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Euphytica **53**: 41–48, 1991.

The reproductive biology of okra. 1. Study of the breeding system in four *Abelmoschus* species

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Received 21 September 1990; Accepted 15 November 1990

Key words: Abelmoschus species, okra, reproductive biology, pollen, ovules

Summary

The breeding system of *Abelmoschus* species is not well known. Although being self-compatibles allogamy reaches 63%. The aim of the study was to place four *Abelmoschus* species on the log P/O scale devised by Cruden (1977). On this scale P = pollen grains per anther × anthers per flower and O = ovules production. Pollens and ovules productions were therefore assessed for 68 accessions of two cultivated species, *A. esculentus* and *A. caillei*, and two wild, *A. manihot* and *A. moschatus*. Extreme log P/O values ranged from 1.69 for the variety Clemson Spineliss (*A. esculentus*) to 2.94 for ORS 278 (*A. manihot*). Averages of wild species *A. moschatus* (2.17) and *A. manihot* (2.19) indicate facultative autogamy. Averages for cultivated species *A. esculentus* (2.00) and *A. caillei* (2.05) suggest more autogamy.

Introduction

The *Abelmoschus* genus (*Malvaceae*) is found throughout the tropical zone. According to Borssum Waalkes (1966) it consists of six species even if the systematic organization appears to be more complex (Charrier, 1984; Hamon, 1988). Two species are cultivated for their unripe pods and sometimes for their leaves. The most widespread and well known is *A. esculentus*. The other species, *A. caillei*, was recently described (Stevels, 1988) and its distribution boundaries (W. and Central Africa) have recently been defined (Hamon & Van Sloten, 1989).

Abelmoschus species are self-fertiles but they express allogamy levels up to 63% (Martin, 1983). Hermaphrodites flowers display entomophilous features such as large and coloured petals, nectar, extrorse dehiscent anthers, ornamented sticky pollen grains, visible coloured stigmata. Ornduff (1969) and later Wyatt (1983) proposed a list of typical morphological characters distinguishing autogamous from allogamous plants. They are reported in Table 1 where features of okra are marked by an asteriks (*). Okra appears to be in a middle position and difficult to classify.

Cruden (1977) examined a great number of species and considered that pollen production (P) and ovule production (O) are closely correlated with the breeding system. He proposed, based on the parameter log P/O, a scale of evaluation which runs from 0.65 (cleistogamy) to 3.65 (xenogamy).

In this paper we examine the breeding system of four *Abelmoschus* species. Two species are only cultivated (*A. esculentus* and *A. caillei*). Two (*A. moschatus* and *A. manihot*) have wild and cultivated forms but the studied accessions, mainly collected in Thailand (Hamon et al., 1987) are only wild.

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Material and method

Material

In total, 68 accessions were used: 11 accessions of *A. esculentus*, 10 of *A. caillei*, 25 of *A. moschatus* and 22 of *A. manihot* (Table 2). The material was from the world okra collection managed by ORSTOM under the auspices of the International Board for Plant Genetic Resources (IBPGR). Most of the wild samples were collected in Thailand (Hamon et al., 1987).

General structure of okra flowers

Flowers of the *Abelmoschus* species are axillary and solitary. They are large, hermaphrodite and ephemeral. The style is centrally positioned, several centimetres long and protected by the stamen column. Anthers (about 100) are arranged in superposed concentric circles on the latter. Stigmas are grouped in cultivated forms; they are located above the anthers and are not necessarily in contact with the stamens at anthesis. Growth of the upper part of the stamen column causes contact mechanically between the upper stamens and the edge of the stigma. The ovary is inferior and comprises at least five carpels each containing ten to twenty ovules. Some cultivated forms display up to twelve carpels. The flower bud, to open on a given day, is easy to identify on the preceding day as it swells considerably. Anthesis takes place at the end of the night. The flower is open at dawn, remains open all morning and closes in the middle of the afternoon. It is wilted in the evening and the petals fall the next day. When a plant begins to flower (about two months after sowing), it continues to produce two or three flowers a week for one to two months. Young pods grow immediately. Harvesting for consumption is carried out three to four days after flowering. Growth then slows and the final fruit

Table 1. Characteristics that often differ between outcrossing species and their selfing derivates (according to Wyatt, 1983).

Characteristics of outcrossers	Characteristics of selfers
Self-incompatible	* Self-compatible
Flowers many	Flowers fewer
Pedicels long	* Pedicels shorter
Sepals large	* Sepals smaller
Corolla rotate	* Corolla funnelform, cylindric, or closed
* Petals large	Petals smaller
Petals emarginate	* Petals entire
* Nectaries present	Nectaries reduced or absent
Flowers scented	Flowers scentless
Nectar guides conspicuous	Nectar guides absent
Anthers long	* Anthers shorter
* Anther dehiscence extrorse	Anther dehiscence introrse
Anthers distant from stigma	* Anthers adjacent to stigma
* Pollen grains many	Pollen grains fewer
Pollen presented	Pollen not presented
* Pistil long	Pistil shorter
Stamens longer or shorter than pistil	* Stamens equal in length to pistil
Style exerted	* Style included
* Stigmatic area well defined, papillate	Stigmatic area poorly defined, less papillate
Stigma receptivity and anther dehiscence – non synchronous	* Sigma receptivity and anther dehiscence synchronous
* Many ovules per flower	Fewer ovules per flower
Many ovules not maturing to seed	* All ovules maturing to seed
Some fruits not maturing	* All fruits maturing
Narrow distribution	* Wide distribution

NB: 20 Features of okra are marked by a (*).

size (10 to 45 cm) forms a varietal criterion. However, full maturity necessary for seed production is only attained after a month.

Experimental site and period

The work was carried out at the ORSTOM station at Adiopodoumé, 20 km south of Abidjan (Côte d'Ivoire). The climate is the humid tropical type with two rainy seasons. Trials were carried out in an open field from October to December. This period of the year is the best for good plant growth and the period of least virus pressure (Fargette, 1986). Several hundred plants from the same accession were sown over a period of a month to produce an unlimited quantity of flowers.

Estimation of the breeding system

Flower buds were collected during the evening before anthesis. The stage is easy to identify by sudden, strong swelling. Buds were collected from plants at the intense flowering phase. The anthers were removed and counted per flowers in twenty flowers per accession. The number of ovules per flower was counted by dissecting the carpels one by one. The anther population, previously obtained, was mixed. Thirty anthers were taken at random and the number of pollen grains was counted in each one.

Data representations

The value of log P/O, (P is the number of pollen grains per anther multiplied by the number of anthers per flower and O is the number of ovules per flower) was calculated for each accession. Number of pollen grains per anther, anthers per flower and ovules per flower were also analysed using principal component analysis (NDMS program, Noirot et al. 1990).

Table 2. Okra accessions used for the study of the breeding system

A. esculentus (cultivated)(11)	Clemson Spineless (Commercial seeds) ORSTOM – IBPGR accessions from: Benin (1347, 1355, 1467, 1506); Togo (803, 958, 1043, 1051, 1159); Sudan (2137).
A. caillei (cultivated) (10)	ORSTOM – IBPGR accessions from: Benin (1572); Guinea (2415); Ivory Coast (372, 407, 451, 520); Togo (890, 1002, 1026, 1260).
A. moschatus (wild)(25)	ORSTOM – IBPGR accessions from: USDA Puerto Rico (280, PI 1193); Thailand(3230, 3231, 3232, 3233, 3234, 3235, 3236, 3237, 3239, 3240, 3242, 3244, 3245, 3246, 3247, 3249, 3250, 3253, 3255, 3256, 3275, 3280, 3292, 3293.
A. manihot (wild)(22)	ORSTOM – IBPGR accessions from: USDA Puerto Rico (278, PI 1191 – 592) Thailand (3241, 3243, 3257, 3258, 3260, 3262, 3263, 3264, 3265, 3269, 3271, 3273, 3274, 3276, 3277, 3281, 3285, 3286, 3288, 3290.

Collecting Missions: Guinea (Hamon et al. 1985)
Sudan (Hassan et al. 1983)
Thailand (Hamon et al. 1987)
Togo and Benin (Hamon & Charrier 1983)

Results

Sexual reproductive resources

Table 3 shows the average number of anthers per flower, pollen grains per anther and ovules per flower for each species. Minima, maxima and coefficients of variation (CV) are mentioned for each variable. Calculated values of the ratio log P/O are shown in the right margin.

Average numbers of ovules per flower per species varied from 84 (*A. esculentus*) to 144 (*A. moschatus*). In cultivated forms CV are greater than in wild (25% and 10%). The smallest ovule number (40) was found in one accession of *A. manihot* (ORS 278). The highest number (174) was observed in one accession of *A. moschatus* from Thailand (ORS 3246). Average numbers of anthers per flower varied between 105 (*A. manihot*) and 117 (*A. esculentus*). This is fairly stable since the majority of accessions possesses slightly over a hundred anthers per flower. The minimum observed (79) was on a variety of *A. caillei* (ORS 1026) and the maximum (150) on a variety of *A. esculentus* (ORS 1159). The general average number of pollen grains per anther, depending on the species, ranges from 72 (*A. esculentus*) to 203 (*A. moschatus*). The lowest number (61) was observed in *A. esculentus* (Clemson Spineless) and the maximum in *A. manihot* (ORS 278). Comparing with cultivated species, wild species produce more than twice pollen grains per anther.

Concerning log P/O, cultivated species have identical averages (2.00 and 2.05). In the same way, wild species have equivalent ratios (2.17 and 2.19). Calculated values for wild species are slightly greater than for cultivated. At the individual level, the lowest ratio (1.69) was observed in the U.S. variety Clemson Spineless (*A. esculentus*), the highest (2.94) was found for the accession ORS 278 (*A. manihot*). It should be noted that 90% of accessions have ratios comprise between 2.00 to 2.50. In Fig. 1, built from Cruden's estimates (1977), are plotted *Abelmoschus* species averages. Corresponding reproductive behaviour is situated near facultative autogamy for wild species and go towards obligate autogamy for cultivated.

Principal component analysis

The study of the three variables (pollen, anthers, ovules) is performed by examining them simultaneously in a principal component analysis. Simple correlation coefficients are statistically significant (5% level) for pollen-anthers (− 0.34) and pollen-ovules (0.42) but not for anthers-ovules (− 0.15). The factorial plane (1*2) is shown in Fig. 2. It accounts for 84% of total variation.

Along axis 1, the small number of pollen grains per anther is combined with a small number of ovules. Along axis 2, the high number of ovules is combined with a large number of anthers per flower. This spatial representation gives an interesting image of the multispecific context. The three point scatters defining respectively *A. moschatus, A. manihot* and the cultivated forms are strongly individualised along axis 1. Accessions of *A. manihot* lie in a central position between cultivated forms (positive values on axis 1) and *A. moschatus*. Only two exceptions are noted: one, an *A. manihot* accession (ORS 592) is located in the scatter defined by *A.*

Table 3. Sexual reproductive resources in four *Abelmoschus* species

Species		Anther per flower				Pollen per anther				Ovule per flower				Log. (P/O)
Name	Status	Mean	C.V. (%)	Min.	Max.	Mean	C.V. (%)	Min.	Max.	Mean	C.V. (%)	Min.	Max.	
A. esculentus	Cult.	117	12	100	150	72	10	61	86	84	25	62	124	2.00
A. caillei	Cult.	115	20	79	142	92	20	69	112	93	27	60	130	2.05
A. moschatus	Wild	106	7	93	122	203	15	127	265	144	8	127	174	2.17
A. manihot	Wild	105	23	118	126	156	16	104	385	105	12	40	145	2.19

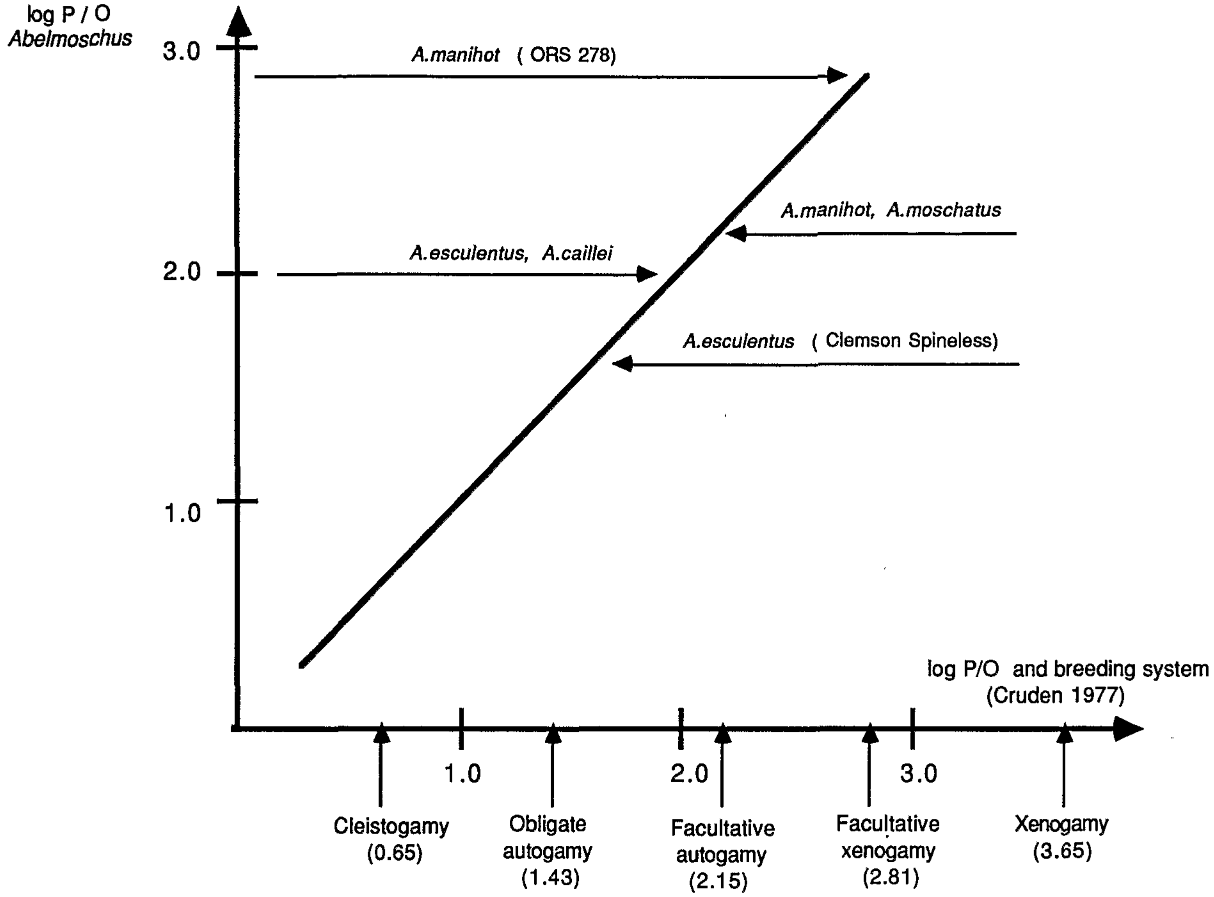

Fig. 1. Estimation of *Abelmoschus* breeding system according to Cruden parameter

moschatus; the other is an *A. caillei* accession (ORS 520) which lies in the scatter defined by *A. manihot*. This factorial plane allows a good discrimination between species and shows the effect of domestication.

Discussion and conclusion

Okra breeding system estimated for four species was assessed using the log P/O criterion defined by Cruden (1977) for other plants. Estimated values placed the genus in a middle position between cleistogamy and xenogamy. The real position is near a facultative autogamy mode.

These observations are in line with those outlined in Table 1 related to the use of morphological characters (Wyatt, 1983). Martin (1983) reported large variations in allogamy rates for *A. esculentus*. The maximum (60%) noted by this author is in accordance with our results, meanwhile, allogamy is often lower and reaches only a few per cents. Okra flowers are self-compatible. So at least four conditions must be fulfilled for self-fertilization to be effective: 1) ovules must be receptive, 2) anthers must be dehiscent, 3) the amount of pollen required for fertilization must have been deposited on the stigmata, and 4) it must have had the time to fertilize ovules.

Flowers open at dawn, so it is possible that all these conditions may be fulfilled in a fair number of cases. An other explanation could be the lack of allopollen arrival during the day, leading to full self-fertilization. Okra flowers have a style in a central position surrounded by a stamen column. Pollen grains are sticky and are not necessarily, at

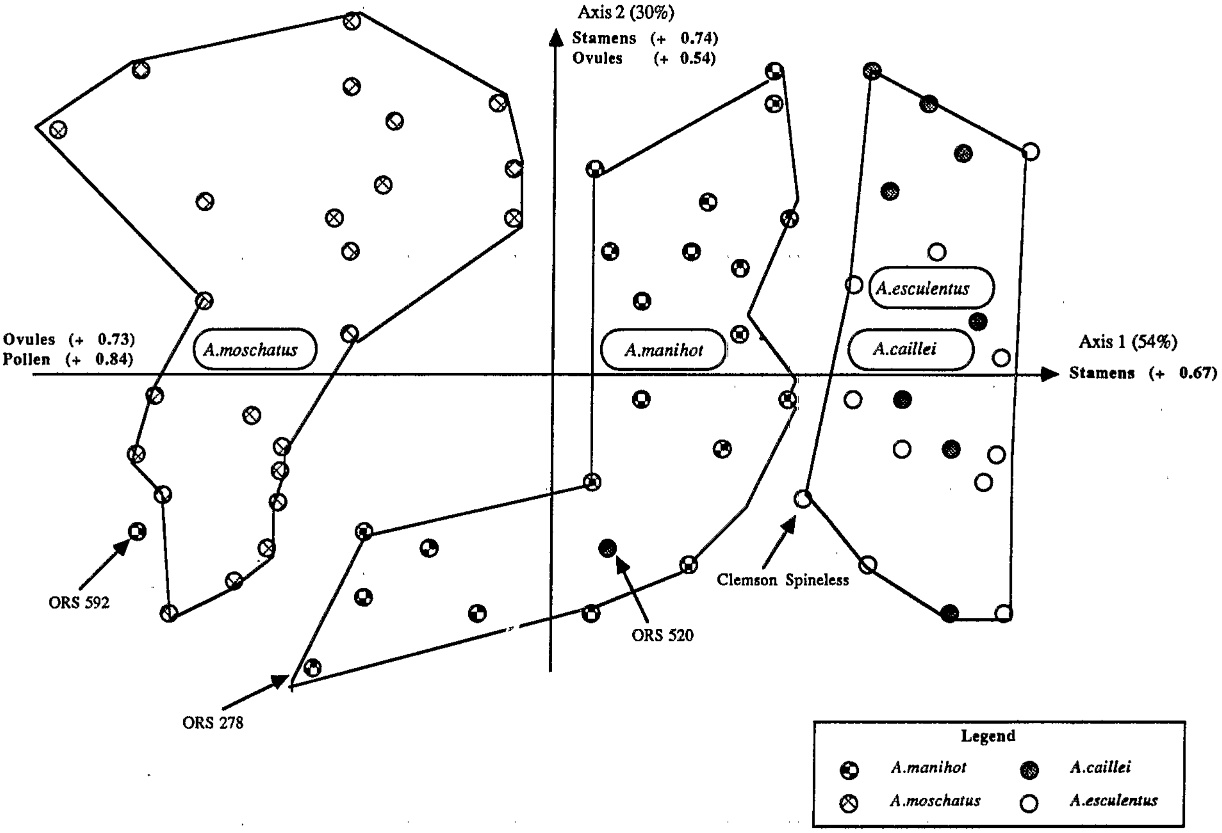

Fig. 2. Multivariate analysis of sexual reproductive resources for four *Abelmoschus* species

the anthesis, in contact with the stigma. Contact takes place, mechanically, during the day by growth of the stamen column. Suppose: 1. there are small differences, between varieties, in the distance separating the upper circle of stamens and the receptive surface of the stigma; 2. the elongation rate of the stamen column is not the same for all. In these conditions first self-pollen grains may arrive, in contact with the stigma, at different times leaving a favourable period for allogamy.

A new question now emerges: how many germinated pollen grains are necessary to ensure total fertilization? Ter Avenesian (1978) considered that an average of seven pollen grains are required for one ovule to be fertilized. As seen above, there are 40 to 160 ovules per okra flower. If this seven is true for *Abelmoschus* 280 to 1120 pollen grains are therefore required. As there are about a hundred pollen grains per anther, the pollen of 3 to 11 anthers could be enough. Anthers are arranged in concentric superimposed circles (5 or 6 anthers per circle). The first value (3) is not limiting but the second (11) may become so. It is clear that enough pollen is produced by each flower but only a small proportion participates in self-fertilization. The percentage of participation may be even smaller in some wild forms leading to a higher level of potential allogamy. Stigmas, which are joined in cultivated species, are more or less separated and located much further from anthers, as can be seen in ornamental *Hibiscus*, and self-fertilization could be more difficult. Going against there is sometimes, like for ORS 278, a smaller number of ovules available, consequently the need of pollen grain germination could be lower.

Wild *Abelmoschus* species have only five carpels (Borssum Waalkes, 1966). Domestication has resulted in a larger number of carpels (up to 11) especially in the cultivated species *A. esculentus* (Hamon, 1988). This should apparently enhance the female reproductive aspect. It has been seen that this is not the case since wild species have frequently more ovules per flower than cultivated forms. The feature which characterizes cultivated forms and which is particularly marked in Clemson Spineless is the small pollen production.

Okra is attractive to a broad range of insects. They are frequently preferentially phytophagous, as can be seen by strongly attacked leaf blades and even torn flowers. Between them, pollen-carriers include bees. Tanda (1985) has shown that in okra plantations bees do not finally disperse pollen between plants for a radius of more than six metres. This is nevertheless enough for allo-fertilization and hybrid production. Everything probably depends on the time at which they are in flowers. Valero et al. (1986) studied, in populations of two *Lathyrus* species, the dynamics of insect movements during the day. These authors show that weevils on the one hand and bees and bumble bees on the other do not display the same behaviour pattern. Weevils (*Bruchus*) make long, repeated visits (up to three hours per flower) generally in the morning, thus contributing to self-fertilization. In contrast bees and bumble-bees make only short and frequent visits and above all during the afternoon. Thus they contribute to allo-fertilization.

In the south part of Ivory Coast, a high density of few active dipters are often seen inside the corolla of okra flowers, just after anthesis. They seem to stay all day long in the flower and could help for self-pollination. In the morning, the external temperature is relatively low (20–24° C), it increases rapidly, at 10.00 hr, to 28–30° C. Simultaneously insects, like bees, become more active. So, in our experimental conditions, allogamy may arise if the self-pollination is not fully effective at 10.00 hr. This is really in accordance with breeding system but does not explain the pollination mechanism.

In the second part of our study we attempt to explain how the self-pollination takes place during the morning and how it is possible to use it to improve controlled hybrid production.

Acknowledgement

We are grateful to Dr. P. Hamon (Université Montpellier III, France) who critically corrected the manuscript. A part of this work has been financed by the International Board for Plant Genetic Resources (IBPGR).

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