Isolation of Chemically Induced Mutants in Borage (*Borago officinalis* L.)

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ABSTRACT: γ-Linolenic acid (GLA, 18:3 Δ6,9,12) has been reported to be helpful in the treatment of a wide range of disorders. Borage (*Borago officinalis* L.) is an annual plant of renewed interest because the seeds are an important source of GLA. The failure to retain mature seeds until harvest limits the total seed and GLA yield per plant and is the major limiting factor for the commercial production of borage. In the course of a mutagenesis program, an agronomically good line of white-flowered borage (RG-001) was treated with ethyl methane sulfonate. As a result of this program, several types of mutants were identified in the M2 generation of plants: a chlorotic mutant (type A); a mutant with increased number of sepals, petals, and ovules but reduced fertility (type B); and mutants with closed flowers (type C1) or partially opened flowers (type C2) that had increased seed retention. The type C mutants are the first reported borage plants with a nonshattering habit. After crossing type B plants with normal plants, a new mutant (type B1) was obtained with higher fertility and higher seed production per flower than those from normal plants. These mutants could be used to develop borage lines that would be superior to those currently available as a source of GLA.
JAOCS 75, 281–283 (1998).

KEY WORDS: EMS-induced mutation, γ-linolenic acid, seed retention, seed shattering.

Borage (*Borago officinalis* L.) is an annual plant that has been used from ancient times for culinary and medicinal purposes. In the book *History of Plants,* published in 1597, John Gerad quoted as "an old verse" the following sentence: "Ego Borago gaudia semper ago" (I, Borage, bring always courage), confirming the old belief in the properties of the leaves and flowers of borage to take away sorrow and melancholy (1).

Recently, interest in borage has been renewed because its seeds contain a high percentage of γ-linolenic (all-*cis*-6,9,12-octadecatrienoic) acid (GLA). This fatty acid is an intermediate of indispensable compounds in the body, such as prostaglandin E1 and its derivatives (2). Normal synthesis of GLA from linoleic acid *via* Δ6-desaturase can be decreased or blocked in humans by several factors, such as stress, diabetes, obesity, alcohol, and aging. Under these circumstances, supplementation of the diet with vegetable oils that contain this fatty acid is being used for the treatment of health problems related to deficiencies in essential fatty acids and prostaglandins (3).

Although seeds of many plants contain GLA (4–6), the most common commercial sources of GLA for pharmaceutical uses are evening primrose (*Oenothera biennis* L.) and borage. Borage seed appears to be the richest known plant source of GLA, with 16 to 28% GLA in the oil and a total seed oil content of 27 to 37% (5–8). Additional advantages of borage, in comparison with evening primrose, are its annual life cycle and its larger seeds, which make harvest and oil extraction easier (9).

Large-scale commercial production of borage is difficult because of its indeterminate growth habit, nonuniform seed ripening, and its shattering habit: a large percentage of ripened seed shatters and falls to the soil before and during harvest. Therefore, typical seed yields of borage under European conditions range from 100 to 500 kg/ha, which represents about half of the potential seed yield (5,10).

Despite considerable natural variability for growth characteristics found in germplasm collections of wild and cultivated borage, the lack of variation in seed shattering is still the major challenge in research work on this semidomesticated species (5,8). Galwey and Shirlin (9) carried out a program of selection in borage for GLA, oil content and seed production, including mutagenesis with 2×10^{-4} M or 2×10^{-3} M aqueous solution of sodium azide, or 0.1 or 1% aqueous solution of ethyl methane sulfonate (EMS) for 2 h, but they produced no mutant phenotypes in subsequent generations.

In 1991, a breeding program was initiated with the objective of developing borage lines that are well adapted to Mediterranean conditions, and with improved characteristics for GLA production: high oil content in the seed, high GLA content in the oil, and reduced seed shattering. After evaluation of a germplasm collection of 210 wild and cultivated species of borage (8), a trial was carried out to increase the natural genetic variability by inducing chemical mutagenesis with EMS. This paper describes the detection and isolation of two mutant lines with improved seed production traits.

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EXPERIMENTAL PROCEDURES

In November 1993, approximately 15,000 seeds of white-flowered borage, belonging to an accession with high self-fertility (RG-001), were exposed to a solution of 1% (vol/vol) of EMS for 16 h under continuous stirring. Seeds were then thoroughly rinsed with tap water for 4 h. Immediately after mutagenesis, M1 seeds were planted in the field in rows 5 m long with 1-m spacing between rows at the experimental farm of the Agricultural Research Center of Córdoba. Approximately 1 wk before flowering, 1000 plants from the EMS-treated seeds (M1 generation) were bagged both to force self-pollination and to make the harvesting of M2 seeds easier. Plants from RG-001 untreated seeds were used as a control.

Seeds of each bagged plant were sown in a single row in November 1994 to produce the M2 generation and, before flowering, five plants of each row were bagged and self-pollinated to obtain M3 seeds.

In the M2 generation, plants with modified morphological traits in relation to the control were both self-pollinated and crossed with untreated plants to recover the mutant traits and to study the genetic modifications involved in these mutations. For each mutant type, about 50 self-pollinations and about 40 crosses with normal plants were made. In November 1995, samples of the resulting seeds were germinated on Petri dishes and cultivated in pots, one seed in each, under controlled pollination conditions to produce 20 plants from each self-pollination and from each of the crosses between mutants and normal plants. During the flowering period (April–May 1996), 10 flowers per plant were inspected to analyze the segregations in the resulting progenies. Fifty pollinated flowers from each mutant type were chosen at random to calculate the variability in the number of petals, sepals, ovules, and seeds per flower, and to apply statistical analysis. Significant differences between means were determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

Mutant isolation. Three different types of mutants were obtained in the M2 generation of plants: Type A: Chlorotic or yellow-leaved plants, with no pollen in their flowers. Type B: Plants with modified flowers: larger than normal, with a higher number of petals, sepals, and ovules (almost double that in the original plants). In most flowers, the style and stigma were malformed. Type C: Plants with flowers closed (C1 type) or partially opened (C2 type). In these two mutant types, the petals did not follow the general rule of falling 2 d after opening, but instead they remained on the flower, dried, and stayed on the plant to the end of the life-cycle. This modification seems to affect shattering of the seeds. Type C1 plants can be considered as indehiscent plants because their mature seeds were fully retained by closed petals. Although the seed retention in C2 plant types was not as strong as in the C1 plants, partially opened petals and seeds remained on the plant well after it had dried at the end of its life cycle.

Because each one of these mutants was located in single specific rows, we were able to hypothesize the genetic cause of these mutations and the prospect of these mutants to increase the yield of GLA per plant.

Mutant multiplication. As described above, the mutants showed different levels of fertility among them when they were both self-pollinated and crossed with untreated plants: Type A was of poor physiological quality and could not be reproduced. Type B mutant progeny showed reduced fertility after both self-pollination and crossing to controls. This may be due to visible pistil malformations and/or meiotic abnormalities, caused by the EMS treatment. These meiotic abnormalities were observed under the microscope as pollen grains of variable size, but they were generally double that of pollen from untreated plants. When fertilization was achieved, there were as many as eight seeds per flower, double the maximum productive capacity (four seeds) of a normal flower. Type C1 had low fertility when it was self-pollinated, possibly owing to alterations in the style of the unopened flowers. In this mutant, the normal process of style elongation that occurs during anthesis is blocked. When flowers were manually opened before anthesis and pollinated, they showed normal fertility. Type C2 had fertility similar to that of normal plants.

Segregants. The self-pollinations of mutants and the crosses with normal plants enabled us to recover all types of mutants identified in the M2 generation. In addition to these mutants, a new mutant type (B1) was obtained after crossing type B plants with normal plants, with floral characteristics intermediate between the two, and with the following advantages: the flowers have about double the number of ovules, a normal-shaped style, and higher fertility and higher seed production per flower than normal plants.

Table 1 shows the mean and range of the number of petals, sepals, ovules, and seeds per flower in the different mutant types of borage. Although the flowers of type B plants have the highest number of ovules, the seed production per flower was significantly lower than in normal plants. Mutant plant type B1 showed the highest seed production per flower due to the combination of high number of ovules per flower and high fertility.

Although an insufficient number of plants from each progeny were studied to test genetic ratios and to establish genetic conclusions, our preliminary results can be explained by the hypothesis that, on the one hand, B and B1 are the results of quantitative changes in the borage genome and, on the other hand, C1 and C2 mutants are produced by localized mutations in the borage genome.

These hypotheses need to be confirmed by means of cytogenetic and molecular techniques before the genetic changes produced in B, B1, C1, and C2 mutants are fully understood.

B1 and C2 mutants could be used to obtain higher production of seeds per plant and therefore of GLA. B1 mutants would allow us to increase the number of seeds per flower, and C2 mutants managed to obtain a higher yield of seeds because these plants retain their mature seeds until harvest, eliminating the seed loss due to seed shattering that characterizes normal plants.

TABLE 1
Variability in Flowers and Seeds in Untreated and Mutant Borage Plants[a]

		Untreated plants	Mutant plants			
			Type B[b]	Type B1[c]	Type C1[d]	Type C2[e]
Petals	Mean[f]	5b	8a	5.1b	5b	5b
	Range	5	7–9	5–6	5	5
Sepals	Mean	5c	8a	5.2b	5c	5c
	Range	5	7–9	5–7	5	5
Ovules	Mean	4c	18.3a	7.9b	4c	4c
	Range	4	16–25	7–8	4	4
Seeds per flower	Mean	2.8b	2.2c	4.5a	1.2d	2.4b,c
	Range	0–4	0–8	0–7	0–4	0–4

[a]Number of flowers inspected in each mutant type and in untreated plants: 50.
[b]Type B: plants with flowers larger than normal and often with the style and stigma malformed.
[c]Type B1: plants with normal-shaped style and high fertility.
[d]Type C1: plants with flowers closed and seed retention.
[e]Type C2: plants with flowers partially opened and seed retention.
[f]Means within the same row with the same letter are not significantly different. Significance was calculated by Duncan's multiple range test ($\alpha = 0.05$ level).

These results are a starting point for future breeding work of borage, designed to develop lines that are superior to those currently available as a source of GLA.

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[Received November 14, 1996; accepted August 7, 1997]