SELF-INCOMPATIBILITY LEVEL IN SOME BRASSICACEAE SPECIES

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Abstract. The Brassicaceae family is characterized by a self-incompatibility system but it includes both incompatible and compatible species. In this study, self-incompatibility level and floral morphology have been examined in five Brassicaceae species (*Capsella bursa-pastoris* L., *Eruca vesicaria* L., *Lepidium sativum* L., *Lobularia maritima* L. and *Sinapis arvensis* L.). The pollen tube count after self-pollination showed that: *Eruca vesicaria* and *Sinapis arvensis* are self-incompatible, *Capsella bursa-pastoris* and *Lepidium sativum* are self-compatible and *Lobularia maritima* is partially self-compatible. Moreover, the flowers of these species show considerable variation; the floral organs of compatible species are generally smaller than those in self-incompatible species, these differences are associated with the different rates of self-compatibility. Our results give an overview of the level of self-incompatibility and its relationship with flower structure of some Brassicaceae species.

Keywords: *floral variation, pollination, reproductive system, ovule production, seed production*

Introduction

The Brassicaceae represent one of the largest and most diverse families of flowering plants. It includes many economically and ecologically important plants, including wild species (*Arabidopsis*, C*apsella* for example) and cultivated species such as vegetables (cabbages, turnips, radish, rutabaga, rocket), oilseed species (rapeseed and camelina) and ornamental plants (*Alyssum* and wallflowers).

The flowers of Brassicaceae are mainly hermaphrodite as in most angiosperms, that is to say they have male and female organs in the same flower. This proximity of the sexual organs would favor the evolution of self-fertilization and thus be detrimental for the evolution of Brassicaceae by a constant increase in inbreeding depression. However, the plants have selected a self-incompatibility mechanism, allowing the female organ to recognize and reject pollen from the same flower or a genetically related flower. The consequence is to avoid inbreeding depression and to favor genetic mixing (Barrett, 1988).

In self-incompatible (SI) Brassicaceae species; the recognition and rejection of the SI pollen is genetically controlled by a single multiallelic S locus containing two genes, *S*-receptor kinase (*SRK*) (Stein et al., 1991) and *S*-locus Cystein-rich or *S*-locus protein 11 (*SCR/SP11*) (Suzuki et al.,1999; Takayama et al., 2000). The SI pollen phenotype is determined sporophytically by the diploid genotype of the parent plant. Therefore, the rejection of the self-pollen occurs when the male and female determinants of respectively the pollen and the pistil tissues have the same S-allelic specificity (de Nettancourt, 2001). In this family, the pollen rejection occurs at the stigma surface by

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preventing the pollen germination (Kitashiba and Nasrallah, 2014) due to the interaction between the male and female proteins, the SCR/SP11 ligand produced by the pollen and the SRK receptor present on the stigma. Indeed, the self SRK-SCR interaction triggers a signal transduction cascade within the stigma epidermal cell that prevents pollen hydration, germination or pollen tube penetration.

Outcrossing is advantageous because it can produce a variable progeny required for adaptation in the face of a wide range of environmental and avoidance of inbreeding depression. However, the transition from outcrossing to self-fertilization is considered the most common evolutionary transition in Brassicaceae. This transition occurred repeatedly in many plant families likely because selfing provides reproductive assurance when pollinators are scarce (Schoen et al., 1996). The loss of self-incompatibility is often accompanied by changes in the morphology of the flower; such as the reduction in the size of the petal in *Capsella rubella* (Fujikura et al., 2018), a decrease in the number of pollen grains in *Arabidopsis thaliana* (Tsuchimatsu et al., 2020), a lower pollen/ovule ratio and loss of herkogamy or dichogamy in *Solanum lycopersicum* (Chen et al., 2007), a decrease in the scent in *Capsella rubella* (Sas et al., 2016), a less nectar production in *Arenaria uniflora* (Wyatt, 1984), a changes in the intensity of petal colors in *Camissoniopsis cheiranthifolia* (Button et al., 2012).

All these studies focus on incompatible or compatible species. However, the precise level of SI, particularly partial self-incompatibility, is not considered when analyzing the relationship between SI and floral morphology.

Brassicaceae that have lost the SI system are clearly self-fertile. However, SI species generally exhibit varying levels of incompatibility, giving them flexibility in their reproductive system and better adaptation to their environment (Hadj-Arab et al., 2010, 2019).

Thus, considering the strength of the SI phenotype when analyzing the relation with the floral morphology could provide more information on reproductive strategies that allow species to better adapt to their environment.

The objective of this study is (1) to determine the level of the self-incompatibility of some Brassicaceae species, and (2) to search the possible link of their floral structure with the level of self-incompatibility.

To address these concerns, we based our analyses by examining multiple floral parameters and conducting controlled pollination experiments on five Brassicaceae species chosen for their different floral dimensions: *Capsella bursa-pastoris* L., *Eruca vesicaria* L., *Lepidium sativum* L., *Lobularia maritima* L. and *Sinapis arvensis* L.

Material and methods

Plant material

This study is based on five Brassicaceae species. One population per species was sampled in Algeria (*Table 1*). For each population, 10 individuals were harvested taking into account a sufficient spacing between the plants in order to avoid individuals originating from the same plant.

Determination of the self-incompatibility level

Inflorescences were harvested from plants growing in their natural environment. The collected inflorescences were put in water-filled bottles, and all mature flowers and fruits were removed, leaving only the flower buds.

Table 1. *Origin of the five Brassicaceae studied species and the characteristics of the collecting sites*

Unit	Species	Localities	Coordinates
C	*Capsella bursa- pastoris L.*	Taksebt	36°33’35”N/4°11’32”E
E	*Eruca vesicaria L.*	Adrar	27° 49’N/00°11’E
L	*Lepidium sativum L.*	Adrar	27° 49’N/00°11’E
M	*Lobularia maritima L.*	Bab-Ezzouar	36°72’14.0”N/3°18’37.7”E
S	*Sinapis arvensis L.*	Bab-Ezzouar	36°72’14.0”N/3°18’37.7”E

The next day, the open flowers undergo self-pollination (SP) at the anthesis stage. Three to seven flowers of each individual are pollinated by their own pollen or by pollen from flowers of the same individual. The pollinations are carried out manually by rubbing the dehiscent anthers on the stigma so as to cover it with pollen. All flowers were pollinated under the same conditions in the same day.

Self-incompatibility phenotypes were determined by counting the pollen tubes that penetrate into the pistil tissues by using fluorescence microscopy after aniline blue staining according to the method of Martin (1959). The pistils were removed from the flowers 24 h after pollination, fixed in ethanol/acetic acid (3:1), treated in aqueous solution of a sodium hydroxide (6 N) for 15 h, and stained for 4 h in aniline blue solution (100 mL of distilled water, 0.707 g of potassium phosphate tribasic K3PO4 and 0.1 g of aniline blue). The number of pollen tubes that penetrated into the upper part of the pistil was determined using a fluorescence microscope. The mean number of pollen tubes allowed us to measure the level of self-incompatibility of the individuals. Three groups of phenotypes were defined: self-incompatible: 0–5 pollen tubes, partially self-compatible: 5.1–25 pollen tubes, self-compatible > 25 pollen tubes.

To assess the effectiveness of plant pollination under natural conditions (NP), three to seven more flowers (previously pollinated) per individual are collected and fixed at the time of harvesting individuals. The same protocol (cited above) was carried out on the pistils of NP. The number of pollen tubes that germinated in the stigma was determined using a Zeiss Axiostar fluorescence microscope.

Morphological characterization

In order to estimate the floral diversity of the studied species and to understand the impact of floral polymorphism on the self-incompatibility level, we carried out a study of the different floral organs in 50 individuals belonging to the different species studied. In total, eleven floral characters were evaluated and measured on 3 flowers of each individual per species. These morphological characters were chosen based on the local flora of Quézel and Santa (1962), from the literature, as well as on our own observations. Furthermore, the flower color was noted as a qualitative character. The parameters were primarily measured using millimeter paper, while measurements of nectar gland and stigma were obtained using a micrometer slide. The descriptor list is presented in *Table 2* and in *Figure 1*.

In order to elucidate the morphological character relationships with self-incompatibility level, a Principal Component Analysis (PCA) and Hierarchical Ascending Classification (CAH) were applied across all studied species.

Table 2. *List of evaluated floral characters*

Trait code	Trait name
FD	Flower diameter (mm)
SD	Stamen distance (mm)
CD	Corolla tube diameter (mm)
CL	Corolla tube length (mm)
PL	Petal blade length (mm)
PW	Petal blade width (mm)
SSL	Short stamen length (mm)
RSP	Ration of LSL/PiL (Large stamen length (mm)/Pistil length (mm))
SS	Stigma surface (mm2): Stigma length (LS)* Stigma width (lS)
NS	Nectar gland surface (mm2): Nectar gland length (LN* Nectar gland width (lN)
CO	Color of flower (1: white; 2: bright yellow; 3: light yellow; 4: slightly pink)

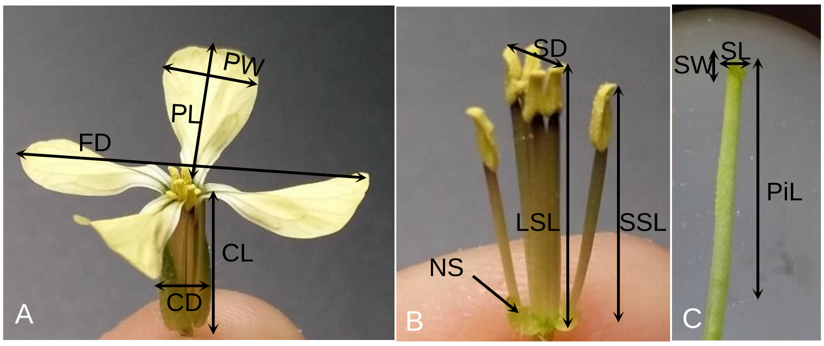

Figure 1. *Location of the quantitative characters measured on the flower. A – complete flower, B and C– fertile sexual organs. See Table 2 for descriptions of the acronyms*

Ovules/seeds counting

In order to estimate the reproductive fitness, we counted the number of ovules and seeds from 10 individuals per species. Ovule counting was performed on three flowers per individual at anthesis stage. For seed counting, 10 siliquae per individual are sampled at maturity, which occur two to three months after flowering.

Statistical analysis

The descriptive analyses (mean, standard deviation and coefficient of variation) were calculated for all the measured characters. Kruskal–Wallis and Dunn tests were used for the comparison analyses ($\alpha = 0.05$), using the R software version 4.3.2 (RTeam, 2023). The multivariate analyses (ACP and CAH) were carried out using the STATISTICA Software Package 6.0.

Results

Self-incompatibility level

After self-pollination, the average number of pollen tubes in the stigma is very different between species, it varies between 0.13 and 63.83 tubes depending on the species (*Table 3*). In *Capsella bursa-pastoris* and *Lepidium sativum* the average number of pollen tubes is > 25, these two species are self-compatible. In *Sinapis arvensis* and *Eruca vesicaria*, the self-pollination led to no pollen tube development; they are self-incompatible since their self-pollen has been rejected. *Lobularia maritima* has a low mean number of pollen tubes, it does not exceed 14 tubes per pistil, this species is therefore partially compatible. The illustration of the pollen tube number in the stigma of the different species is given in *Figure 2*.

Table 3. *Average number of pollen tubes in the style after self-pollination (average of 3 to 7 flowers per individual and ten individuals per species)*

Ind.	***Eruca vesicaria* L.**	***Sinapis arvensis* L.**	***Capsella bursa-pastoris* L.**	***Lepidium sativum* L.**	***Lobularia maritima* L.**
M	**0.18 a**	**0.13 a**	**63.83 b**	**45.12 b**	**13.70 ab**
SD	0.57	0.40	9.57	21.39	8.80
CV	**3.16**	**3.08**	**0.15**	**0.47**	**0.64**

Ind: individual; M: mean; SD: standard deviation; CV: coefficient of variation. The different letters after the mean values indicate significant differences among species, and the same letter indicate no significant difference (α = 0.05), according to Dunn’s test

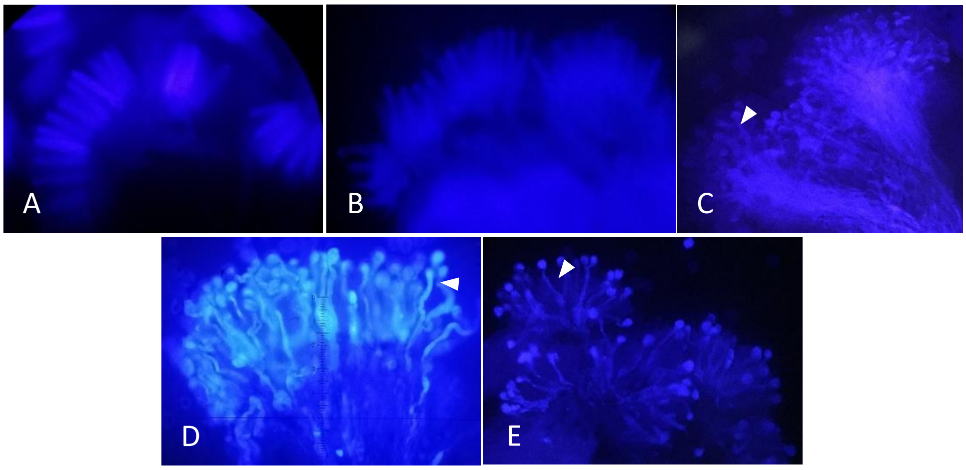

Figure 2. *Fluorescent micrographs of the pollen grains germinating in the stigma following self-pollinations. Note that on self-incompatible Eruca vesicaria (A) and Sinapis arvensis (B), no pollen germinate; self-compatible Lepidium sativum (C) and Capsella bursa-pastoris (D), large number of pollen tubes penetrate in the stigma; partially compatible Lobularia maritima (E), lower number of pollen tubes penetrate in the stigma. A pollen tube growing down the stigmatic papilla is indicated by a white arrow*

The pollinations results show variable levels of self-incompatibility according to the species and according to pollination treatment (*Figure 3*). The distribution of individuals according to the levels of self-incompatibility indicates that the most *Eruca vesicaria* and *Sinapis arvensis* individuals, are self-incompatibles. These two species do not present any compatible individuals. On the contrary, the *Capsella bursa-pastoris* individuals are distributed only in the compatible class; this species does not present any incompatible individual. The most *Lepidium sativum* individuals are distributed in the compatible class (90%) and it contains 10% of partially compatible individuals.

Lobularia maritima is partially compatible, it shows 14% of incompatible individuals and 72% of partially incompatible individuals but it also contains compatible individuals (14%).

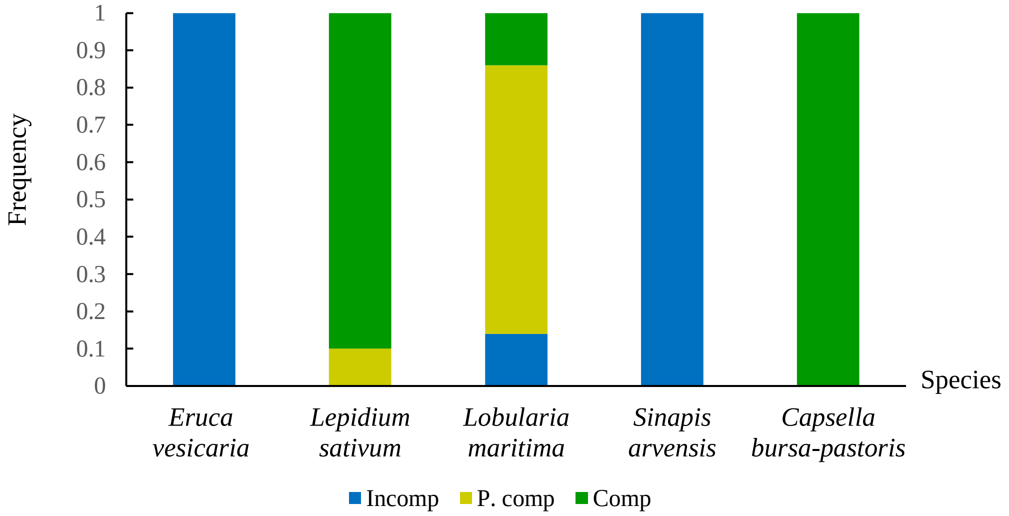

Figure 3. *Distribution of individuals according to the levels of self-incompatibility. Incomp.: incompatible; P. comp.: Partially compatible; Comp.: compatible*

Reproductive fitness in natural conditions

The *in-situ* pollination efficiency for all studied species is estimated by counting ovules, seeds and pollen tubes in the pistil following pollination under natural conditions. The results are grouped in *Table 4*.

The natural pollinations show that in all five species, the pollen grains have germinated indicating a large reproductive fitness under natural conditions. *Lobularia maritima* species shows the lowest number of pollen tubes with an average of 36.45 pollen tubes/pistil. The average number of ovules counted per species varies from 2 to 22.87 ovules/flower and the average number of seeds produced per species varies from 1.98 to 22.80 seeds per siliqua. The rate of ovules producing seeds is > 88.60% for all species. This reflects the efficiency of pollination in natural conditions.

Table 4. *Average number of ovules, seeds and pollen tubes under natural conditions in the Brassicaceae studied species*

Species	Pollen tube number	Ovule number	Seed number	% of seeds produced
Capsella bursa-pastoris	54.53 **a**	22.87 **a**	22.80 **a**	99.73
Eruca vesicaria	89.03 **b**	20.07 **ac**	17.79 **ac**	88.63
Lepidium sativum	71.61 **ab**	2 **b**	2 **b**	100
Lobularia maritima	36.45 **a**	2 **b**	1.98 **b**	99
Sinapis arvensis	86.13 **b**	10.27 **bc**	9.13 **bc**	88.90
P value	9.53E-06	2.05E-09	1.30E-09	
Significance	**s**	**s**	**s**	

The different letters after the mean values indicate significant differences among species, and the same letter indicate no significant difference, according to Dunn's test
s: significant difference based on Kruskal–Wallis test, α = 0.05

Flower morphological diversity

The values of the statistical parameters: means, standard deviations and coefficients of variation calculated for each flower character and each species are given in *Table 5* the significance of differences is given in *Appendix.* Based on the averages of the quantitative characters, we note that *Eruca vesicaria* and *Sinapis arvensis* have the highest averages for almost all the characters and *Capsella bursa-pastoris* has the lowest values. The two species *Eruca vesicaria* and *Sinapis arvensis* are characterized by a large flower size (FD = 23.1–15.42 mm; CD = 3.73–4.47 mm; CL = 10.57–4.40 mm; PL = 10.45–6.78 mm; PW = 7.3–4.95 mm, respectively). *Capsella bursa-pastoris* and *Lepidium sativum* are distinguished by a small flower size (FD = 2.22–2.58 mm; CD = 1.28–1.70 mm; PL = 1.55–1.67 mm; PW = 1.00–1.03 mm). *Lobularia maritima* has an intermediate flower size between.

Stamen spacing varies or not among species. The anthers are close to each other in *Capsella bursa-pastoris* (SD < 1 mm), *Lobularia maritima* and *Eruca vesicaria* (SD = 1.13 mm). They are distant in *Lepidium sativum* (SD = 1.65 mm) and *Sinapis arvensis* (SD = 2.55 mm).

In *Lobularia maritima* the anthers exceed clearly the stigma (RSP = 2.16 mm), the other species present anthers at the same level as the stigma (RSP ~1 mm).

The size of the nectar gland is larger in *Eruca vesicaria* and *Sinapis arvensis* compared to the nectar glands of *Lobularia maritima* (SN = 0.31 mm^2 and 0.14 vs 0.02 mm^2 respectively). The nectar glands are insignificant (tiny) in *Capsella bursa-pastoris* and *Lepidium sativum.*

The average values of the stigma size are also variable between species. The stigma is larger in *Sinapis arvensis* and *Eruca vesicaria* (SS ~ 0.3 mm^2) compared to the three other species (SS $\leq$ 0.03 mm^2).

The coefficients of variation reveal differences according to the species and according to the characters. Some characters are not variable in some species but have great coefficients of variation in other species. For example, SD (distance between stamens) which does not vary in *Capsella bursa-pastoris* (CV = 0.11) has a coefficient of variation greater than 0.50 in *Eruca vesicaria* and greater than 0.25 in *Lobularia maritima*. *Lepidium sativum* and *E. vesicaria* show a variation for stigma surface (SS) comparing to the other species.

Overall, most of the morphological characters of the flower are variable between species. *Lobularia maritima* shows the highest variability, particularly in stamen distance (SD), ratio of stamen to pistil length (RSP), and Stigma surface (SS) where coefficients of variation exceed 0.25.

The color of the flower varies according to the species; the flowers of *Lobularia maritima* and *Capsella bursa-pastoris* are white, those of *Sinapis arvensis* and *Eruca vesicaria* are yellow and the flowers of *Lepidium sativum* are slightly pink.

The phenetic relationships between the studied species and their link with reproductive system was carried out by two multivariate methods, Principal Component Analysis (PCA) and Hierarchical Ascending Classification (CAH). These analyses separate the compatible species from the self-incompatible species based on some floral characters. The PCA is carried out from the raw data matrix of the 11 floral characters. Together, the two first components explained 78.08% of the total variation with 62.48% and 15.69% for Fact 1 and Fact 2 respectively. The values of contribution of the characters to the formation of Fact 1 and Fact 2 are represented in *Table 6*.

Table 5. *Mean, standard deviation and coefficient of variation of intraspecific floral traits*

Species	**Characters**										
	FD	**SD**	**CD**	**CL**	**PL**	**PW**	**SSL**	**RSP**	**SS**	**NS**	**CO**
Capsella bursa-pastoris	**2.22** ± 0.22	**0.85** ± 0.09	1.28 ± 0.29	1.23 ± 0.24	**1.55** ± 0.11	**1.00** ± 0.00	1.23 ± 0.16	**1.02** ± 0.07	**0.02** ± 0.00	**0.00** ± 0.00	1.00 ± 0.00
	0.10	***0.11***	*0.23*	*0.20*	*0.07*	*0*	*0.13*	*0.07*	*0*	*/*	*0.00*
Eruca vesicaria	**23.1** ± 1.45	1.13 ± 0.61	3.53 ± 0.37	**10.57** ± 0.67	**10.45** ± 0.86	**7.3** ± 0.78	**11.6** ± 0.73	1.17 ± 0.12	0.3 ± 0.05	**0.31** ± 0.07	3.00 ± 0.00
	0.06	***0.54***	*0.10*	*0.06*	*0.08*	*0.11*	*0.06*	*0.10*	*0.17*	*0.23*	*0.00*
Lepidium sativum	2.58 ± 0.25	1.65 ± 0.39	**1.70** ± 0.38	1.18 ± 0.24	1.67 ± 0.27	1.03 ± 0.11	1.97 ± 0.13	1.04 ± 0.23	0.03 ± 0.01	**0.00** ± 0.00	4.00 ± 0.00
	0.10	*0.24*	*0.22*	*0.20*	*0.16*	*0.11*	*0.07*	*0.22*	*0.33*	*/*	*0.00*
Lobularia maritima	5.08 ± 0.43	1.13 ± 0.33	1.93 ± 0.32	**0.98** ± 0.05	2.05 ± 0.30	1.98 ± 0.28	**1.18** ± 0.20	**2.16** ± 0.63	0.03 ± 0.01	0.02 ± 0.00	1.00 ± 0.00
	0.08	***0.29***	*0.17*	*0.05*	*0.15*	*0.14*	*0.17*	***0.29***	***0.33***	*0*	*0.00*
Sinapis arvensis	15.42 ± 2.18	**2.55** ± 0.49	**4.47** ± 1.05	4.40 ± 0.67	6.78 ± 0.57	4.95 ± 0.24	5.13 ± 0.48	**1.02** ± 0.13	**0.32** ± 0.10	0.14 ± 0.02	2.00 ± 0.00
	0.14	*0.19*	*0.23*	*0.15*	*0.08*	*0.05*	*0.09*	*0.13*	*0.31*	*0.14*	*0.00*
P value	2.53E-09	1.55E-06	2.98E-08	1.47E-08	7.92E-09	1.20E-09	3.68E-09	7.26E-06	7.03E-08	2.12E-06	5.84E-10
Significance	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**

FD: Flower diameter; SD: Stamen distance; CD: Corolla tube diameter; CL: Corolla tube length; PL: Petal blade length; PW: Petal blade width; SSL: Short stamen length; RSP: Ration of LSL/PiL; SS: Stigma surface; NS: Nectar gland surface; CO: Color of flower. mean ± standard deviation. In italics, the coefficient of variation; s: significant difference based on Kruskal–Wallis test, $\alpha = 0.05$

Table 6. *Loading of the floral characters on the first two Fact axes of five Brassicaceae species. Loading higher than 0.6 are indicated in boldface*

Abbreviations	**Characters**	**Fact 1**	**Fact 2**
FD.	Flower diameter	**0.988299**	-0.055043
SD.	Stamen distance	0.318372	0.185252
CD.	Corolla tube diameter	**0.830459**	-0.060338
CL.	Corolla tube length	**0.929270**	0.071471
PL.	Petal blade length	**0.981100**	0.006031
PW.	Petal blade width	**0.982921**	-0.076993
SSL.	Short stamen length	**0.936831**	0.105511
RSP.	Ration of LSL/PiL	-0.198377	**-0.830103**
SS.	Stigma surface	**0.906088**	0.024316
NS.	Nectary surface	**-0.703391**	**0.637578**
CO.	Color of flower	0.283846	**0.746137**

The distribution of the characters on the circle of the correlations is given in *Figure 4A*. It shows that the majority of the characters strongly contribute to the formation of the Fact 1. The most significant characters are: Flower diameter (FD), Corolla tube diameter (CD), Corolla tube length (CL) Petal blade length (PL), Petal blade width (PW), Small stamen length (SSL), Stigma surface (SS). With respect to Fact 2, only two characters are significant: Stamen/pistil ratio (RSP), Color of flower (CO). The character of nectar gland surface (NS), contributes to the formation of Fact 1 and Fact 2. The distribution of individuals on the plane 1–2, allows to observe the relationships and similarities between individuals and species (*Figure 4B*).

In this analysis, three sets are distinguished: The first set (S1) groups the self-incompatible species distributed into two subsets in the positive part of a Fact 1; the first subset contains the individuals of *Eruca vesicaria* and the second groups the individuals of *Sinapis arvensis*. These two species are characterized by a large flower. The second set (S2), located in the positive part of Fact 2 regroups the individuals of the two self-compatible species *Capsella bursa-pastoris* and *Lepidium sativum* distributed in two groups, they are distinguished by insignificant nectar glands and reduced flower size. The third set (S3), groups the individuals of the partially self-compatible *Lobularia maritima* in the negative part of Fact 1 and Fact 2. *Lobularia maritima* is isolated from the other two groups by particularly smaller pistil than the stamen and intermediate flower size.

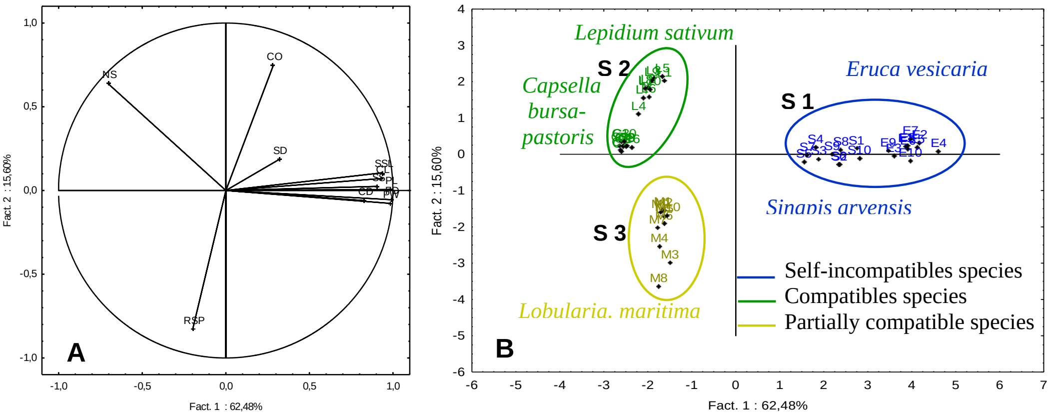

Figure 4. *Principal component analysis of five Brassicaceae species based on 11 floral characters. A – Loading of the 11 variables on the main components (see Table 1 for abbreviations), B – Global point cloud of 50 individuals representative of all species (Set S1: E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, S1, S2, S3, S4, S5, S6, S7, S8, S8, S9, S10; Set S2: C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, L1, L2, L3, L4, L5, L6, L7, L8, L9, L10; Set S3: M1, M2, M3, M4, M5, M6, M7, M8, M9, M10). The circles correspond to the grouped individuals according to their self-incompatibility level*

A hierarchical ascending classification (CAH) was also carried out from the matrix of morphological data. The dendrogram (*Figure 5*) was constructed from the Euclidean distances of individual mean values reveals the same groups identified in the ACP analysis. It reveals a distribution into three sets of individuals. It is noticed that the first set includes the self-incompatible species: *Eruca vesicaria* and *Sinapis arvensis*. The second consists of the compatible species: *Capsella bursa-pastoris* and *Lepidium sativum*. The third set is located in an intermediate position between the other two sets, it contains the individuals of the partially self-compatible *Lobularia maritima.*

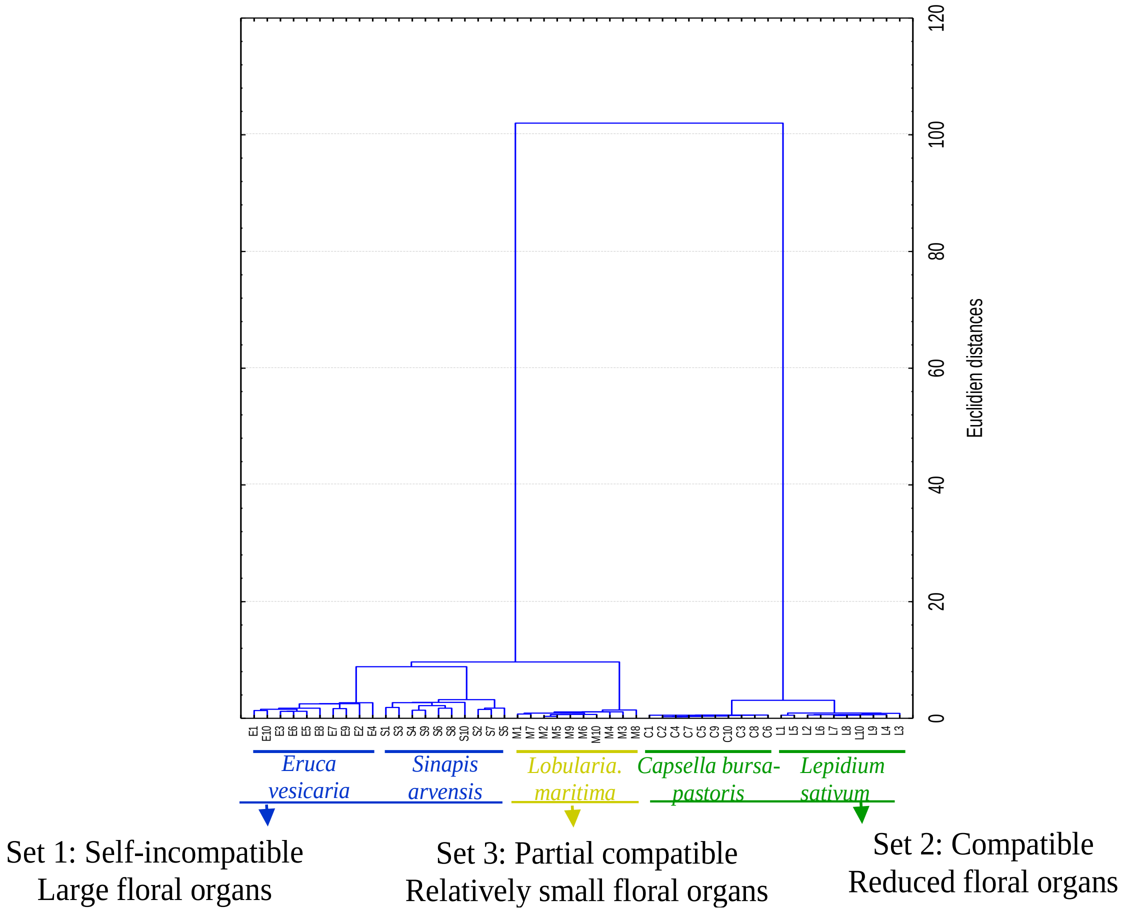

***Figure 5.** Dendrograms showing the relationships between five Brassicaceae species, based on 11 floral traits*

Discussion

The pollination allowed us to determine the level of self-incompatibility of *Capsella bursa-pastoris*, *Eruca vesicaria*, *Lepidium sativum*, *Lobularia maritima* and *Sinapis arvensis* species.

In *Eruca vesicaria* and *Sinapis arvensis*, the self-pollen was rejected on the stigmatic surface for almost all self-pollinations. This results from a blockage or rapid cessation of the growth of the pollen tubes on the surface of the stigma. These two species would therefore be very self-incompatible. Several studies have already reported the self-incompatibility of these two species (Ford and Kay, 1985; Sun et al., 2005; Sihag, 2023).

In *Capsella bursa-pastoris* the most of the plants accept their own pollen and the pollen tubes penetrate the style and the ovary. The loss of self-incompatibility in *Capsella* is well-documented (Guo et al., 2009) and is attributed to its allopolyploid nature, as polyploidization can induce the breakdown of the SI mechanism (Montalt et al., 2022). The loss of SI in this allotetraploid species is due to the loss of the male determinant: the SCR gene (Bachman et al., 2019).

The majority of *Lepidium sativum* individuals accept also their own pollen and the pollen tubes penetrate the style, this was shown by Bateman (1955). However, according to Sampson (1962) *Lepidium sativum* is a moderately crosspollinated species with a self-incompatibility system. In this species there must be self-incompatibility and compatibility according to populations and individuals, as has been described in many species. Although being one of the important vegetable crops with considerable medicinal value, our knowledge about its self-incompatibility system is limited. The self-compatibility of this population may be due to the non-functional of the S-locus or the regulatory genes.

Lobularia maritima exhibits a partially compatible system. While Gómez (2000) reported this species as self-compatible, our observations reveal a more complex situation where both self-compatible and self-incompatible individuals coexist within population. This partial self-compatibility allows for a mixed reproductive system combining outcrossing and autonomous selfing, which provides reproductive assurance when pollen availability is limited. The selfing experiments confirmed this mixed-mating, demonstrating that *Lobularia maritima* is not completely self-incompatible, it presents a degree of self-compatibility (Borgen, 1984).

Variable levels of self-incompatibility are frequent and have already been described in the Brassicaceae family. Within the genus *Brassica*, for example, *B. rapa* showed populations that consist of both selfing and self-incompatible plants (Aïssiou et al., 2018) and the closely related species, *B. oleracea*, revealed partially self-compatible cultivars while the wild cabbage exhibited a great fraction of self-incompatible plants (Hadj-Arab et al., 2010, 2019). Durant et al. (2020) believe that most SC populations or species result from relatively recent shifts from SI to a SC status with a mixed-mating or selfing reproductive system. This may be the case of the *Lobularia maritima* population analysed in this study as it contains many partially compatible individuals in addition to the incompatible ones. The partial self-incompatibility conferring a mixed matting system is a successful strategy as reported by Rios-Carrasco and Vasquez-Santana (2022). Self-incompatible weeds such as *Eruca vesicaria* and *Sinapis arvensis* create populations with greater genetic diversity through increased gene flow. Conversely, *Capsella bursa-pastoris*, exhibits self-compatibility enabling individual plants to establish new populations independently, facilitating rapid colonization and local adaptation, but with limited diversity.

According to the extensive studies on self-incompatibility in Brassicaceae, it would seem that there is a close correlation between the reproductive system of a species and the visibility of its flowers (Tsuchimatsu and Fujii, 2022). These two characters are related as the self-compatible species generally have smaller and more discreet flowers than the self-incompatible species (Bateman, 1956). Goodwillie et al. (2010), demonstrated that the variation in floral dimensions is often associated with changes in reproductive strategies, including shift in pollinator type or shift from outbreeding to selfing.

Large flowers with large petals create natural landing zones that facilitate insect pollinator visits. These broad surfaces make it easier for insects to guarantee cross-pollination (case of *Eruca vesicaria* and *Sinapis arvensis*). *Capsella bursa-pastoris* and *Lepidium sativum* have a very small flower and are compatible so they would reproduce preferentially by selfing. *Lobularia maritima* presenting a mixed reproductive system (selfing/out-crossing) showed relatively intermediate floral characters comparing to the other species but forms a compact inflorescence that can look like a solitary showy flower, this dense arrangement effectively attracts insect pollinators. The outcrossing rate is positively associated with the product of flower size and number (Goodwillie et al., 2010).

The floral size reduction, which includes reduction in petal size is commonly associated with the transition from outcrossing to selfing as part of the so-called “selfing syndrome” (Sicard and Lenhard, 2011).

The difference in the size of the flower observed in the species, is also observed in the surface of a stigma (SS) and a nectar gland (SN). *Eruca vesicaria* and *Sinapis arvensis* show higher values for these characters than *Lobularia maritima*, *Capsella bursa-pastoris* and *Lepidium sativum*. Their large stigma surface enables them to capture more pollen grains, increasing the likelihood of successful pollination (Kobayashi et al., 2006). Similarly, this makes them more attractive to pollinators because they offer more nectar rewards. This increased attractiveness can lead to more frequent visits by pollinators. In many genera, self-incompatible species typically display adaptations favoring insect pollination, such as nectaries and conspicuous flowers, whereas the self-compatible species have partially or completely lost these adaptations and have evolved devices to ensure the success of self-pollination (Lloyd, 1965). In *Arenaria uniflora*, for example, Wyatt (1984) reported that the nectaries are extremely reduced and perhaps non-functional in selfing populations.

The floral color plays a crucial role in modulating plant–pollinator interactions. In this study, self-incompatible species (*Sinapis arvensis* and *Eruca vesicaria*) unlike compatible species (*Lepidium sativum* and *Capsella bursa-pastoris*) attract pollinators due to their important nectar glands and then probably copious nectar production but also for their bright colors (yellow). This allows species to increased pollen transfer between flowers, enhancing seed production and leads to genetic diversity. In *Camissoniopsis cheiranthifolia*, Button et al. (2012) observed parallel changes in both petal size and coloration: self-fertilizing populations typically exhibited smaller petals with decreased brightness and chroma.

The degree of separation of the stamens from the stigma determines the fitness to self-pollination (Toräng et al., 2017). A position of the stigma higher than the anthers tends to reduce self-pollination and to increase the rate of cross-pollination (Kobayashi et al., 2009; Nasrallah, 2023). Our results indicate that self-incompatible species, *Eruca vesicaria* and *Sinapis arvensis*, have stamens and stigma almost in the same level, thus ensuring self-pollination in environments that are pollen limited leading to self-fertilization. Potentially in certain conditions, such as during flower ageing (Hadj-Arab et al., 2010).

Self-compatibles species: *Capsella bursa-pastoris* and *Lepidium sativum* have stigma and anthers at the same height, this arrangement facilitates self-pollen deposition. *Lobularia maritima* has the anthers higher than the stigma, this position promotes the deposition of self-pollen.

The number of ovules showed a difference according to the species, this parameter allowed us, after counting the seeds, to estimate the number of seeds produced relative to the number of ovules. After counting the seeds, all species studied showed that the majority of the ovules produce seeds (>80%) in the natural conditions. This means that pollination was effective, leading to successful fertilization of ovules. This is confirmed by the number of pollen tubes penetrating the style which exceeds 36 pollen tubes in all species, under natural conditions (NP)

These results identify the key morphological traits associated with the level of self-incompatibility, which represents an important foundation for future studies. Using molecular marker analysis across populations could confirm the morphological results and would certainly provide valuable additional insights of their genetic diversity.

It would be valuable to expand this research with a broader sampling particularly the partial self-incompatible *Lobularia maritima* populations to better understand how these plants combine different reproductive strategies.

Conclusion

This study reveals a great variation of the SI level and a variation of the floral traits depending on the species. Some studied floral traits differentiate the self-compatible and self-incompatible species, they would be adaptations corresponding to the strategies of allogamy and autogamy. The number of seeds produced under natural conditions indicates the efficiency of pollination under natural conditions and successful fertilization of ovules for all studied species. These findings provide an overview of the importance of the floral structure on the reproductive system.

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APPENDIX

Results of Dunn comparison test

Species	Characters									
	Flower diameter FD	**Stamen distance SD**	**Corolla tube diameter CD**	**Corolla tube length CL**	**Petal blade length PL**	**Petal blade width PW**	**Short stamen length SSL**	**Ration of stamen/Pistil length RSP**	**Stigma surface SS**	**Nectar gland surface NS**	**Color of flower CO**
	P value	***P value***	***P value***	***P value***	***P value***	***P value***	***P value***	***P value***	***P value***	***P value***	***P value***
Capsella bursa-pastoris - Eruca vesicaria	1.32E-05***	1 ns	4.81E-05***	0.000351563***	1.98E-07***	2.01E-07***	1.57E-06***	0.654493895 ns	5.85E-05***		0.000595024***
Capsella bursa-pastoris - Sinapis arvensis	0.009638103**	0.010629983*	1 ns	1 ns	1 ns	1 ns	0.304586053 ns	1 ns	1.47E-05***		0.16002207 ns
Capsella bursa-pastoris - Lobularia maritima	0.77464887 ns	1 ns	0.332630842 ns	1 ns	0.262585211 ns	0.142874928 ns	1 ns	0.000226521***	1 ns		1 ns
Capsella bursa-pastoris - Lepidium sativum	1 ns	2.72E-06***	6.02E-07***	0.105240102 ns	0.000479696***	0.000556423***	0.002119405**	1 ns	1 ns		1.90E-07***
Eruca vesicaria - Sinapis arvensis	1 ns	0.517206612 ns	0.011815538*	7.87E-05***	8.12E-06***	4.95E-07***	0.020663004*	0.419445899 ns	1 ns	0.031767312*	1 ns
Eruca vesicaria - Lobularia maritim	0.021363705*	1 ns	0.145254136 ns	2.07E-07***	0.006947918**	0.015710468*	3.07E-07***	0.16628119 ns	0.004531094**	9.57E-07***	0.000595024***
Eruca vesicaria- Lepidium sativum	3.40E-08***	0.001369002**	1 ns	1 ns	1 ns	1 ns	1 ns	0.579724388 ns	0.007870545**		1 ns
Sinapis arvensis- Lobularia maritima	1 ns	0.481172232 ns	1 ns	1 ns	1 ns	0.219119127 ns	0.140209759 ns	9.47E-05***	0.001501852**	0.031767312*	0.16002207 ns
Sinapis arvensis - Lepidium sativum	0.000121105***	0.617270866 ns	0.000433788***	0.038557663*	0.007146853**	0.00107722 **	1 ns	1 ns	0.002715559**		0.013189686 *
Lobularia maritima - Lepidium sativum	0.045071864*	0.001207577**	0.010039793*	0.000565727***	0.653380056 ns	1 ns	0.000641524***	0.000178083***	1 ns		1.90E-07***

P: significance level; *p≤0.05; **p≤0.01; ***p≤0.001; ns: non-significant

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