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Is self-compatibility the main breeding system in borage (*Borago officinalis* L.)?

Abstract The polygenic postzygotic self-incompatible system reported in borage (*Borago officinalis* L.) is refuted. The behaviour of pollen tubes after self- and cross-pollination, analysis of the crossability coefficient, seed set after continuous generations of self-pollination and the effects of inbreeding depression in borage were studied. Evidence of self-compatibility was established. The influence of protandry in a self-compatible system is also discussed.

Key words Self-compatibility · Self-pollination · Inbreeding depression

Introduction

Borage (*Borago officinalis* L.) is a plant species present throughout the temperate areas of the world as a wild plant. In some countries it is cultivated. For example, in Spain, mainly in the Ebro Valley, it is an important vegetable crop. In other regions of Spain and in some areas of Europe and America, borage is cultivated for the commercial production of seeds, which are used in pharmaceuticals because the seeds of borage are rich in gamma linolenic acid, an essential and unusual fatty acid (Wolf et al. 1983; Whipkey et al. 1988).

Cultivated borage of the Ebro Valley comes from wild populations selected for and maintained by farmers. These populations are white-flowered and well-adapted to the environmental conditions. Local seed companies sell only seed from wild varieties, and no breeding programmes are carried out to improve traits which are required by farmers.

Detailed knowledge of the reproductive biology of *Borago officinalis* is not readily available, and the little data that has been reported are contradictory. It is known that borage is an allogamous plant with an entomophilous pollination system. Bees mainly, attracted by nectar, take pollen grains and scatter it about neighbouring flowers (Alvarez and Villa 1992). East (1940) reported borage to be self-incompatible with some populations as facultative outbreeders. Crowe (1971) studied these populations and showed that the incompatible reaction was expressed after fertilization and that the genetical basis was polygenic. Such a system had not been described before in nature.

In 1989, Mayo and Leach, applying a computing model to study the multifactorial self-incompatible system described by Crowe (1971), reported that mutation rates would have to be extremely high to maintain the level of variability needed in small populations according to the Crowe system (1971). In 1990, Leach et al. observed that the number of seeds produced by plants were a function of the pollination form (bee pollination, natural pollination in the absence of bees, artificial cross-pollination between unrelated plants, artificial cross-pollination between related plants, artificial self-pollination). They also argued that the ranking of observed seed set could be a consequence of inbreeding depression – the rate decreasing with endogamy level – and concluded that there was no evidence that would suggest a multifactorial or simple self-incompatibility system. Furthermore, they suggested the possibility of self-compatibility in borage. However, it was still not clear from their data which was the main reproductive system in borage.

The work we are presenting in this paper deals mainly with the reproductive system in borage. We tried to assess whether the reproductive system in borage was controlled by a postzygotic polygenic self-incompatibly as Crowe (1971) claimed or whether the species is self-compatible as Leach et al. (1993) suggested.

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For this purpose four experiments were designed:

1) ultraviolet microscopy of pollen-tube growth after cross and self-pollination;
2) application of the method described by Anderson et al. (1989) in order to identify and clarify the reproductive barriers in borage;
3) study of the response of borage to continuous self-pollination;
4) study of the effects of inbreeding depression in borage plants.

Material and methods

Plant material

The following four populations were used in our experiments:

– a population of wild blue-flowered borage from Sweden, named 362, kindly supplied by The Gene Bank of Zaragoza (Spain);
– a local white-flowered population from a farmer of Luceni (Zaragoza), named 184;
– a breeding line of white-flowered borage obtained after a mass selection at the SIA of Zaragoza, actually commercially present as cv. Movera;
– I-155, a white-flowered breeding line developed of the CIA of La Rioja (Spain).

We selected these genotypes because we believe they represent a significant sample of the variability that could be present in this species.

All plants were grown in pots inside a greenhouse from November to May 1993–1997.

Pollen-tube behaviour after cross- and self-pollination using ultraviolet microscopy

Flowers from plants of all genotypes were self- and cross-pollinated. Twelve flowers per genotype and pollination type were collected at intervals of 4 h during 1 day and manipulated according to Johansen (1949) as modified by Montaner (1997). Pistils were kept in aniline blue for a minimum of 12 h and observed by an ultraviolet light microscope at a 487905 setting (excitation: BP 395–440; suppression: FT 460; emission: LP 470). In the case of cross-pollination, a test of compatibility between populations was made.

Pollen-tube growth in self- and cross-pollinated flowers was compared to determine the effects of a possible prezygotic self-incompatibility system in this species. Moreover, the pollen-tube growth process from pollen-grain germination to the arrival of the ovule in the pollen tube was observed under these conditions.

Pollen-tube growth data were recorded as the following scale: 0, pollen germination in stigma; 1, pollen tubes in the middle of style; 2, pollen tubes in the ovary; 3, pollen tubes in ovules.

Applying coefficient of crossability to distinguish between self-incompatibility and other reproductive barriers

In order to apply the method described by Anderson et al. (1989) to borage a diallel cross involving the above lines and populations was performed. Male (MCC) and female (FCC) coefficients of crossability were calculated and represented in a scatter diagram.

Response of borage to continuous self-pollination

Eight plants per line or population and 200 flowers per plant were self-pollinated in an insect-proof greenhouse following the pollination method described by Montaner et al. (1994). This process was performed for five consecutive generations. For each generation of self-pollination the following parameters were determined in order to assess the level of self-fertility: (1) percentage of flowers bearing at least one seed (%S) and (2) total of seeds produced/total number of ovules (S/O) (four ovules per flower were assumed).

Using these parameters we were able to compare the self-fertility of different generations of inbreeding using a variance analysis.

Effects of inbreeding depression in borage plants

Plants of S0 and S5 generations were grown and compared for inbreeding depression using a complete randomized block design with five plots per generation and six plants per plot (exceptions are market out in the text).

The following parameters were determined as indicators of inbreeding depression. We chose these parameters because they have been described as being the most discriminatory in the species (Susín and Alvarez 1994).

1) Viability of seeds: ten seeds per plot and four plots per generation were examined.
2) Viability of seedlings after transplanting. For that purpose the total number of survival plants were recorded and compared between generations.
3) Length of leaves petioles. The length of all the petioles per plant was determined but for the analysis only the plot mean was taken into account.
4) Plant weight.

Results

Pollen-tube behaviour after cross- and self-pollination applying ultraviolet microscopy

No differences were detected between self- and cross-pollinated flowers for any genotype. Results obtained by ultraviolet microscopy showed that, under our experimental conditions, pollen-tube growth had the same behaviour independent of pollen origin. In both cases the time from pollen germination in the stigma to arrival at the ovary was the same. Twelve hours after pollination pollen tubes had reached the ovary in 90% of the analysed flowers, independent of the origin of the pollen.

Applying coefficient of crossability to distinguish between self-incompatibility and other reproductive barriers

Table 1 shows the %S obtained from a diallel cross of all the genotypes used in this study. These data allowed us a calculate male (MCC) and female (FCC) coefficients of crossability (Anderson et al. 1989). Figure 1 indicates that borage is a self-compatible crop.

### Answer of borage to continuous self-pollination along several generations

Results for five generations appear in Table 2. This table shows the percentage of flowers bearing at least one seed

Table 1 Mean percentage of flowers bearing at least one seed in a diallel cross (%S) between genotypes expressed in the table

Diallel cross	Genotypes			
	184	362	I-155	Movera
184	72.74	66.22	74.36	62.40
362	58.00	55.41	72.02	76.01
I-155	60.21	80.31	70.51	78.31
Movera	60.03	70.00	68.05	55.00

Table 2 Mean percentage of flowers bearing at least one seed (%S) and total of seeds/total number of ovules (S/O) for five consecutive generations of selfing of populations 184 and 362 and lines I-155 and Movera. Data followed by different letters are significantly different at the 5% level according to the Fisher LSD test

Generation	%S	S/O
184		
S1	72.8±6.4a	0.33±0.05
S2	73.6±4.8a	0.37±0.03
S3	55.5±6.7b	0.29±0.05
S4	69.7±1.8a	0.35±0.06
S5	70.8±2.8a	0.41±0.02
362		
S1	54.2±4.5a	0.27±0.02
S2	28.3±7.2b	0.11±0.04
S3	50.5±2.4a	0.24±0.04
S4	56.8±3.8a	0.34±0.18
S5	62.5±4.7a	0.30±0.05
I-155		
S1	72.4±4.9	0.33±0.04
S2	76.1±5.2	0.35±0.02
S3	74.5±3.6	0.46±0.07
S4	83.6±7.8	0.38±0.02
S5	72.4±7.6	0.33±0.09
Movera		
S1	55.5±5.17a	0.29±0.03a
S2	62.7±2.36a	0.30±0.02a
S3	54.7±5.81a	0.28±0.09a
S4	87.5±9.42b	0.45±0.12b
S5	77.5±4.71b	0.42±0.09b
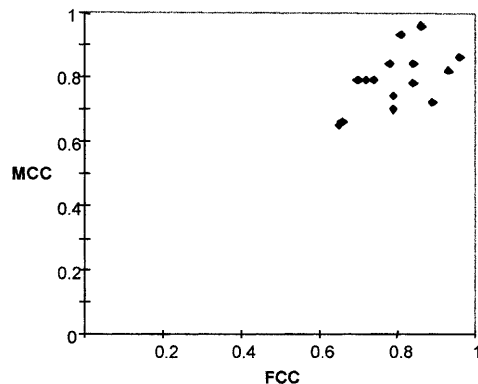
Table 3 Means and standard deviation of percentage of viability of seeds, viability of seedlings, length of leave petioles and weight of S_0 and S_5 generations of 184 and 362 populations and I-155 and Movera lines. Values observed are means of five plots per generation and 6 plants per plot, except for viability of seeds where values observed are means of four plots per generation and 10 seeds per plot

	184	362	I-155	Novera
Percentage of viability of seeds				
S0	95.7±7.8	81.4±13.4	87.1±11.1	98.5±3.7
S5	97.1±4.8	80.0±10.0	80.3±8.2	91.4±8.9
	ns[a]	ns	ns	ns
Viability of seedlings				
S0	30	30	26	30
S5	29	30	28	28
	ns	ns	ns	ns
Length of leave petioles (cm)				
S0	17.6±2.0	13.6±2.3	16.9±1.4	18.7±1.4
S5	16.5±1.1	14.8±0.8	18.6±1.5	18.4±1.6
	ns	ns	ns	ns
Weight (g)				
S0	360±89.0	323±26.5	290±169.5	405±130.1
S5	296±64.6	288±76.6	237±62.4	301±37.8
	ns	ns	ns	ns

[a] ns, No significant differences after comparing both generations

(%S) and total of seeds/total number of ovules (S/O) for five consecutive generations of selfing. The analysis of this data showed that differences between generations were observed only for Movera.

Effects of inbreeding depression in borage plants

Results of all assays designed to evaluate inbreeding depression appear in Table 3. No significant differences were observed for the characters analysed.

Discussion

Pollen-tube behaviour after cross- and self-pollination applying ultraviolet microscopy

Results of this assay provided us with information on two aspects of borage reproductive biology. First, we can now describe the pollen tube unfolding from pollen germination to reach the ovules. Secondly, the nature of reproductive system can now be studied because prezygotic self-incompatibility could be detected by analysing morphological or growing pollen-tube abnormalities.

Under our experimental conditions, it took 12 h to detect the pollen tubes reaching the ovules. Nevertheless, Quinn et al. (1989) saw pollen tubes reaching the micropile 7 h after pollination. In our opinion these differences are a consequence of environmental conditions, namely temperature. In another assay we studied the influence of temperature on pollen-tube growth (unpublished data) and

Fig. 1 Plot of male-crossability coefficient (*MCC*) against female coefficient of crossability (*FCC*) of a diallel cross from 16 crosses of 362, I-155, 184 and Movera plants

verified that plants growing at 22.5°C have pollen tubes reaching the ovules 6 h after pollination. The different speed is not a special feature in borage. Species like *Zea mais* (Herrero and Johnson 1980) *Cydonia oblonga* (Sedgley and Buttrose 1987), tomato (Williams and Knox 1985; George et al. 1984), *Rhaphanus raphanistrum* (Young and Stanton 1990) and others all show the influence of temperature on the prefertilization process.

Leach et al. (1993) stated that outcrossing in this species was stimulated because self-pollen is less competitive than cross-pollen. We did not detect any difference between self- and cross-pollinated flowers with respect to the patterns of pollen tubes. In our opinion outbreeding in *Borago officinalis* L. could be maintained by protandry, mechanisms cited by Quinn et al. (1987) in this species or by those detected in floral biology experiments reported here. The same growing behaviour confirms that a prezygotic self-incompatibility system can be rejected in borage. Crowe (1971) came to the same conclusion. She said that the failure of the majority of compatible pollinations to produce the full complement of four seeds suggested that the incompatibility reaction could be expressed after fertilization, resulting in the abortion of potential seeds.

Applying the coefficient of crossability to distinguish between self-incompatibility and other reproductive barriers

Taking into account that the reproductive system described by Crowe (1971) needs a high level of homozygosity to express self-incompatibility and that there are several generations required to reach that level, we decided to apply male and female coefficients of crossability to distinguish self-incompatibility and other reproductive barriers, following the methodology developed by Anderson et al. (1989).

The area in the "Anderson's" diagram (Fig. 1) shows that borage is a self-compatible or pseudo-compatible species (Anderson et al. 1989). Results of this method agree with Leach et al. (1990) and Mayo and Leach (1993), who reported that borage was not self-incompatible. Nevertheless according to Mayo and Leach (1993) Anderson's method did not have any discriminatory power for borage because the points distribution within Anderson's diagram do not define any reproductive system. These authors attribute the non-validity of this method to the presence of maternal effects. Our data did not show any maternal effects because reciprocal crosses data (Table 1) did not give evidence of them.

In our opinion, differences in the discriminatory power of Anderson's method between our results and Mayo and Leach conclusions (1993) are due to the parameter used to calculate the crossability coefficients. The latter authors used seed set [as number of seed harvested/maximum potential number per flower (four seeds per flower)]. If we consider that the number of seeds produced per flower is strongly related to nourishing conditions, as has been reported by Leach et al. (1993) in borage or in Boraginaceae-like *Cryptantha flava* (Casper and Wiens 1981; Casper 1984) or *Anchusa officinalis* (Andersson 1988, 1990), the parameter used by Mayo and Leach (1993) could be greatly influenced by environmental conditions. However the parameter %S used by us, which considers only flower success, independently of the number of seeds, is less dependent on the nutritional conditions of the plant and, consequently more suitable for calculating crossability coefficients.

If borage is really a self-compatible crop as is shown in Anderson's diagram, the species could allow self-pollination although it is necessary to determine the effects of inbreeding depression on the vigour and viability of inbreed plants.

Response of borage to continuous self-pollination along several generations

Anderson's method showed that the borage reproductive system might be pseudo-compatible also self-compatible. At a practical level, pseudo-compatibility and self-compatibility are the same but there are theoretical differences between them. Pseudo-compatibility is a very frequent feature in self-incompatible plants, and it depends on the level of heterozygosity of the S-genes controlling the system. Self-compatibility is a permanent breakdown of self-incompatibility (Østerbye 1986).

If we consider that theoretically the level of homozygosity in the S5 generation is around 95% (Sanchez Monge 1974) and take into account the results show in Table 2, the self-compatibility hypothesis is reinforced at least for three of the four populations we used. It is known that often in nature and especially in crops, self-incompatible plants can fix self-compatible genes (Mayo and Leach 1987) and that self-compatible genotypes can be selected within a self-incompatible species, as happened in sugar beet (Cochet and Soreau 1989) or almond tree (Socias i Company et al. 1995).

In our case, we discard the last hypothesis because we are using different borage populations and lines (white-flowered ones from Spain and a violet-flowered wild population of Swedish origin). In our experiments all of them showed evidence of self-compatibility, so our results reinforce the hypothesis of borage as a self-compatible species. In the case of cv. Movera there was an increase in %S and S/O in generations S_4 and S_5, but this behaviour could be due not to pseudo-compatibility but to the later flowering time for the three first generations. Movera is a late-flowering line so flowering proceeds later in the year when temperatures are higher. High temperatures affect fertility (Montaner 1997).

Effects of inbreeding depression in borage plants

Results of Table 3 do not show any difference between S0 and S5 for any of the analysed traits. This fact, together with the fertility data shown above, allow us to postulate borage as a self-compatible crop with no significant levels of inbreeding depression.

If borage is self-compatible and protandrous, why does *Borage officinalis* maintain protandry? That is a question that we could answer if we consider the evolutionary origin of this family. We have reviewed the mating systems of several members of the Boraginaceae family, and we note that there are different reproductive types (Casper and Wiens 1981; Casper 1984; De Jong et al. 1986; De Jong and Klinkamer 1989; Klinhamer and De Jong 1987, 1990; Philipp and Schou 1981; Weller and Ordonuff 1989, 1991). It has been reported that it is unusual to find more than one type of system within a family, and the system is as old, if not older, than the families themselves (Crowe 1964). In the case of the Boraginaceae the coexistence of several reproductive systems suggests a recent origin of this family from an evolutionary point of view. In this context we think that the mating system of borage may not be completely stabilized. Notwithstanding, the necessity for insect pollination, protandry and differences in self-compatibility degree between populations could be an indication of outcrossing as the primitive state of the mating system in borage. Moreover, borage could be like the many angiosperms that have adopted self-pollination as the last evolutionary trend (Stebbins 1974). In these groups of plants the system may still be evolving. To support our idea, perhaps it is necessary analyse phylogenetically several populations of *Borago officinalis* and related genera.

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