Potential of Using Recessive Macromutant Lines in Sunflower Hybridization

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Abstract

A recessive macromutant line was selected from and individual plant segregated during B line fixation. This new line, named as BO, has a peculiar morphology which was fixed and maintained by selfing. Its **rfrf** genotype was proved by crossing with cms female lines. Morphology is characterized by 1) flat, strongly fasciated stem, 2) extremely short internodes, 3) very high number of small leaves, 4) thin petioles, 5) deformed bud and inflorescence, 6) very poor pollen production. Nevertheless, line BO has a good combining ability for yield and oil content and resistance to stem diseases. With the aim to improve pollen production and introduce **Rf** gene(s), crossings were made with branching restorers and new lines were selected from their progenies. The restorers named as BBO show resistance to Sclerotinia, Phomopsis, Marcrophomina, Phoma, Alternaria and with cms females give uniform hybrids of normal monohead morphology. In the back-crosses (cms A x BBO) x BBO 50:50 % segregation of normal and mutant types show a monogenic recessive determination of morphology. Several hybrid combinations gave superior achene yield to the controls and 48-52 % oil content and had yellowish stem at maturity with almost no symptoms in our performance trials.

Key words: morphology, restorer, disease resistance, hybrids, performance

Introduction

Sunflowers are grown on about 400.000 hectares in Hungary. About 70 % of this acreage is dominated by varieties of Cereal Research Institute developed in the framework of own and a joint breeding programme with foreign companies. Result of official state trials of a great number of foreign and domestic hybrids showed different levels of performance and different reactions to diseases present in Hungary, such as Sclerotinia sclerotiorum, Phomopsis helianthi, Macrophomina phaseolina, Phoma macdonaldii and Alternaria. In rainy years, these diseases (mainly white rot and brown stem canker) cause heavy yield losses. At individual and plant population level complicated interaction with fast - evolving pathogens are realized — as a final result — in the oil content, achene and oil yield. It would be an irrealistic aim for a breeder to insist on creating a totally symptomless crop; in the practice, genotypes (lines, hybrids) with a complex, good field resistance to the main diseases are needed. Resistance to Sclerotinia is partial and polygenic (VEAR and TOURVIEILLE, 1985; TOURVIEILLE and VEAR, 1986) and no hybrids exist with absolute resistance to this parasite. Natural and official infections showed the partial and polygenic character of resistance to Phomposis as well (TOURVIEILLE and PELLETIER, 1988). Although brown stem canker (Phomopsis/Diaporthe helianthi) may chemically be controlled (REGNAULT, 1988), it poses serious problems in many countries (ESTRAGNAT, 1992). A marcromutant of peculiar morphology was obtained from segregation during selection of a B line and found to be symptomless in natural conditions during attack of stem diseases on other genotypes (FRANK, 1992). The aim of present

study was to investigate performance and reaction to diseases of hybrids obtained by crossing different females with resistant restorer forms, derived from the above mentioned mutant.

Materials and methods

In 1984, in the selfed progeny of a B line, one plant of a very peculiar morphology was found and maintained by selfing and sibbing until now. This line named 'BO' showed no disease symptoms on their stem and later on it was used as pollen donor in manual crossings with female testers with the aim to find out the presence of **Rf** gene(s). In 1986, after gibberellin treatment (50 ppm during 24 hous) 'BO' line was used as female for crossing with normal branching restorers for introduction of **Rf** gene(s): In the F_2 progenies of these crosses large number of individual plants greatly differing in height, leaf number, position, size and colour, stem thickness, number of lateral branches and heads, form of canopy, flowering date and duration, 1000-achene mass, oil content, reaction to stem diseases were segregated and maintaned by selfing. Those with the best characters were tested with females for the presence of **Rf** gene(s), then fixed by selfing and sibbing. Experimental single-cross and three-way cross hybrids were produced by manual crossings and in an isolated plot (open pollination) with females of GKI, Rustica and Dekalb. Performance trials were conducted at Szeged-Kiszombor (and in France) in randomized block design (3-replications), with spacing 70 cm between rows and 35 cm between plants (80 plants/plot). Health state of hybrids in natural conditions was evaluated by bonification on scale 1-9, where 1=worst, 9=best (with no symptoms). Artificial infections were carried out with agar disks containing mycelium of Sclerotinia on heads cut off at physiological maturity and covered with plastic bags in a cave. Evaluation of reaction to infection was made by calculating surface of 4 spots as a % of the whole surface of the head. Sclerotinia test on heads was also made by Dekalb Argentina S.A., at Bragado, Argentina. Phomopsis mycelium test was made in the middle of wounded stems at stage 4.1. Length of spots (cm) was measured 2, 4, 6, and 8 weeks after infection. Hybrids of performance trials were harvested and achene yield calculated at 10 % humidity, oil content was measured by NMR at 0 % humidity.

Results and discussion

The teratological morphology could be fixed by selfing. The original 'BO' macromutant plant is characterized by 1) flat, strongly fasciated stem with many thin ribs, 2) extremely short internodes, 3) great number of small leaves 4) very thin petioles, 5) strongly deformed bud and inflorescence, 6) poor pollen production (Table 1). Disk florets and achenes are normal. 'BO' plot surrounded by other lines in the nursery gave 90 % true-to-type morphology after open pollination, thus, it is highly self-compatible. In crossings with female lines (CH-0, HA 300, DPG 34 etc.) 100 % monohead F_1 plants with normal morphology were obtained, but they were male sterile which proves the **rfrf** genotype. Female F_1 plants back-crossed with 'BO' gave rise to 50:50 % normal and 'BO' types in BC_1 which proves the monogenic recessive determination of the teratological morphology. It suggests pleiotropy of the recessive gene because no further segregation was observed (the characters remained together as a 'package').

During selfings and maintenance it was observed that even in rainy periods when strong attack of Sclerotinia, Phomopsis, Macrophomina, Phoma, Alternaria was found in other A,

B and R lines, stems of 'BO' line were almost symptomless and this fact drew our attention to its potential use in breeding for disease resistance, since the first male-sterile hybrid combinations also showed less or no disease symptoms on the stems.
Oil content of 'BO' line in selfed and sibbed material is low (Table 2) and it may hardly be improved. Conversion of 'BO' into **cms** analogue is difficult because of strongly distorted head and its use in seed production would not be efficient. Therefore, conversion into R form was carried out by crossing 'BO' plants (treated with 50 ppm gibberellic acid during 24 hours) with different branching restorers. In F_2 generation, out of a great number of segregants differering in plant height (from 50 cm up to 2 m), leaf number (from 50 up to 300), leaf size, position and colour, number of lateral branches (from 2-3 up to 20-30) shorter types (60-120 cm) with many lateral inflorences and showing no disease symptoms were selected and selfed, then individually crossed (in couples) with females for testing the presence of **Rf** gene(s). Plants that gave rise to 100 % male fertile hybrids were chosen for further selfing and lines were named 'BBO'. Morphological characterization is given in Table 1. These lines have a distorted main head with poor pollen production, whereas lateral inflorescences produce abundant pollen assuring perfect pollination during 14-24 days. Oil content could be increased from 27-30 to 38-48 % (Table 2). Stems of these lines remain yellowish-green even at full maturity and show resistance to stem diseases mentioned above. (Cms A x BBO) x BBO back-cross generation segregated 50:50 % normal monohead and 'BBO' plants that proves again the monogenic recessive determination. This morphology may well be used for distinction and inbred line protection.

Experimental hybrids (Sc. and Tc.) were produced with different females and evaluated in performance trials. Those with yield at the level of controls (Ex-399, Viki, HNK-173) were tested for Sclerotinia on the head (Table 3) in Argentina. FB 292 was the best for ratio of diseased plants and latency. Out of numerous performance trials carried out in Szeged-Kiszombor and France, results of one trial are presented in the Table 4 (in comparison with Viki as a standard: %V). Those under GR and GD codes cover hybrids with restorer 'BBO 327'. Eight hybrids were superior to Viki for oil yield. For health state in natural conditions they showed similar good field resistance to stem and head diseases. High achene yield and oil content show a good potential of hybrids for practical use. Artificial infections with Phomopsis in the field on these hybrids showed a moderate size of spots 2 and 4 weeks after inoculation (Table 4). Although infection by wounding is a drastical event for the plant, no broken plants were found in these hybrids. Naturally, the presence of symptoms might have been expected because the females carry certain susceptibility. Artificial infection of other hybrid combinations on cut heads by Sclerotinia showed from 0-100 % rotting of head surface: out of 43 hybrids thirteen may be considered resistant (or at least tolerant), not exceeding 9.6 % rotting of the head tissue (0-9.58 %; table is not presented).

Conclusion

Maintainer line 'BO' and restorers 'BBO' derived from it, resistance of which to stem diseases is presumably determined by a pleiotropic recessive gene, may practically be used for creation of Sc. and Tc. hybrids with good field resistance and high performance expressed in oil content, achene yield and oil yield.

Acknowledgements

The authors thank Dr. Anibal Fernandez, sunflower breeder and his technicians for the Sclerotinia test carried out at the Research Station of Dekalb Argentina., S.A., Bragado, Argentina. Dr. Éva Péter, Mrs. G. Pálvölgyi and Mrs. J. Juhász sont gratefully acknowledged for the Sclerotinia and Phomopsis test made in Szeged, as well as Mrs. Aranka Takács for typing and arrangement of the manuscript.

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Legends to the figures

Fig. 1. Macromutant fixed 'BO' line in bloom.

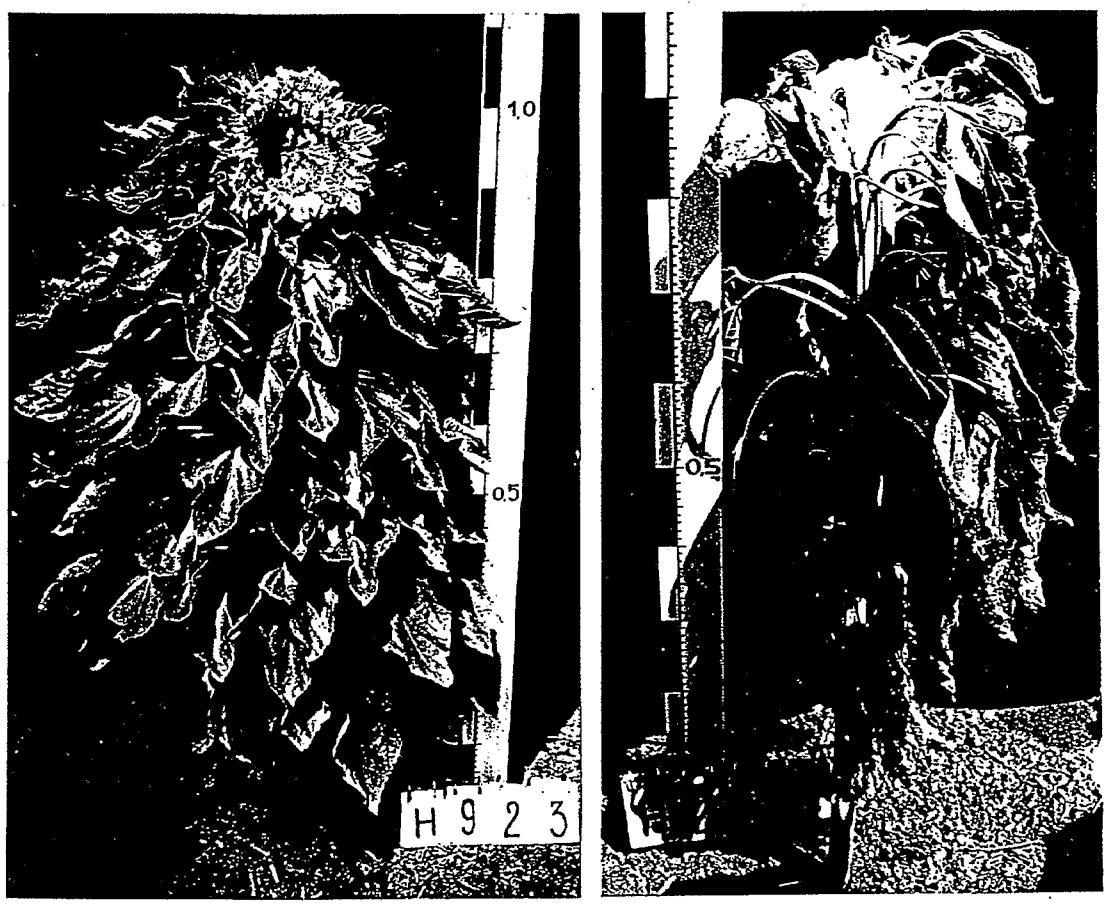
Fig. 2. Healthy 'BO' monohead plant (B line, rfrf) at stage 5.1.1.

Fig. 3. Branching, short stem restorer 'BBO' (RfRf) in bloom.

Fig. 4. Uniform plot of 'BBO' line in bloom for selfing.

Fig. 5. Plot of a taller 'BBO' line with healthy stems and heads.

Fig. 6. Symptomless plants (stems) of an F_1 hybrid (at physiological maturity) containing genome of a 'BBO' line as a male parent.

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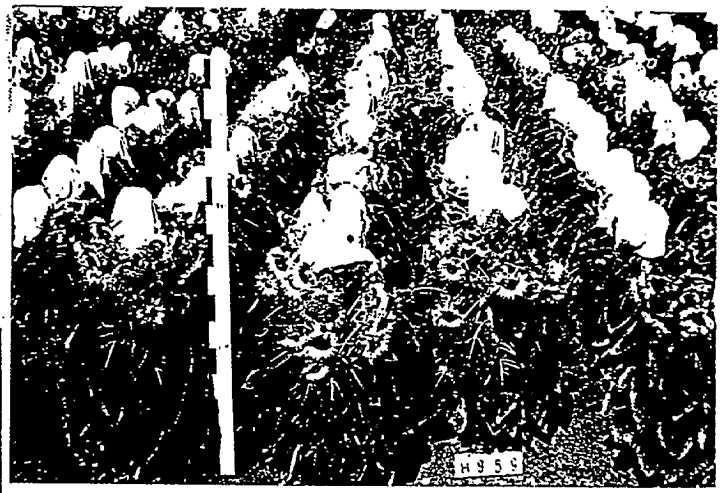
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Table 1

CHARACTERIZATION OF NORMAL AND MUTANT LINES

CHARACTER		NORMAL B LINES	BO (rfrf)	BBO (RfRf)
Plant height,cm		90-140	110-120	80-100
Stem	diameter, mm	30-45	35-45	25-35
	cross section	round	elliptic	elliptic
	ribs	weak → strong	very strong	very strong
	internode length, cm	8-25	1,5-5	1,5-5
	reaction to stem diseases	susceptible→resistant	resistant	resistant
Leaves	length, cm	20-38	15-20	12-18
	width, cm	20-36	10-15	8-12
	size	small → very big	small	small
	shape	triangular → cordate	triangular	triangular
	colour	light → dark green	light → dark green	light → dark green
	position	erectoid → bending	horizontal → bending	horizontal→bending
	number	15-30	100-200	70-200
Leaf petiole	thickness	very thin → very thick	thin	very thin → thin
	length, cm	10-30	10-15	8-15
Inflorescence principal	shape	regular	deformed	deformed
	diameter, cm	15-30	20-25	15-20
	pollen production	poor →abundant	very poor	very poor
	ray flowers: shape	oval, with smooth edge	oval, with curled edge	oval, with curled edge
	ray flowers: colour	pale yellow → orange	yellov	yellow
	stigma: colour	yellow → purple	yellow	yellow
	stigma: no.of lobes	2; rarely 3	2; often 3-4	2; often 3-4
	duration of bloom, days	7-10	7-10	7-10
Lateral inflorescences	duration of bloom, days	-	-	14-24
	arrangement on the stem	-	-	at the top
	diameter, cm	-	-	5-10
	shape	-	-	normal, round
	pollen production			abundant
Achenes	size	medium → big	medium	small
	shape	oval	oval	elongated
	colour; presence of stripes	grey → black; stripes present or absent	black, striped	black, striped
	1000-achene mass, g	40-55	45-48	25-30
	oil content, %	35-53	35-38	38-45

Table 2.

DEVELOPMENT OF OIL CONTENT DURING INBRED LINE FIXATION

Line	Oil content, %						
	1991	1992		1993		1994	
	selfed	selfed	sibbed	selfed	sibbed	selfed	sibbed
BO	30-33	33-34	33-34	34-35	35	36-38	35
BBO 325/22	32-35	31-35	42.90	40-46	36-42	44	44
BBO 325/22	31-34	32-36	40.50	32-44	32-42	42-45	44
BBO 325/23	30-36	31-34	41.98	37-47	39.42	43	43
BBO 325/24	31-33	33-35	39.27	39-45	39-43	-	-
BBO 326/7	31-33	31-33	39.28	34-41	38-41	39-42	42
BBO 326/9	30-34	30-33	38.25	39-43	34-39	36-37	39
BBO 326/15	29-33	31-34	39.51	39-41	38-39	-	-
BBO 326/16	30-33	30-34	36.25	-	-	-	-
BBO 327/1	27-30	30-34	40.58	38-44	36-42	42	42
BBO 327/3	29-32	27-34	41.17	37-41	37	37-38	38
BBO 327/4	27-29	27-33	38.59	35-39	37	-	-
BBO 327/8	27-31	28-33	29.18	40-41	38-39	42	42
BBO 327/9	30-33	33-35	39.58	38.42	37-41	38-42	41-42
BBO 327/14	27-32	28-33	40.13	38-44	38-39	38-40	40-42
BBO 333 SJB	32-39	32-41	37-40	41	38-40	38-39	38-39
BBO 335 SJB	35-40	37-40	-	39-41	42	41-42	39-41
BBO 361	34-38	35-38	-	39-46	45-46	43-48	48

Table 3.

WHITE HEAD ROT ON EXPERIMENTAL HYBRIDS OBTAINED BY CROSSING NORMAL FEMALE LINES WITH „BBO" RESTORERS

(artificial infection)
Dekalb Argentina, Bragado, 1994

Code of Hybrid	No of inoculated plants	Diseased plants		Latency, days
		no	%	
FB 192	45	35	77.8	36.2
FB 292	37	24	64.9	40.0
FB 392	41	36	87.8	40.4
FB 492	29	28	96.6	38.7
FB 5192	46	45	97.8	34.3
DAP 58193	37	34	91.9	32.2
DAP 58293	42	42	100.0	32.6
DAP 53093	40	31	77.5	36.6
DAP 53193	35	35	100.0	31.4
DAP 57693	42	36	85.7	33.7
DAP 57793	42	39	92.9	33.7
DAP 58093	45	41	91.1	30.9
G 100 susceptible check	38	38	100.0	26.0
ACA tolerant check	42	38	90.5	35.9

Table 4.

PERFORMANCE OF EXPERIMENTAL HYBRIDS CONTAINING RESTORER LINE BBO-327 AS A MALE PARENT
Szeged (Hungary), 1995

Code of Hybrid	Cross	Oil yield		Oil content		Achene yield		Health state*	Phomopsis art.inf.**		
		kg/ha	% V	%	% V	kg/ha	% V	1-9	2	4	6
Master	Sc.	2441	116.5	52.8	103.5	4617	112.5	6.33	2	7	14
Sonrisa	Sc.	2348	112.2	53.3	104.5	4403	107.3	7.00	3	7	15
GR-4203	Sc.	2308	110.2	50.1	99.2	4564	111.2	8.00	2	3	20
GR-4201	Sc.	2273	108.5	52.9	103.7	4296	104.6	7.67	4	8	14
GR-4206	Sc.	2268	108.3	52.1	102.1	4352	106.0	7.33	2	8	11
GR-4202	Sc.	2243	107.0	50.2	98.4	4466	108.8	8.00	4	7	12
GD-42012	Tc.	2195	104.8	52.1	102.1	4211	102.6	7.33	3	7	14
GD-4207	Tc.	2175	103.8	51.6	101.0	4217	102.7	7.33	3	7	14
GR-4204	Sc.	2148	102.5	50.0	98.1	4291	104.5	7.67	1	4	12
EX-399	Sc.	2125	101.4	51.7	101.3	4108	100.1	7.00	2	4	16
GD-42014	Tc.	2106	100.5	50.5	99.0	4168	101.5	7.33	3	7	13
VIKI st.	Sc.	2094	100.0	51.0	100.0	4104	100.0	7.67	2	6	12
GD-4208	Sc.	2093	99.9	50.5	98.9	4144	100.9	7.00	-	-	-
GD-42013	Tc.	2089	99.7	50.8	99.5	4113	100.2	7.00	3	7	13
GR-4205	Tc.	2054	98.1	51.4	100.7	3995	97.3	7.33	2	8	14
GD-42015	Tc.	2032	97.0	51.3	100.6	3960	96.5	7.33	2	5	11
GD-42011	Tc.	1938	92.5	51.4	100.8	3964	91.7	7.00	2	4	7
GD-42011	Tc.	1922	91.7	50.6	99.1	3798	92.5	7.33	2	4	7
GD-42010	Tc.	1844	88.0	51.7	101.3	3568	86.9	7.00	2	6	17
HNK-173	Sc.	1832	87.4	49.9	97.9	3664	89.2	7.00	2	4	10
LSD 5%		117.0		1.6		194					

* health state with natural infections, scale 1-9, where 1=worst, 9=best
** length of spots developed 2, 4 and 6 weeks later after artificial infection of stems by Phomopsis helianthi