

## INTERFERTILITY IN THE GENUS MORICANDIA DC.

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### Abstract

The objective of this work was to outline a more natural taxonomy of the genus *Moricandia* DC. The possibility of obtaining experimental hybrids using most of the species of this genus was studied using a diallelic crossing table. *M. moricandioides* and *M. foetida*, both endemic of the Iberian Peninsula, were found to have strong reproductive isolation. Hybrid seeds were obtained from the remaining species; however, as in *M. suffruticosa* x *M. arvensis* and *M. spinosa* x *M. nitens*, alterations in pollen fertility and pollen morphology studied under SEM, were found, showing important reproductive disorders. Thus, it is proposed to maintain this genus comprised of eight species: *M. arvensis* (L.) DC., *M. moricandioides* (Boiss.) Heywood, *M. foetida* Bourg ex Coss., *M. suffruticosa* (Desf.) Coss, *M. spinosa* Pomel, *M. nitens* (Viv.) Dur. & Batt., *M. sinaica* (Boiss.) Boiss and *M. foleyi* Batt. *M. arvensis* (L.) DC. var. *robusta* Batt. must be included in the typical *M. arvensis*(L) DC.

### Introduction

The genus *Moricandia* DC. (*Brassicaceae-Cruciferae*) is found in the Mediterranean, Irano-Turanian and Saharo-Sindian regions. It is comprised of eight species adapted to tolerate drought and arid conditions. One of the species of this genus, *M. arvensis* (L.) DC., behaves as an aggressive weed in adequate environmental conditions of high temperatures and calcareous soil, extending to the circummediteranean area. In the Iberian Peninsula it infests agricultural fields in extensive areas in Levante and the southeast in both irrigated and non-irrigated conditions.

The taxonomy of this genus has traditionally been controversial. Specimen determination is difficult, due to the reduced morphological differences found among the different species. This has led to the description of high number of "taxa". The situation is even more complicated, as part of the variability of this genus is located in deserts and steppes where access has been difficult until recently.

Moreover, the karyology of the genus *Moricandia* was virtually unknown until 1970, as only the chromosome number of *M. arvensis* was known (MANTON, 1932). VALDÉS-BERMEJO (1970) reported the chromosome numbers of the Iberian species and later SOBRINO-VESPERINAS (1978) studied a large part of the variability of the genus and detected a euploid series.

In a previous study, the possibility of obtaining intergeneric and interspecific crossings in some genera of the tribe *Brassicaceae* was considered. It was found possible to obtain F1 experimental hybrids within the genus *Moricandia* (SOBRINO-VESPERINAS, 1988).

The obtention of experimental hybrids using a wide sample of the genetic variability of the genus was studied in order to test the possibility of genetic transfer within

*Moricandia* and to provide data on its reproductive behaviour. Thus, some taxonomic and phylogenetic aspects of this genus may also be clarified.

### Material and Methods

The starting material was obtained from seed samples of the genus *Moricandia* kept at the *Cruciferae* Germplasm Bank in the Departamento de Biología Vegetal at the Escuela Técnica de Ingenieros Agrónomos de Madrid (GÓMEZ-CAMPO, 1990). In all cases (except one) the seeds were collected directly from their natural habitats, as indicated in Table 1. Six F1 experimental *Moricandia* hybrids, obtained by the author, were also used (Table 2).

This collection of "taxa" widely represents the genetic variability of the genus, both at the specific and infraspecific levels. However, it should be noted that *M. foleyi* Batt., an endemic species from a small area in the north of Africa, could not be included.

The seeds were sown in two seasons in 10-cm-diameter pots on sterilized soil and placed in a greenhouse with a controlled minimum temperature ( $t > 10^{\circ}$  C). They were later transplanted to 24-cm-diameter pots.

Taxon	Reference No.	Location
<i>M. arvensis</i> (L.) DC.	GC-0863	Jumilla (Murcia) Spain
<i>M. arvensis</i> (L.) DC.	GC-2990	Trafaute. Morocco
<i>M. arvensis</i> (L.) DC. var. <i>robusta</i> Batt.	GC-3660	N. of Biskra. var. Algeria
<i>M. arvensis</i> (L.) DC. var. <i>garamantum</i> Maire	GC-3670	Tamanraset (Hoggar) Algeria
<i>M. sinaica</i> (Boiss.) Boiss.	GC-3748	Behbahan. Iran
<i>M. foetida</i> Bourg. ex Coss.	GC-4073	Tabernas (Almeria) Spain
<i>M. moricandioides</i> (Boiss.) Heywood	GC-3482	Jaén. Spain
<i>M. spinosa</i> Pomel	GC-1845	Drahu. Algeria
<i>M. nitens</i> (Viv.) Dur. et Barr.	GC-2129	Egypt
<i>M. suffruticosa</i>	GC-1833	Maillot. Algeria

Table 1. "Taxa" of the genus *Moricandia* DC. used in the experimental crossings.

With the plant material described in Table 1, a diallelic crossing table of 8 x 10 was devised. *M. sinaica* and *M. foetida* were eliminated as female parents, as poorly developed plants were obtained from these two species. However, they could be used as male parents since they produced pollen normally. Ten flowers were emasculated for each of the experimental crossings. The flowers were emasculated at the floral bud state, then the selected pollen was added and finally the flowers were isolated in labelled cellophane bags. Fruits and seeds were collected when they were ripe and the following ratios were calculated: viable seeds per hybrid flower, fruits per hybrid flower and viable seeds per fruit.

The F<sub>1</sub> experimental hybrids (Table 2) were sown and grown in the same previously described conditions along with the parents in order to check their identity and study their morphological characteristics and fertility.

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(GC-1833) <i>M. suffruticosa</i> x (GC-0863) <i>M. arvensis</i>
(GC-1845) <i>M. spinosa</i> x (GC-2129) <i>M. nitens</i>
(GC-0863) <i>M. arvensis</i> x (GC-1845) <i>M. spinosa</i>
(GC-1833) <i>M. suffruticosa</i> x (GC-1845) <i>M. spinosa</i>
(GC-1845) <i>M. spinosa</i> x (GC-1833) <i>M. suffruticosa</i>

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Table 2. F<sub>1</sub> experimental hybrids of the genus *Moricandia* used as starting material.

## Results and discussion

### Experimental Crossings

Most of the experimental combinations assayed in the diallelic table produced viable seeds (Table 3), although relevant differences were found among the different crossings. Compared to the other "taxa", *M. suffruticosa* had the lowest variation of the seeds per hybrid flower ratio in the different crossings, when used as a female parent. In this species, seeds were obtained in all of the combinations assayed. This species was followed by *M. spinosa*, which only did not produce seeds in hybridisation with *M. foetida* and *M. moricandioides*. Precisely these species had the highest ploidy levels and produced the greatest number of viable hybrid seeds. *M. moricandioides* had the lowest number of hybrid combinations. Similar behaviour was observed when *M. moricandioides* was used as a male parent, as no viable seeds were produced except with *M. suffruticosa*.

When the "taxa" were used as male parents, they behaved very differently than when used as female parents. This suggests that the pollen-stigma incompatibility barriers and/or chromosome imbalances of the hybrid embryo are important in the isolation of these species. The greatest number of viable hybrid seeds were obtained with the African population determined as *M. arvensis* (GC-2990), when used as a male parent.

Although it has been proven experimentally that the two crossing directions are not equivalent, the chances of hybridisation between two "taxa" can be related to the sum

	GC 0863	GC 2990	GC 3660	GC 3670	GC 4073	GC 3748	GC 3482	GC 1845	GC 2129	GC 1833
GC 0863		6,45	12,3	0	0	0,35	2,55	12,4	3,14	4,80
GC 2990	0		0,2	2,80	0	4,30	0	0	5,10	5,92
GC 3660	3,55	0,15		0	0	0,63	0	0,2	0	0,09
GC 3670	0	1,17	0		0	1,17	0,90	0	0	0
GC 4073	-	-	-	-		-	-	-	-	-
GC 3748	-	-	-	-		-	-	-	-	-
GC 3482	0	0	0	0	0	2,13		0	0	0
GC 1845	5,63	10,1	8,00	9,00	0	4,33	0		1,33	7,70
GC 2129	0	15,8	0	6,13	0,83	2,50	0	1,18		0
GC 1833	1,55	1,38	5,27	2,13	1,40	1,93	0,33	5,08	0,84	

Table 3. Viable seed number per hybrid flower obtained in experimental hybridisation among 10 taxons of the genus *Moricandia* DC.

of the indices of both crossing directions. It can be considered that in nature, the chances of the pollen of a taxon reaching the stigma of another taxon are the same as the chances of the latter's reaching the former's stigma.

Viable seed production ability is notable between the European population of *M. arvensis* (GC-0863) and *M. arvensis* var. *robusta*. Based on this and their morphological identity, we propose that they be classified in one taxonomic group. There is also a notable crossing ability of *M. sinaica* with *M. nitens* and with African populations initially determined as *M. arvensis*.

The experimental crossings showed that two species, *M. moricandioides* and *M. foetida*, are almost completely reproductively isolated from the rest of the species of the genus studied and between each other. The species closest to them is *M. suffruticosa* which is fairly infertile with the remaining "taxa". The remaining species, in turn, form a group in which gene flow is possible: *M. arvensis* var. *arvensis*, *M. arvensis* var. *garamantum*, *M. nitens*, *M. sinaica* and *M. spinosa*. Thus, it can be suggested that *M. suffruticosa* is the species used, most closely resembling the original stock of the genus *Moricandia*. However, *M. foleyi* Batt., endemic of a small area in the north of Africa, has a larger number of primitive morphological features and according to POGGIO (pers. com.), it has a chromosome number of  $2n=14$ . As previously mentioned, this species could not be included in the experimental hybridisations.

Floral timing poses no problem in *Moricandia*. The different ploidy levels were not the main obstacle in obtaining F1 experimental hybrids, as the species which remain almost totally reproductively isolated, *M. moricandioides* and *M. foetida*, have the same chromosome number as *M. arvensis*. However, karyological barriers were important in some cases in this genus. All of the species assayed have the same base chromosome number  $x=14$  and a chromosome series with chromosome numbers  $2n=28$ ,  $2n=56$ ,  $2n=84$  has been reported (SOBRINO-VESPERINAS, 1978).

Geographic barriers determine the isolation of some interfertile *Moricandia* species, but in some cases this isolation is not complete and introgression or introgressive

hybridisation may occur. This seems plausible among *M. suffruticosa*, *M. spinosa* and *M. arvensis* in the north of Africa. However, the introgression of *M. moricandioides* over *M. foetida*, as suggested by HEYWOOD (1964), seems less likely due to the strong reproductive isolation between the two species.

#### Study of the experimental hybrids

*Germination and morphology.*- Seed germination of assayed F1 experimental hybrids (Table 2) was average to high (50%-100%). Intermediate morphological characteristics were observed when the hybrids were compared to parents. This is specifically shown in the shape of the cotyledons.

Abundant viable seeds were produced by F1 hybrid fruits, although both seed length and seed number per fruit were less than in parents in identical environmental conditions. *M. suffruticosa* x *M. arvensis* was the only combination which did not produce fruits nor seeds, as shortly after pollination the young fruits died.

*Interfertility.*- Parental pollen fertility, studied using the carmine acetic technique, was between 90 % and 99 %, while a much more irregular behaviour was shown in the experimental hybrids. Thus, pollen fertility was high in *M. suffruticosa* x *M. spinosa*, despite chromosome number differences. In *M. suffruticosa* x *M. arvensis*, which combine very different ploidy levels, pollen fertility only reached 54% and the pollen grains varied in size. In a more detailed study using SEM, the pollen of *M. suffruticosa* x *M. arvensis* showed strong anomalies from the tricolpate type of the parents common in the *Cruciferae* family. Sterile grains were present as well as carmine acetic positive grains of average and large size. In the combination *M. spinosa* x *M. nitens*, which also have different ploidy levels, a high percentage of carmine acetic positive pollen was found. However, all of the pollen grains had a consistent malformation in the form of a double ring when observed under SEM. Nevertheless, this did not alter viable seed formation.

#### Proposed nomenclative changes

Due to the viable seed production ability and morphological identity of the European population *M. arvensis* (GC-0863) and *M. arvensis* var. *robusta* (GC-3660) from the north of Africa, we propose that they be classified in one taxonomic group.

*Moricandia arvensis* (L.) DC., *Reg. Veg. Syst. Nat.* 2: 626 (1821).

*Moricandia arvensis* (L.) DC. var. *robusta* Batt. in Batt. & Trabut, *Fl. Syn.* 34 (1902).

### Taxonomic aspects

Fertility barriers among the different *Moricandia* species appear to be non-existent in some cases, whereas in others they emerge abruptly. There is also an intermediate group in which relationships are difficult to interpret. Thus, it is proposed to maintain a total of eight species for this genus each with their own characteristics in accordance with SCHULZ (1936) and the obtained results. This is contrary to the criteria established by MAIRE (1967), who classified *M. suffruticosa*, *M. spinosa* and *M. nitens* under *M. arvensis*. The recognized species are as follows: *Moricandia arvensis* (L.) DC., *M. moricandioides* (Boiss.) Heywood, *M. foetida* Bourg. ex Coss., *M. nitens* (Viv.) Dur. et Batt., *M. suffruticosa* (Desf.) Coss., *M. spinosa* Pomel, *M. sinaica* (Boiss.) Boiss., *M. foleyi* Batt.

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