNS 12 x NARI 34 showed significant positive standard heterosis for linoleic acid. Other than these crosses, less inbreeding depression for oil content and fatty acid composition was found in the backcross populations. It provides a platform for improving the safflower breeding program by identifying genotypes and crosses with high yield and good content of oil and fatty acid and hence, heterotic hybrids with *per se* performance for high oil content with good amount of fatty acid could be produced for commercial use utilizing cytoplasmic genetic male sterility (CGMS) system. The parents of identified highly heterotic hybrids can be converted into A and R lines to exploit heterosis in safflower.

Authors' contribution

Conceptualization of research (RS); Designing of the experiments (RS, SM, YLD); Contribution of experimental materials (RS); Execution of field/lab experiments and data collection (YLD, SM); Analysis of data and interpretation (YLD, SM); Preparation and editing of the manuscript (YLD, RS, SM).

Acknowledgment

The senior author is thankful to UGC for providing the funds for conducting research work through the NFSC fellowship under the Ministry of Social Justice and Empowerment.

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