Int. J. Dev. Biol. 45 (S1): S43-S44 (2001)

Short Report

Inbreeding depression in *Rosmarinus officinalis L.*

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ABSTRACT *Rosmarinus officinalis* plants are self-compatible but automatic self-pollination is prevented by strong protandry. Flowers of *R. officinalis* set abundant seed when cross-pollinated by hand. Nevertheless, the seed set by self-pollination is clearly lower and similar in amount to those obtained in open-pollination. Insect-mediated geitonogamy is possible, and appears to be responsible for the lower seed set in open-pollination. Seed set by male-sterile plants indicates that gynodioecy could be considered as a strategy to increase reproductive output in *R. officinalis*. Two different steps can be distinguished in the halting of seed selfing: early abortion of the embryo/endosperm just after fertilisation, leading to small and empty seeds; and late abortion of the embryo, leading to empty but apparently normal seeds.

Introduction

Lamiaceae have four ovules per flower, thus they produce no more than four seeds per fruit. These seeds are dispersed as 4 nutlets. Self-compatibility appears to be a constant feature of breeding systems in Labiatae (Owens & Ubera-Jiménez, 1992). There is still no wholly convincing evidence that self-incompatibility occurs in any species in the Labiatae. Protandry is typical of Lamiaceae and separation of maturation timing of anthers and stigma requires that a vector serve as intermediary in pollination. Nevertheless, species that have many flowers open at one time, with some in the male and some in the female phase, are especially liable to geitonogamy (cross-pollination between two flowers on the same plant due to de behaviour of the pollinator). Geitonogamy leads to a high rate of self-pollination (Cruden *et al.,* 1984). Gynodioecy is particularly common in the Labiatae. Species are defined as gynodioecious when their populations comprise plants with hermaphrodite flowers (male fertile, MF) and plants whose flowers are functionally female, with the male organs reduced and sterile (male sterile, MS). Since MS plants must be outcrossed, the reproductive output is enhanced (Horovitz, 1980). Gynodioecy is controlled in *R. officinalis* by cytoplasmic (mitochondrial) genes (Hidalgo-Fernández *et al.,*1999). The main goal of this report is to describe inbreeding depression in *R. officinalis* by self-pollination.

Material and Methods

Seed set in self and cross-pollination was tested by hand pollination over mature stigmas in caged plants. A total of 190 and 179 flowers were self- and cross-pollinated respectively. Each test was carried out in three different plants. A prior test of pollen tube development was carried out to rule out inhibition of pollen tube growth. Seed development in self and cross fertilization was studied by fixing the ovary/fruit in FAA after 24 and 48 hours and after 3, 6, 8, 14 and 21 days of pollination. Samples were embedded in paraffin and sectioned on a Jung Autocut microtome (10 µm). Sections were stained with safranine and fast-green. Total seed numbers produced in both types of pollination were counted and weighed. Total number of seeds produced in open pollination were estimated in both MF and MS plants to compare these results with those obtained by manual pollination.

Results

Table I shows total set of seeds produced in hand and open-pollination. The results obtained in selfing experiments show that 97 flowers did not initiate seeds, whereas in allogamy, only 24 flowers did not produce seeds. Most of the fruits in allogamy yielded 2 or 3 seeds. Reproductive success in self and cross pollination can be estimated as Sx100/Fx4, S being the total number of seeds produced and F the total number of flowers pollinated. The reproductive success in allogamy was 54.88 and seed weight was 13.8 µg. Reproductive success was 21.97 in autogamy and seed weight was only 8.3 µg. Comparing seed set for hand self-pollination and MF open-pollination we can see that most of the flowers produced only 1 or 2 seeds (81.7% and 73.7% respectively). In less than 20% of the cases we found 3 or 4 seeds per flower. Meanwhile, cross-pollination and MS open pollination show the same output. Three or 4 seeds were produced in 54.2% of cross-pollinated flowers. A total of 62.9% flowers produced 3 or 4 seeds in MS open-pollination.

Figure 1 shows different stages of the development of the embryo in self and cross-fecundation. Fig. 1a shows normal development of embryo, endosperm, nucellus and hypostase after 48 hours of the cross-fecundation. Fig. 1b shows halted embryo and endosperm development at 3 days of self-fecundation. The hypostase has not been differentiated although it is evident in the normal development 24 hours before, so the halt in development must take place before

TABLE I

SEED SET BY HAND AND OPEN POLLINATION

	Hand pollination				**Free-pollination**			
nº of seeds	allogamy		autogamy		Male-sterile		Male-fertile	
per flower	nº flowers	(%)*	nº flowers	(%)*	nº flowers	(%)	nº flowers	(%)
0	24	-	97	-	-	-	-	-
1	25	(16.1)	40	(43.0)	143	(13.8)	484	(34.2)
2	46	(29.7)	36	(38.7)	242	(23.3)	559	(39.5)
3	60	(38.7)	13	(13.9)	435	(41.8)	319	(22.5)
4	24	(15.5)	4	(4.3)	220	(21.1)	53	(3.7)
total flowers	**179**		**190**		**1,040**		**1,415**	

* The percentage in hand pollination has been calculated without the value of 0 seed/flower in order to compare these values with the data obtained in open pollination.

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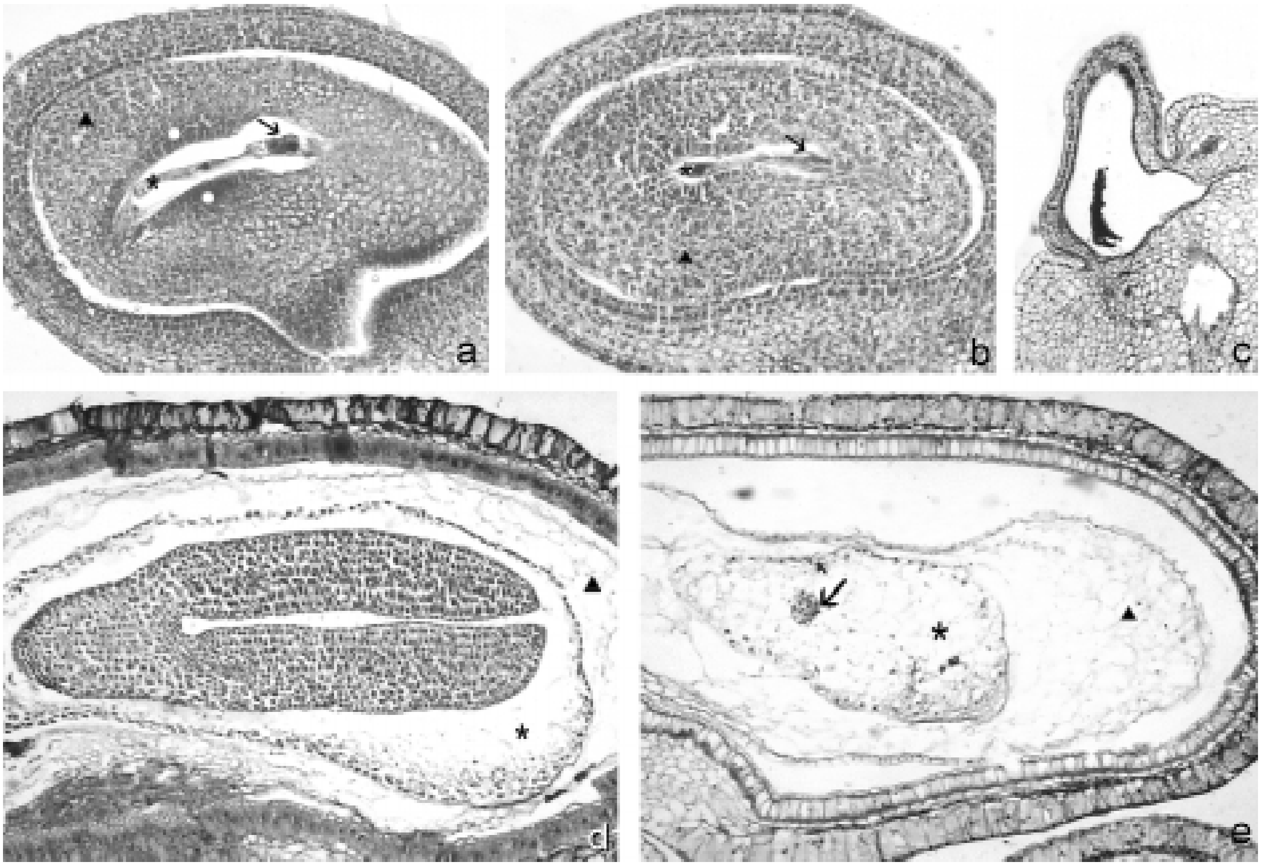

Fig. 1. Different stages of seed development in self and cross-pollination. (a) *Normal embryo after 48 hours of cross-pollination. X200.* **(b)** *Aborted embryo after 3 days of self-pollination. X200.* **(c)** *Aborted seed after 21 days of self-pollination. X80.* **(d)** *Normal development of a 21-day embryo. X50.* **(e)** *Late abortion of embryo in self-pollination. X50. Endosperm (asterisk). Nucellus (triangle). Embryo (arrow), Hypostase (circle).*

48 hours. The early abortion of endosperm/embryo leads to small, empty seeds (Fig. 1c) similar to those that remain unpollinated. Fig. 1d shows the normal development of the embryo, endosperm and nucellus after 21 days of cross-fecundation. An embryo aborted at a late stage of development is shown in Fig. 1d. The nucellus, endosperm and integuments are developed but the embryo seems to be collapsed. Notice the difference in shape between the normal testa in Fig. 1d in comparison with the testa of the aborted seed in Fig. 1e.

Conclusions

Seed set by self-pollination shows strong inbreeding depression, whereas seeds corresponding to cross-pollination are more abundant and heavier. *R. officinalis* produces complex flowers with a strong protandry that do not avoid self-pollination. Insect-mediated geitonogamy seems to be the reason why a high rate of self-pollination occurs in open pollination. Similar conclusions are described for other protrandric species (Garnock-Jones & Molloy, 1982). Seed set by MS plants indicates that gynodioecy provides additional cross-pollination in wild populations. Two different events lead to the abortion of the embryo in self pollination: 1.- early abortion just after pollination, in which no seed is produced and 2.- late abortion producing empty fruit (vain fruits). In the first case, neither the endosperm nor the embryo are developed and the integuments remain like those of unfertilized fruits. Nevertheless, in the second type of abortion, the nucellus, endosperm, integuments and embryo are developed, but suddenly the embryo is aborted leading to a vain seed. Due to the embryo halting, testa maturation seems to be incomplete. The lower weight in the self-pollination seed set compared to the weight in cross-pollination could be due to a higher proportion of these vain seeds in the former.

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