5

Euphytica **53**: 49–55, 1991.

The reproductive biology of okra. 2. Self-fertilization kinetics in the cultivated okra (*Abelmoschus esculentus*), and consequences for breeding

Serge Hamon & Jean Koechlin
Laboratoire de Ressources Génétiques et d'Amélioration des Plantes Tropicales. ORSTOM B.P. 5045. 34032 Montpellier Cedex 1, France

Received 21 September 1990; accepted 15 November 1990

Key words: Abelmoschus esculentus, okra, reproductive biology, pollination, breeding

Summary

The use of Cruden's index (1977), for studying okra sexual reproductive allocations, indicates a facultative autogamy mode. The purpose of the research described is to obtain more accurate information on the self-fertilization process. For this, self-pollen grain germination was stopped at different times of the day. Twelve varieties of *Abelmoschus esculentus* were used. Self-fertilization kinetics – expressed by the setting rate – displayed an increase between 7.00 to 16.00 hr. Study of obtained progenies, by stimulated allogamy, confirmed the process and showed that allo-pollen grains deposited on a stigma after midday had only a very little chance of contributing to fertilization. As a result, the polymorphism of the flower structure and particularly the distance between anther and stigmas, insect types and movement habits play major roles in governing okra allogamy. We also show that self-fertilization kinetics can be used to improve controlled hybrid production.

Introduction

Okra is a tropical vegetable grown throughout the intertropical and Mediterranean area. Little work has been done on the floral biology of the cultivated okra, *Abelmoschus esculentus*. The flower structure combines hermaphrodism and self-compatibility. The 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. So, conditions are favourable for autogamy but, as the process is not mechanically obligatory, opportunity is left for allogamy. Hamon & Koechlin (1991) show that the use of the log P/O, defined by Cruden (1977), positions okra reproductive mode near facultative autogamy.

Concerning the allogamy level, results reported in the literature give contradictory information. Venkatazramani (1953) mentioned anther dehiscence well after anthesis and suggested strongly allogamous behaviour. In contrast Purewal & Randhawa (1957) presumed that there was very strong autogamy. They showed that pollen grain germination begins a few minutes after the contact with stigmas. In their opinion, because the style is thought to be receptive before flower opening, there would be enough time for self pollination to take place before anthesis. This opinion is shared by Srivastava & Sachan (1973) and Chandra & Bhatnagar (1975).

Observed allogamy levels have given very varied results. Chauhan et al. (1968), Choudhury & Choomsai (1970), Shalaby (1972), Mitidieri &

Vencovsky (1974) and Martin (1983) recorded a broad range of variation with levels varying from 0 to 60%.

To account for observed differences, authors mentioned various environmental parameters such as: plant spacing; insect population density; ecological condition at a given site. We are sure that cultivated okra is not strictly autogamous nor allogamous. However, none of the present approaches has been able to give clear information on how pollination actually takes place.

Here we present a simple and original method, using interruption of self-fertilization at different moments, to demonstrate how fertilization kinetics takes place under natural conditions. We confirmed the kinetic profile by forced pollination, at specific times and the study of progenies. In addition, the better understanding of okra breeding gives possibilities of applications for improving hybrid production in some difficult combinations.

Material and methods

Material

Studies were carried out on twelve *Abelmoschus esculentus* varieties. They were all pure lines, morphologically very different and easy to identify. Accessions and their origins are listed in Table 1.

Experimental site and period

The work was carried out at the ORSTOM station at Adiopodoumé (20 km south of Abidjan, Côte d'Ivoire). Experimental conditions were the same as described in Hamon & Koechlin (1991). Several hundred plants from the same accession were sown, over a period of a month, to produce an unlimited quantity of flowers.

Interruption of pollen germination

Pollen germination was stopped in open flowers by cutting the upper part of the style (zone immediately below the stigmas). Thus, in the absence of other phenomena, only germinated pollen tubes, which had passed this point, were able to fertilize ovules. Style cutting was carried out systematically on each variety at 7.00 hr, 9.00 hr 11.00 hr, 14.00 hr and 16.00 hr. For each combination (time × variety) twenty flowers were simultaneously treated. Setting was considered to have taken place when a fruit developed one week after the operation. The setting rate was thus the percentage of flowers producing a fruit after pollination. Setting rate, number and weight of seeds per fruit were recorded.

Artificial allogamy

Flowers whose pollen was to be used were bagged on the preceding day to ensure purity. In bags, anthesis took place normally. Receiving flowers were not emasculated so they can begin to self-fertilize. The availability for allogamy was assessed by placing an excess amount of foreign pollen on the stigmas. To compare with stoppages of pollen germinations, operations were made at the same times. The pollen was chosen in such a way that hybrids could be easily identified in progenies. Varieties used as markers in the trials were: ORS-803,

Table 1. Origin of studied accessions

ORSTOM number	Country of origin	Collector number	Latitude	Longitude
772	Burkina Faso	P 37	14.10 N	01.50 W
803	Burkina Faso	P 135	12.00 N	03.50 W
1043	Togo	HCS 166	09.20 N	00.15 E
1051	Togo	HCS 174	09.30 N	00.15 E
1159	Togo	HCS 237.A	10.50 N	00.05 E
1347	Benin	HAH 82	10.05 N	02.45 E
1355	Benin	HAH 89	10.55 N	02.55 E
1467	Benin	HAH 190	10.40 N	01.20 E
1506	Benin	HAH 223.B	09.30 N	01.40 E
1551	Benin	HAH 255.A	07.00 N	01.45 E
2137	Sudan	HSD 55	13.30 N	33.35 E
Clemson Spineless		Standard cultivar		

Collecting missions: Burkina Faso — Perret (1981) unpublished; Togo, Benin — Hamon & Charrier (1983); Sudan — Hassan et al. (1983)

in which the red stem colour dominates different shades of green; ORS-278, which belongs to *A. tetraphyllus* since the obtaining of F1 hybrids is not limiting with *A. esculentus* (Hamon & Yapo, 1985). Thus we can express proportions of natural autogamy and artificial allogamy. To disturb the natural process, as little as possible, treated flowers were not rebagged. Because of the varietal purity and combinations chosen, possible contamination by foreign pollen would be immediately identified in the progeny.

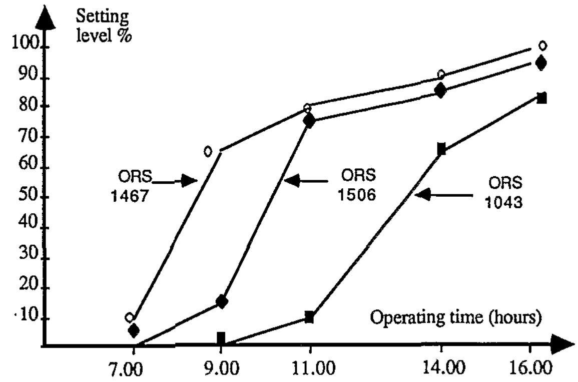

Fig. 1. Variability of fruit setting for three okra varieties.

Results

Blocks in the natural pollination process

The self-fertilization process was stopped artificially in a set of twelve okra cultivars by cutting the style at various times of the day from 7.00 hr to 16.00 hr. At 7.00 hr the average setting rate was very small (1%). It then increased to about 22% at 9.00 hr and 46% at 11.00 hr. Stopping of pollination at 14.00 hr permitted 75% setting. A ceiling about 90% setting was reached towards 16.00 hr. This final percentage is very close to the real setting rate recorded in okra under natural conditions.

This general evolution conceals underlying diversity. For example, the individual behaviour of three varieties are shown in Fig. 1.

We observe that ORS-1467 set fastest, pollen grain germinated earlier and (or) tubes grow most quickly. It was an exception since it was the only one able to set after stoppage at 07.00 hr. ORS-1506 was in an intermediate position and corresponds to the behaviour of most varieties. ORS-1043 and 'Clemson Spineless' were among varieties displaying the slowest setting. The seed composition of the pods show that no completely mature fruit contained less than 30 seeds. This leads to assume a minimum seed formation for setting. ORS-1467, with a rapid setting, appeared to be capable of fruit set with less than 50 seeds. 'Clemson Spineless', which is very slow, requires much greater filling (90 seeds).

Principal component analysis

The analysis was carried out with simultaneous use of the setting rate, the number and weight of seeds per fruit. The factorial plane (1 × 3 – 65% of total inertia) is shown in Fig. 2. Variables are shown by arrows whose direction and length are related to their correlation with factorial axis. Points corresponding to a given time of cutting, independent of the variety, are represented by a small ellipse which becomes darker with cutting time. The cultivar number is shown in the centre. First axis separates the point scatter corresponding to the cutting carried out at 14.00 hr (negative values of axis 1) from those performed between 7.00 hr and 9.00 hr (positive values on axis 1). Setting rate and number of seeds per fruit increased during the day. Nevertheless, the 1000 seed weight (non-albuminous) fell. This shows a decrease in embryo weight. Third axis separates the point scatter corresponding to the cutting carried out at 9.00 hr (positive values for axes 1 and 3) from that of 11.00 hr (negative values on axis 3 and positive values on axis 1). The setting rate increased between these two moments and 1000 seed weight decreased at the same time. The points for the cuts performed at 11.00 hr are the most dispersed in the plane. Indeed, varieties ORS-1355 and ORS-1043, which displayed late setting and had heavier seeds, lie off-centre. ORS-1467, which was very fast, was the only variety able to set from 7.00 hr onwards. Its 11.00 hr point lies within the scatter formed by the varieties at 14.00 hr.

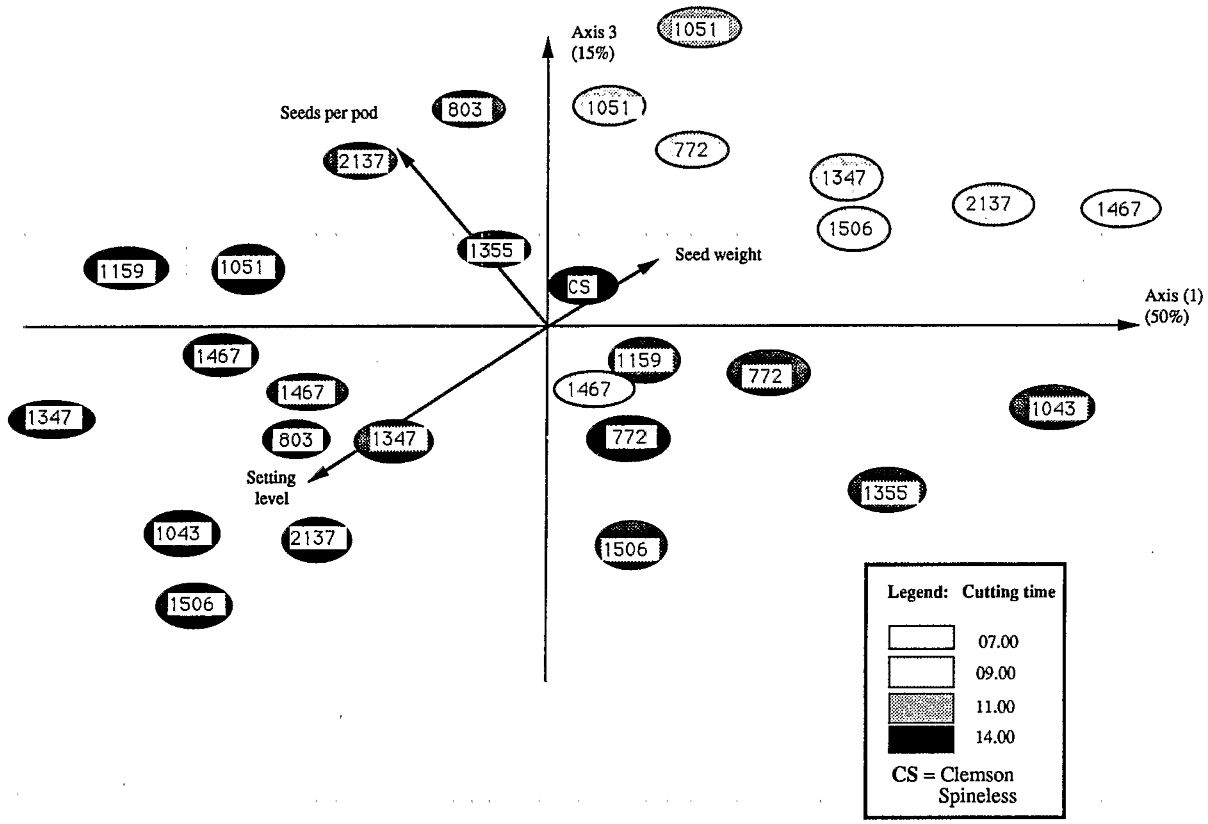

Fig. 2. Multivariate view of okra pod setting.

Artificial allogamy

Several plants of the variety ORS-1506 (green) were pollinated by ORS-803 (red stem colour dominant) every hour from 8.00 hr to 12.00 hr. The relative proportion of hybrids for each situation is shown in Table 2. 37.5% hybrids was obtained at 8.00 hr. This rate fell quite fast. It was only 10% at 11.00 hr and almost zero (0.1%) at 12.00 hr.

'Clemson Spineless' was pollinated by accession ORS-278 (*A. tetraphyllus*) between 6.00 hr and 9.00 hr. Results are shown in Table 2. After pollination at 0600 A.M., we counted an average production of interspecific hybrids of some 70%. There was no distinct variation in the number of hybrids until 8.00 hr. This only began to fall towards 9.00 hr. Production of interspecific hybrids of this combination was greater than that of the intraspecific combination (ORS-1506 × ORS-803).

Discussion and conclusion

The germination of new pollen tubes, stopped by cutting styles in okra varieties, showed that pod setting increases when cutting was carried out later during the day. However, considerable behaviour differences were observed between varieties. Controlled cross pollinations, which are equivalent to induced allogamy, were performed at different times in the morning. Hybrids production decreases with time. The interspecific combination using a slow-setting variety gave 70% hybrids when pollen had been deposited at 7.00 hr.

These results show that okra self-fertilization is a progressive phenomenon. At anthesis, self-fertilization is far from complete and only a few ovules are then fertilized. Ovules continue to be fertilized during the morning and allogamy may be possible. The possibility of allogamy decreases rapidly and a pollen grain deposited on the style after midday has no impact on progeny. These results differ with those of Srivastave & Sachan (1973) and Chandra & Bhatnagar (1975) who worked in India and who consider that autogamy is obligatory in okra since it takes place before the flower opens.

Mulcahy (1983) stressed that flowers of plants making preferential autogamy tend to self pollinate before or during opening. Exposure, of still receptive stigmas, to potential pollinators occurs only after self pollination. In okra, stigmas are exposed to allopollination at anthesis, and only anthers in the upper ring come into contact with stigmas. The moment of contact depends on the initial distance between these and the rate of elongation of the stamen sheath. This makes easier to understand observed differences between cultivars.

Reasons mentioned above are probably accompanied by a diversity of the number of ovules which must be fertilized to ensure setting. In a given variety, the number of ovules available depends on the number of ovules per carpel and on the number of carpels per fruit. These are varietal characteristics. One locule contains an average of 10 to 20 ovules, and a variety with 5-locule fruits such as ORS-1506 will have 50 to 100 ovules. Another with 8 locules, such as ORS-803, will possess 80 to 160 ovules. The number of seeds per fruit increases steadily when cutting is performed later. However, it is limited for cultivars which have low initial potential (e.g. ORS-1506). To these fluctuations are added those related to the intrinsic requirements of varieties (minimum seed number) for setting.

Table 2. Hybrid production and pollination time

Pollinating time	Female by male		Female by male	
	ORS 1506	ORS 803	Clemson	ORS 278
	S/P	%H	S/P	%H
07.00			92.6	68.1
08.00	83.9	37.5	79.2	70.0
09.00	86.6	25.4	69.1	60.0
10.00	80.8	12.5		
11.00	85.6	09.6	73.0	19.2
12.00	82.8	00.1		

S/P Seed per pod
%H Percentage of hybrids

All the wild *Abelmoschus* species bear fruits with five carpels (Borssum Waalkes, 1966). This character is still present in many cultivars. In cultivated species, it would appear that selection of fruit size has led sometimes to an increase in the number of carpels per fruit. Under these conditions, there is perhaps a risk, in the long term, of producing varieties in which setting is difficult due to a lack of available pollen.

Because of the sticky pollen, allo-fertilization in okra is necessarily entomophilous. The level of allogamy observed in southern Côte d'Ivoire under natural flowering conditions (Hamon, 1988) is very low (2%). Finally, everything depends on the entomological environment, the dynamics of insect movements and their attraction by okra pollen. It is therefore obvious that comparisons between Africa, India and Puerto Rico (Martin, 1983) have no real significance.

The absence of systematic self-fertilization in okra cultivars combined with a progressive self pollination mechanism can be used in plant breeding for at least two reasons: – the emasculation operation is required for controlled hybrids. The removing of one hundred stamens from each flower is difficult and produces a stress and some varieties do not withstand this action very well. As a consequence, a high abscission level has been observed in numerous crosses (Hamon, 1988); – the potential contribution, for plant breeding, of hardy genes present in spontaneous species requires no further demonstration. In the case of the genus *Abelmoschus*, not all interspecific hybrid production is as easy as the combination of 'Clemson Spineless' crossed with *A. tetraphyllus* (Charrier, 1984).

The method that we described has the advantage of being simple to use. The only restrictive condition is that of being able to separate hybrids from self fertilized seeds. The technique obtains a high rate of hybrids when the combination is compatible. In addition, it enables setting in difficult combinations. Indeed, if the combination gives only a few hybrids, the flower will drop for lack of fertilized ovules; whereas if not emasculated it will be less fragile since self-fertilization will make setting possible. For example, after about a hundred emasculations, each taking about ten minutes (i.e. 16 hours in all), 1000 hybrids would be obtained from 'Clemson Spineless'. The setting rate is approximately 10% after emasculation with approximately 100 seeds per fruit (Hamon, 1988). If, between 6.00 and 7.00 hr, the same number of flowers is pollinated – which is perfectly feasible – approximately 7000 hybrids will be obtained since the average rate of production of interspecific hybrids is 70%. The comparative gain is on a different scale since if it is related to the same amount of time spent (16 hours), the multiplication coefficient is about 100. We used this technique to obtain a few *A. esculentus* × *A. moschatus* hybrids with wild accessions collected in Thailand by Hamon et al. (1987). These hybrids could not be obtained in any other way.

In conclusion, we have precised the okra reproductive biology for which flowers are self-compatibles. Unlike claims by numerous authors, self-fertilization is far from terminated at anthesis and increased progressively during the morning. So, even if there is no allopollen, enough ovules have been fertilized to assure pod setting.

This mechanism gives a strong potential for allogamy but in most cases only allopollen arriving before midday could have a chance to contribute to a progeny. The number of fertilizable ovules per flower and the relative distance between upper anthers and stigmas are genetic characters which can induce polymorphism in self-fertilization rates. These small differences in flower structure and different ecological conditions (temperature and insects), at a given site, permit to explain observed differences.

As we have demonstrated, the self-fertilization process can be judiciously exploited to easily obtain large numbers of controlled hybrids simply by depositing pollen in the morning without prior emasculation.

Acknowledgement

We are grateful to Dr. P. Hamon (Université de 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).

References

Borssum Waalkes, J. van, 1966. Malesian Malvaceae revised. Blumea 14 (1): 1–251.

Chandra, S. & S.P. Bhatnagar, 1975. Reproductive biology of *A. esculentus* 1. Reproductive behaviour – floral morphology anthesis and pollination mechanism. Acta Bot. India 3: 104–113.

Charrier, A., 1984. Genetic resources of the genus *Abelmoschus* Med. (Okra). International Board for Plant Genetic Resources. Roma 61 pages.

Chauhan, G.S., S.K. Rajput, J.S. Gaur, & Kushava, 1968. Blossom biological studies in three species of the genus *Abelmoschus*. J.N.K.V.V. Res. J. 2 (1): 9–12.

Choudhury, B. & M.L.A. Choomsai, 1970. Natural cross-pollinisation in some vegetable crops. Indian J. Agric. Sci. (40) (9): 805–812.

Cruden, R.W., 1977. Pollen – ovule ratios; a conservative indicator of breeding system in flowering plants. Evolution 311: 32–46.

Hamon, S. 1988. Organisation évolutive du genre *Abelmoschus* (Gombo). Co-adaptation et évolution de deux espèces de gombo cultivées en Afrique de l'Ouest *A. esculentus* et *A. caillei*. Edit. ORSTOM, T.D.M. No. 46, 191 pages.

Hamon, S. & A. Charrier, 1983. Large variation of okra collected in Benin and Togo. Plant Genet. Resources Newsl. 56: 52–58.

Hamon, S., & A. Yapo, 1985. Perturbations induced within the genus *Abelmoschus* by the discovery of a second edible okra species in West Africa. Acta Hortic. 182: 133–144.

Hamon, S. & J. Koechlin, 1991. Reproductive biology of okra. 1. Study of the breeding system in four *Abelmoschus* species. Euphytica 53: 41–48.

Hamon, S., N. Chomchalow, C. Chantaraprasong, & S. Chomchalow., 1987. Collecting *Abelmoschus* germplasm in Thailand. IBPGR Newsletter for South East Asia 11 (2): 2–6.

Hassan, M.S., A.A. Geneif, M.K. Ahmed, & S.A. Hussein. 1983. Horticultural crops collected in Sudan. Plant Genet. Resources Newsl. 56: 33–40.

Martin, F.W., 1983. Natural outcrossing of okra in Puerto Rico. J. Agric. Univ. of Puerto 67: 50–52.

Mitidieri, H. & R. Vencovsky, 1974. Polinizaçao cruzada do quia beiro em condiçoes de campo. Rev. Agric. (Piracicaba) 49 (1): 36.

Mulcahy, D.L., 1983. Pollen selection. An overview. In pollen biology and applications for breeding. Edit. Elsevier, Amsterdam: 15–17.

Purewal, S.W. & S.W. Randhawa 1957. Studies in *Hibiscus esculentus* (lady's finger). 1. Chromosome and pollination studies. Ind. J. Agri. Sci.: 129–136.

Shalaby, G.I., 1972. Natural cross-pollination in okra. J. Agric. Sci. 3 (1): 381–386.

Srivastava, L.S. & S.C.P. Sachan, 1973. Studies on flora biology of okra (*Abelmoschus esculentus* L. Moench). Allahabad Farmer 47 (1): 63–65.

Venkatazramani, K.S., 1953. Some observations on blossom biology and fruit formation in *Hibiscus esculentus*. J. Madras Univ. Sect. B 22 (2): 183–200.