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Interfertility and siliqua morphology of hybrids in the genus *Coincya* Rouy (*Cruciferae, Brassiceae*)

Abstract

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A series of crosses was performed to investigate relationships between 7 taxa of the genus *Coincya* Rouy: *C. hispida* var. *hispida*, *C. hispida* var. *deflexa*, *C. leptocarpa* f. *leptocarpa*, *C. leptocarpa* f. *calatrava*, *C. longirostra*, *C.pseudoerucastrum* subsp. *pseudoerucastrum* and *C. pseudoerucastrum* subsp. *cintrana*. Several viable seeds were obtained from the hybrid crosses performed and the number of seeds per pollinated flower was counted for each cross. Pollen fertility was determined in 18 of the crosses, and the stability of meiosis was estimated in some of the F1 hybrids. Fruit morphology and seed production in 9 of the crosses was compared to those of the parents. Data corresponding to interfertility, pollen fertility and fruit morphology were used to draw taxonomic conclusions.

Introduction

Traditionally, the genus *Coincya* Rouy (syn. *Hutera* Porta) has only included *C. rupestris* and *C. leptocarpa,* two species strictly endemic to the central and SE-Iberian Peninsula. Based on fruit and, to a lesser extent, leaf morphology this genus shows clear differences from *Rhynchosinapis* Mayek. Both the genera bear heterocarpic fruits with seeds located in the valves and rostrum. However, in *Rhynchosinapis* the length of the fruit is greater than its width and heterocarpy is less pronounced. The exception is *R. longirostra*, where the rostrum is relatively important in seed production. Gómez-Campo (1977a) found phenetic variation in fruit characteristics in a group of endemic species of the genera *Rhynchosinapis* and *Hutera* and concluded that there was no solid argument for their separation. Later, the same author (Gómez-Campo 1977b) referred both genera to *Hutera* including 12 species. A further revision on the grounds of nomenclature priority (Greuter & Burdet 1983) assigned both *Hutera* and *Rhynchosinapis* to *Coincya*. More recently, Leadlay (1993) reviewed *Coincya* in *Flora Iberica* following a systematic study and enumerated 4 species for the Iberian Peninsula.

With the aim of determining the possibility of gene flow and to aid in the clarification of taxonomic relationships, An analysis of the interfertility in a group of 7 taxa belonging

to 4 species according to Greuter & al. (1986) was made. Three different intraspecific categories were used to evaluate differences in the degree of reproductive isolation of the taxonomic levels proposed.

Material and methods

Seeds of seven taxa collected in the wild were obtained from the "Cruciferae Germplasm Bank" of the Dep. de Biología Vegetal, Universidad Politécnica de Madrid: *Coincya hispida* var. *hispida*, *C. hispida* var. *deflexa* Gómez-Campo (under *Hutera*), *C. leptocarpa* (Glez.-Albo) Greuter & Burdet f. *leptocarpa*, *C. leptocarpa* (Glez.-Albo) Greuter & Burdet f. *calatrava* Gómez-Campo (under *Hutera*), *C. longirostra* (Boiss.) Greuter & Burdet, *C. pseudoerucastrum* (Brot.) Greuter & Burdet subsp. *pseudoerucastrum* and *C. pseudoerucastrum* subsp. *cintrana* (Continho) Greuter & Burdet. A series of crosses was performed using the seven parents to obtain ten crosses per combination. Emasculation was performed as previously described by Sobrino (1988). Once mature, the fruits were collected and the number of viable seeds determined in the experimental hybrids obtained. Hybrids were authenticated by comparison with their parents. Both fruit and seed morphology were analysed. Further, pollen fertility was estimated by staining 300 grains per parent and hybrid with carminoacetic glycerine. Meiotic stability was evaluated by staining isolated anthers with 2% orcein and observing 40 meiotic cells under a Zeiss microscope.

Results

Table 1 shows the results expressed as the number of seeds per emasculated and pollinated flower obtained from the experimental crosses. The seeds obtained from crosses in either direction were included to correspond best with conditions in nature. Viable seeds were obtained in most of the combinations. Hybrids such as *Coincya longirostra* ×*C. pseudoerucastrum* subsp. *cintrana* showed high fertility, with nine viable seeds per flower. Only in two of the 21 combinations no seeds were obtained, although the corresponding

Table 1. Viable seeds per hybridized flower in experimental F1 combinations of 7 taxa of *Coincya*.

X / Ξ	9	10	12	13	15	18	19
9	-						
10	6.52	-					
12	2.57	1.86	-				
13	2.70	4.11	1.75	-			
15	6.67	0	1.72	1.79	-		
18	2.13	1.37	0	1.13	1.57	-	
19	0.47	0.66	0.33	1.12	4.40	3.4	-

9.- *Coincya longirostra*. 10.- *C. hispida*. 12.- *C. leptocarpa*. 13.- *C. leptocarpa* f. *calatrava*. 15.- *C. pseudoerucastrum* subsp. *cintrana*. 18.- *C. hispida* var. *deflexa*. 19.- *C. pseudoerucastrum*.

parental species proved to be infertile when crossed with the remaining taxa, ruling out the possibility of reproductive isolation. When *C. longirostra* was used as the female parent, viable hybrid seeds were obtained from combinations with each taxon. The crosses performed between infraspecific taxa of the same species showed no difference from those performed between interspecific taxa.

The pollen fertility was estimated in 18 of the experimental hybrids Table 2. These showed high fertility levels which at times reached similar levels to those of the parental species (e.g. 97% in the hybrid *C. longirostra* ×*C. hispida*). Moreover, no differences in pollen fertility were detected between inter- and infraspecific combinations.

The large number of seeds formed by the hybrid fruits is of particular interest. Each seminal primordium gave rise to perfectly formed seeds. Moreover, the hybrid *C. hispida* ×*C. longirostra* produced a greater number of seeds than any of the parents. This hybrid showed heterosis for fruit length with maximum mean values of 72 mm. However, the longest fruit was that of *C. pseudoerucastrum* subsp. *cintrana* ×*C. pseudoerucastrum* (76.3 mm) which also showed heterosis. The *C. hispida* ×*C. longirostra* hybrid produced a mean number of 38.6 seeds per fruit which was substantially greater than parental values (26.3 and 24.5).

The length of the rostrum in no case reached the maximum values shown by *C. longirostra* and *C. leptocarpa*. However, all fruits produced by the hybrid combinations had a rostrum length over 18 mm and were of intermediate length respect to those of the parents. The most fully developed rostrum, with a rostrum to valve length ratio of 0.90, was observed in the fruits of *C. longirostra* ×*C. leptocarpa* var. *calatrava*. The hybrids produced by *C. longirostra* or *C. leptocarpa* showed longer rostra while those of fruits produced by the parents *C. hispida* and *C. pseudoerucastrum* were shorter. The mean numbers of seeds in the rostra of hybrid fruits were 2.3 to 4.3. These failed to reach maximum values corresponding to *C. longirostra*. In general, the number of seeds in the rostrum was relatively low in the hybrid fruits with the exception of those of *C. longirostra* ×*C. leptocarpa* f. *calatrava* (number of seeds in rostrum /valves ratio = 0.37) and of *C. leptocarpa* f. *calatrava* ×*C. leptocarpa*. The number of seeds in the rostrum was always intermediate with respect to the values obtained in each parent.

Table 2.- Pollen fertility in the 7 parents and 18 experimental hybrids of *Coincya*.

X / Ξ	9	10	12	13	15	18	19
9	97	78	-	77	91	97	86
10	97	95	-	-	-	-	-
12	-	-	97	-	-	-	-
13	97	85	88	94	98	-	-
15	85	-	82	97	94	-	90
18	-	-	-	-	69	74	90
19	-	73	-	-	-	97	96

9.- *Coincya longirostra*. 10.- *C. hispida*. 12.- *C. leptocarpa*. 13.- *C. leptocarpa* f. *calatrava*. 15.- *C. pseudoerucastrum* subsp. *cintrana*. 18.- *C. hispida* var. *deflexa*. 19.- *C. pseudoerucastrum*.

Discussion

High fertility levels corresponded to normal metaphase activity. Configurations of 24 bivalents were detected in five of the hybrid specimens. Only in the combination *C. hispida* var. *deflexa* ×*C. pseudoerucastrum* subsp. *cintrana* anaphase meiotic anomalies were found (12% of the cells showed anaphasic bridges) reducing pollen fertility to 69%.

All the parents showed pollen fertility levels over of 95% with the exception of *C. hispida* var. *deflexa* which, surprisingly, only showed 74% fertility. The morphology of this species may be described as intermediate between that of *C. longirostra* and species with less developed rostra (*C. pseudoerucastrum* or *C. hispida*) suggesting a hybrid origin.

The ease of hybrid production between taxa, copious production of seeds by the F1 hybrids and the high degree of pollen fertility of most combinations suggests the possibility of gene flow in the absence of geographical barriers. These results confirm the initial findings of Harberd & McArthur (1972) and Leadley & Heywood (1990) that there are not reproductive barriers to gene flow in *Coincya*. The fact that the morphological differences observed do not correspond to reproductive barriers is of great interest. It would, therefore, seem that the differences produced in the traditional species have been maintained because of their distribution in mountainous areas which are isolated by extensive plains.

From a taxonomic point of view, this no doubt constitutes a problem in that the entire group is able to contribute to the gene pool. Nevertheless, the morphological differences between species are clear and have fully been characterized. Their classification has, therefore, been approached in two ways. Gómez-Campo (1977b) and Greuter & al. (1986) have maintained the classic nomenclature which includes several different, independent species, while Leadlay (1993) uses a synthetic argument to establish the existence of only two species. The present authors feel that the later approach would be consistent with the high rate of interfertility detected. However, if interfertility is used as the main criterion in the definition of species, there is only one species and morphological differences cannot be taken into account. On these grounds, *C. rupestris* and *C. leptocarpa* would be subordinated to *C. monensis*, despite their profound morphological differences. Further, this type of division would result in the loss of much information with respect to morphological and ecological diversity. It is consequently proposed that the species should be maintained as separate entities albeit with a close phylogenetic relationship. Therefore, the following species endemic to the central and SE-Iberian Peninsula would be: *C. hispida*, *C. longirostra*, *C. pseudoerucastrum*, *C. rupestris* and *C. leptocarpa*.

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