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Y. Takagi · S. M. Rahman

Inheritance of high oleic acid content in the seed oil of soybean mutant M23

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Abstract A mutant line, M23, of soybean [*Glycine max* (L.) Merr.] was found to have two fold increases in oleic acid content in the seed oil compared with the original variety, Bay. Our objective was to determine the inheritance of the high oleic acid content in this mutant. Reciprocal crosses were made between M23 and Bay. There were no maternal and cytoplasmic effects for oleic acid content. The F_1 seeds and F_1 plants were significantly different from either parents or the midparent value, indicating partial dominance of oleic acid content in these crosses. The oleic acid content segregated in the F_2 seeds and F_2 plants in a trimodal pattern with normal, intermediate and high classes, satisfactorily fitting a 1:2:1 ratio. The seeds of a backcross between M23 and F_1 segregated into intermediate and high classes in a ratio of 1:1. These results indicated that oleic acid content was controlled by two alleles at a single locus with a partial dominant effect. Thus, the allele in M23 was designated *ol* and the genotypes of M23 and Bay were determined to be *olol* and *0l0l*, respectively. The oleic acid contents of the F_2 seeds and F_2 plants were inversely related with the linoleic acid content which segregated in a trimodal pattern with normal, intermediate and low classes in a 1:2:1 ratio. Thus, it was assumed that the low linoleic acid content in M23 was also controlled by the *ol* alleles. Because a diet with high oleic acid content reduces the content of low density lipoprotein cholesterol in blood plasma, the mutant allele, *ol*, would be useful in improving soybean cultivars for high oleic acid content.

Key words Soybean mutant · Oleic acid · Linoleic acid · Inheritance · Oil quality

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Y. Takagi (✉) · S. M. Rahman
Laboratory of Plant Breeding, Faculty of Agriculture, Saga University, Saga 840, Japan

Introduction

It is preferable to know the inheritance of a trait before making a choice of an appropriate breeding method and obtaining resources for a breeding program. It has generally been shown that the fatty acid composition of the oil in different crops is simply inherited and controlled by the genotypes of either the embryo or the maternal parent.

Thro et al. (1985) found that the fatty acid composition in the groat oil of oats was controlled by genes acting in an additive manner. The content of the oleic and linoleic acids in the oil of corn was shown to be controlled by a single major gene (Poneleit and Alexander 1965; Widstrom and Jellum 1984) or two independent genes (De la Roche et al. 1971). Knowles and Hill (1964) reported that three alleles at one locus controlled the oleic and linoleic acid contents in the oil of sunflower. Analyses of the distribution of oleic acid content in the oil of backcross and F_2 seeds of sunflower indicated that the content of this acid was controlled by a single partially dominant gene, designated *0l* (Fick 1984). Significant maternal influences were also recognized. Miller et al. (1987) reported that the oleic acid content of this crop was controlled by a major gene, *0l*, with partially dominant action, and a second gene, *ml*. When the *ml* gene is present in a homozygous condition together with the *0l* gene, the oleic acid content of the seed was elevated to 82% or higher.

Previous studies of the fatty acid composition of soybean oil indicated the inheritance of oleic , linoleic , and linolenic acid contents to be quantitative (White et al. 1961; Burton et al. 1983; Hawkins et al. 1983). Recently, however, it was claimed that the linolenic acid content was controlled by a single gene (Rahman et al. 1994 b; Wilcox and Cavins 1985), two genes (Fehr et al. 1992), or more than two genes (Howell et al. 1972). Brim et al. (1968) and Martin et al. (1983) reported that the maternal parent had a significant effect on the fatty acid composition in this crop.

The mutant M23, with a high oleic acid content in its seed oil, was obtained by X-rays (Rahman et al. 1994 a). The objective of the present study was to determine the inheritance of the high oleic acid content of this mutant.

Materials and methods

The lines used for the study were a mutant M23 and its parental variety, Bay. The former was obtained by X-rays from the latter (Rahman et al. 1994 a).

M23 was reciprocally crossed with Bay in a glasshouse (12 h day, 20–30 °C) at Saga University in 1992. The F_1 seeds from these crosses were planted in the same year in the glasshouse to produce F_2 seeds.

The parents and reciprocal F_1 and F_2 hybrids were grown in the field in 1993 to produce the seeds. Reciprocal crosses and a backcross were also made in the field. The seeds from these crosses, and those of the parents and the reciprocal F_1 and F_2 hybrids, were harvested in October 1993, all from field grown plants.

An analysis was made on both a seed and a plant basis by gas chromatography to determine the fatty acid composition, as described earlier by Takagi et al. (1989). The seed based analysis was performed using the parents, F_1, F_2 and BC_1 (M23 × F_1) generation seeds. The plant based analysis was made with the parents, F_1 and F_2 plants, in which 20 seeds from individual plants were pooled for the analysis.

Chi square tests were made to confirm the fitness of the observed segregation ratio to the hypothesized genetic ratio. A single gene model was applied for the segregation of oleic acid content in the F_2 seeds and plants, and BC_1 seeds; namely, a 1:2:1 segregation ratio for the former and a 1:1 ratio for the latter.

Results and discussion

The fatty acid compositions of oil at the seed and plant levels of M23, Bay and their reciprocal F_1s are shown in Table 1. The palmitic, stearic and linolenic acid contents were similar in both parents. The seed and plant level analyses showed that M23 and Bay were significantly different in both oleic and linoleic acid content. The oleic acid content in M23 was two fold higher than that of Bay.

The reciprocal F_1s did not differ significantly for oleic acid content at both seed and plant levels (Table 1). These results indicated that the oleic acid content was controlled by the genotype of embryo, and was not affected by the genotype of the maternal parent.

Oleic acid content in soybean oil was reported previously to be under the control of the maternal genotype (Brim et al. 1968; Martin et al. 1983). The difference between the present and previous findings may be related to the lines used as parents. The lines employed by previous workers were selected from the natural variability in soybean fatty acid composition. The present results are from an induced mutant, developed by X-rays, which may carry a major genetic modification in oleic acid synthesis compared to the original variety.

Since no significant maternal effects could be detected on oleic acid content, the data from reciprocal F_1 seeds and F_1 plants were combined in Figs. 1 and 2, respectively. The results showed partial dominance of oleic acid content (Table 1, Figs. 1 and 2). Reciprocal F_1 seeds (30.1 and 30.9%, respectively) were significantly different from both parents (22.5 and 50.4%, respectively) and also from the midparent value (36.5%). The content of F_1 seeds ranged from 28.1 to 34.3% in the combined data (Fig. 1). Similar results were also obtained from reciprocal F_1 plants (Table 1), in which the oleic acid content ranged from 27.8 to 33.4% in the combined data (Fig. 2).

No consistent differences in oleic acid content were observed between the reciprocal crosses for both the F_2 seeds and the F_2 plants. This indicates no cytoplasmic

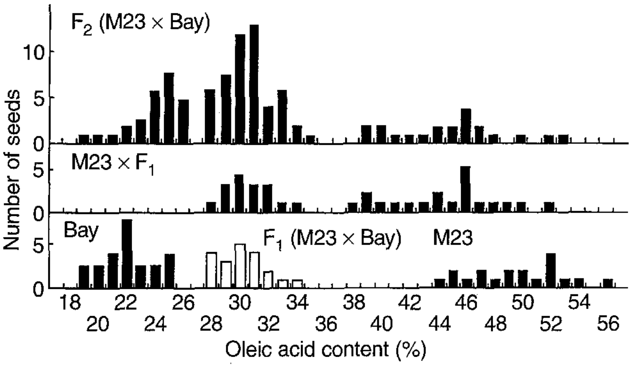

Fig. 1 Distribution of seeds having different oleic acid contents in M23, Bay, and their F_1, F_2 and BC_1 populations

Table 1 Fatty acid composition (%) of soybean oil at seed and plant level of M23, Bay, and of their reciprocal F_1s. Expressed as mean value and standard error

Level	Material	No. of seeds or plants	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Seed	Bay	20	11.4 ± 0.06	2.8 ± 0.06	22.5 ± 0.42	55.3 ± 0.31	8.0 ± 0.13
	F_1 (Bay × M23)	7	10.5 ± 0.14	2.8 ± 0.04	30.1 ± 0.46	48.4 ± 0.49	8.2 ± 0.12
	F_1 (M23 × Bay)	13	10.3 ± 0.12	2.9 ± 0.06	30.9 ± 0.51	47.9 ± 0.52	8.0 ± 0.10
	M23	20	9.3 ± 0.12	2.6 ± 0.06	50.4 ± 0.88	29.4 ± 0.77	8.3 ± 0.18
Plant	Bay	30	11.6 ± 0.06	2.9 ± 0.02	23.3 ± 0.26	54.1 ± 0.20	8.1 ± 0.06
	F_1 (Bay × M23)	10	10.3 ± 0.08	2.8 ± 0.07	30.8 ± 0.46	48.0 ± 0.38	8.1 ± 0.15
	F_1 (M23 × Bay)	20	10.6 ± 0.07	2.7 ± 0.06	29.9 ± 0.27	48.8 ± 0.24	8.0 ± 0.07
	M23	30	9.5 ± 0.04	2.3 ± 0.04	50.2 ± 0.65	30.1 ± 0.64	7.9 ± 0.07

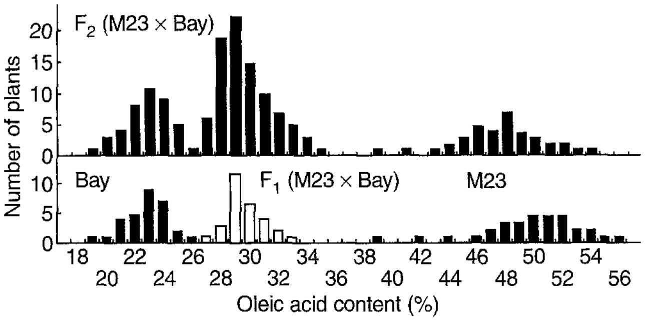

Fig. 2 Distribution of plants having different oleic acid contents in M23, Bay, and their F_1 and F_2 populations

effect on oleic acid content. For this reason the data from reciprocal crosses were combined in Figs. 1 and 2.

In both F_2 seeds and F_2 plants, oleic acid content segregated into three phenotypic classes: normal, intermediate and high. Among the F_2 seeds, it ranged from 19.6 to 26.3% for 27 seeds of the normal class, from 28.2 to 35.8% for 52 seeds of the intermediate class, and from 39.0 to 53.2% for 21 seeds of the high class (Fig. 1 and Table 2). For the F_2 plants, there was some difficulty in separating the normal class from the intermediate class. Here, the two classes were separated at the 26.0% content, which was the highest content found among the plants of Bay. Thus, the oleic acid content of F_2 plants ranged from 19.9 to 26.0% for 42 plants of the normal class, from 27.1 to 35.1% for 88 plants of the intermediate class, and from 39.9 to 54.8% for 37 plants of the high class (Fig. 2 and Table 2). Oleic acid contents were also observed in BC_1 seeds which segregated 16 intermediate class seeds (28.6 to 34.0%) and 20 high class seeds (38.3 to 53.2%) (Fig. 1 and Table 2).

The F_2 data for oleic acid content at both seed and plant levels were satisfactorily fitted to a 1:2:1 ratio for the normal, intermediate and high classes. The data of the BC_1 seeds also satisfactorily fitted a 1:1 ratio (Table 2), suggesting that two alleles at a single locus with a partial dominant effect control the oleic acid content. The high oleic acid content of M23 is due to a recessive allele, whereas the normal oleic acid content of Bay is ascribed to its dominant allele. The intermediate level of oleic acid content seems to be caused by heterozygosity for the two alleles. Thus, the mutant M23 and its original variety Bay are assigned the genotypes *olol* and *OlOl*, respectively. The present mode of gene action controlling oleic acid content in M23, Bay, and their hybrids, is the same as that reported in sunflower by Fick (1984).

Poly unsaturated fatty acids, such as linoleic and linolenic acids in soybean and some other oilseed crops, are known to be formed through a sequential desaturation pathway. Moreover, it has been shown that a mono unsaturated fatty acid, oleic acid, is the precursor for the synthesis of linoleic and linolenic acids in that pathway, and that there is a strong inverse relationship between the oleic acid and linoleic plus linolenic acid contents (Dutton and Mounts 1966; Stymne and Appelqvist 1980). Based on this fatty acid inter relationship, some success has been achieved in selecting soybean lines with high oleic acid and low linolenic acid contents (Howell et al. 1972; Hammond and Fehr 1983).

In the present study, mutant M23 had a greatly increased content of oleic acid and a reduced content of linoleic acid, with no change in linolenic acid content, as compared to those of the original variety, Bay. The same inverse relationship between oleic and linoleic acid contents was also observed in F_2 seeds and F_2 plants from their crosses. Furthermore, high oleic and low linolei acid contents in M23 were shown to be con trolled by a single gene *ol*. The same result has been reported in corn where a single major gene controls both oleic and linoleic acid contents (Poneleit and Alexander 1965; Widstrom and Jellum 1984).

The simple nature of the inheritance of the high oleic acid content in M23 will be important in respect of its use in soybean breeding for high oleic acid content. Because of the lack of maternal and cytoplasmic effects, M23 can be used as either a male or female parent in breeding. Because of the expression of the *ol* gene in the embryo, plants with a high oleic acid content can be selected using F_2 seeds, by analyzing their cotyledonary portion for oleic acid content and growing plants from the embryo containing part. Only the progenies of plants which prove to be homozygous for *ol* should be used for the selection of other characters.

An oil with a high oleic acid content is in greater demand for cooking and for salads than one with a higher percentage of polyunsaturated fatty acids because a diet incorporating high oleic acid could reduce plasma cholesterol, a risk factor for coronary heart diseases (Grundy 1986). The gene, *ol*, may be useful in filling such demand.

Table 2 Numbers of seeds or plants having different oleic acid contents in the F_2 (M23 × Bay) and BC_1 (M23 × F_1) generation

Level	Material	Oleic acid content			χ^2 (1:2:1 or 1:1)	P
		Normal	Intermediate	High		
Seed	F_2	27	52	21	0.88	> 0.70
	M23 × F_1	0	16	20	0.44	> 0.50
Plant	F_2	42	88	37	0.78	> 0.50

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