Structural differences in stigmas of *Arachis* species (Leguminosae) and their probable significance in pollination

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ABSTRACT

The stigmas of the cultivated peanut (*Arachis hypogaea* L.) and some of its wild relatives in section *Arachis*, and one accession from section *Rhizomatosae* of the genus *Arachis* were studied with scanning electron microscopy. Three morphological types were observed and two of them correlated well with the two main growth cycle types observed in section *Arachis*. The annuals possessed large, globular stigmas with many papillae and with no guard hairs; the perennials had small, stigmatic surfaces with prominent guard hairs that over-arched the surfaces. The third stigma type can be described as intermediate in size and extent of hair growth on the surfaces. The implications of the different stigma structures in pollination in *Arachis* are discussed.

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RÉSUMÉ

AKROMAH, R.: *Différences structurelles entre les stigmates de l espèce d'*Arachis *(Leguminosae) et leur importance probable en pollinisation.* Les stigmates de l'arachide cultivée *(Arachis hypogaea* L.*)* et ses quelques relatifs sauvages de la coupe d' *Arachis* et une accession de la coupe de Rhizomatosae du genre *Arachis* étaient étudiés utilisant la microscopie électronique à balayage. Trois types morphologiques étaient observés et deux d'eux corrélaient bien avec les deux types principaux de cycle de croissance observés dans la coupe d'*Arachis*. Les plantes annuelles possédaient des grands stigmates globulaires avec plusieurs papilles et sans des poils de garde. Les plantes vivaces avaient des petites surfaces de stigmate avec des poils de garde prominents qui surélevaient les surfaces. Le troisième type de stigmate peut être décrit comme intermédiaire en dimension et en extension de croissance des poils sur les surfaces. Les implications des différentes structures des stigmates en pollinisation d'*Arachis* sont discutées.

Introduction

The stigmas in flowering plants serve an important role in pollen capture, hydration, and germination. These roles are very much tied to the morphology of the stigma, as this determines the ease with which pollen can be deposited on stigma and retained for hydration and germination. Stigmas vary in their structural features, and stigma characteristics have been used as taxonomic tools in flowering plants (Heslop-Harrison, 1981). Structural features that are important for successful pollination include the size of the stigmatic surface, presence or absence of hairs, and whether or not secretions are produced on the surfaces (Heslop-Harrison, 1976, 1981; Lakshmi & Shivanna, 1985; Lu, Meyer & Pickersgill, 1990).

The size of the stigma correlates well with the number of pollen grains it can accommodate, and the stigmatic hairs, when present, can serve to trap or prevent pollen from getting onto the surface of the stigma (Lu *et al.*, 1990). Heslop-Harrison (1981) also observed that some stigmas produce a sticky secretion to which pollen grains adhere and categorized the stigmas of flowering plants into 'dry' and 'wet' types. She described those without a free-flowing surface secretion but with a hydrated proteinaceous surface pellicle as 'dry' papillate types, and the other category having a free-flowing surface secretion as 'wet' stigmas.

Although stigmas of members of the family Leguminosae have been described as being of the wet papillate type (Heslop-Harrison & Heslop-Harrison, 1984), Lakshmi & Shivanna (1985) and Lu *et al.* (1990) found *Arachis* stigmas to show some deviations. Lakshmi & Shivanna (1985) described the stigmatic papillae of *A. hypogaea* as multi-cellular and multi-seriate, and indicated that they contrast with the unicellular papillae found in other Papilionaceous taxa.

Lu *et al.* (1990) grouped the *Arachis* species into three by size, shape and presence of hairs, and found them to correlate well with growth cycle types. They reported that annuals have elongated stigmas with highly curved surfaces composed of many papillae completely devoid of surrounding hairs, and that can accommodate as many as 15 pollen grains. The stigmas of perennials are relatively small with long hairs that over-arch the stigmatic surfaces and that tend to interfere with pollen capture. These stigmas can accommodate only about three pollen grains. Lu *et al.* (1990) described a third category, which they found only in the D genome species *A. glandulifera* Stalker, as intermediate in size and extent of hair growth; having stigmas that were smaller than those of the annuals but larger than stigmas of the perennials and having very short hairs. Few other legumes (e.g. *Trifolium pratense, Phaseolus coccineus,* and *Vicia faba*) release exudate only when external agents rupture the cuticle of the stigma (Heslop-Harrison & Heslop-Harrison, 1983, 1984; Lord & Heslop-Harrison, 1984).

Section *Arachis*, to which *A. hypogaea* belongs, contains some diploid wild species reported to be resistant to several diseases and pests of peanut such as rust, early and late leaf spots, and rootknot nematodes (Abdou, Gregory & Cooper, 1974; Subrahamanyam, Moss & Rao, 1983; Subrahamanyam *et al.*, 1985; Nelson, Simpson & Starr, 1989). They are, therefore, important potential sources of disease-resistant genes that can be used for breeding for disease resistance in *A. hypogaea.*

However, although barriers to interspecific gene exchange between the wild species and the cultivated species are not well developed, as in many other legume genera such as *Vigna* (Smartt, 1994), very little success has been reported in the use of wild species in peanut improvement. Seed set is low in many interspecific crosses, and attempts to produce fertile synthetic autotetraploids, especially in perennial species, have failed (Singh, 1986, 1988). Stigma structures have been implicated in some of the pollination failures and Lu *et al.* (1990) have suggested, from differences in stigma structure in species belonging to section *Arachis,* that some of the species may require external agents to deposit pollen on the stigma surfaces.

Information on stigma morphology in *Arachis* germplasm is limited, as stigmas of many of the species have not been described. Since the work of Lu *et al.* (1990), new *Arachis* species have been described (Krapovickas & Gregory, 1994) for which no description has been provided on stigma morphology.

The objective of this study was to describe stigmas of the new entries in the *Arachis* germplasm collection at the University of Reading, United Kingdom, to add to current knowledge on stigma morphology in section *Arachis.* The information is an important guide to decisions on ideal directions of crosses involving the wild species, and can indicate where manipulation of flowers is necessary to ensure that the pollen of interest lands on the target stigmas. Besides, it can be useful for assigning species to their correct taxonomic groups.

Materials and methods

The stigma types of the following species belonging to section *Arachis* were determined during summer 1997 by scanning electron microscopy: *A. benensis* 35005, *A. cruziana* 36024, *A. decora* 029882, *A. duranensis* 30075, *A. kuhlmannii* 30035, *A. magna* 30093, *A. valida* 30011, *A. hypogaea* spp. *fastigiata* var. *peruviana,* and one accession belonging to section *Rhizomatosae* (PI 262090). Four accessions that were studied by Lu *et al.* (1990) were included in this study for comparison with the newly described ones. These were *A. batizocoi* 9484, *A. correntina* 7830, *A. duranensis* 10038, and *A. villosa* 22585.

Flowers were collected at anthesis and kept in distilled water until they were worked on, to avoid drying. Under the light microscope, the stigma of each flower was carefully removed with a pair of fine forceps, leaving a few millimeters of style attached. The detached stigmas were fixed in Karnovsky's (Karnovsky, 1965) fixative (4 % paraformaldehyde + 3 % glutaraldehyde in 0.05-*M* potassium phosphate buffer, *p*H 7.0) in sealed bottles at room temperature for 4 h, washed three times in distilled water, and then dehydrated through a graded acetone: water series of 30, 50, 70, 90, 95, 100 per cent. The final 100 per cent acetone treatment was repeated. Each stage of the dehydration lasted 30 min, except for the absolute acetone stage, which was kept for 1 h. The absolute acetone jar contained anhydrous copper sulphate powder at the bottom to absorb moisture; therefore, a Pasteur pipette was used to take aliquots of just the acetone without disturbing the copper sulphate.

The specimens were then dried in liquid carbon dioxide by using a Tousimis Samdri - 780 Critical Point Drier. The dried specimens were mounted on SEM stubs and the base of each specimen on a stub was coated with silver conducting paint, which served to dissipate heat build-up during scanning in the electron microscope. Thirty nanometers of gold was deposited on the specimens by using a Polaron E500 Sputter Coater, after which they were viewed and photographed on a Jeol T20 SEM, operating at 20 KV.

For comparison of specimens, photographs were taken at a standard magnification of × 200 for all the specimens, while variable magnifications were used for detailed observation of each specimen. Three photographs were taken for each specimen.

Results

The stigmas observed in the 13 species and one variety of the cultivated groundnut fell into three morphological Types A, B and C (Fig. 1), and varied in size, shape and extent of hair growth surrounding the stigma surfaces. The

Fig. 1a. The Type A stigma typical of species with an annual growth cycle represented here by the stigma of *A. benensis* 029882 (x 450). Note the elongation of the stigmatic head and the cluster of large *papillae*.
h = hair, pa = papillae, s= stigma, st = style

Fig. 1b. The intermediate type stigma (Type B) represented here by the stigma of *A. cruziana* 36024 (x 516). Note the relative small size of the stigmatic surface and a correspondingly reduced cluster of papillae.
h = hair, pa = papillae, s= stigma, st = style

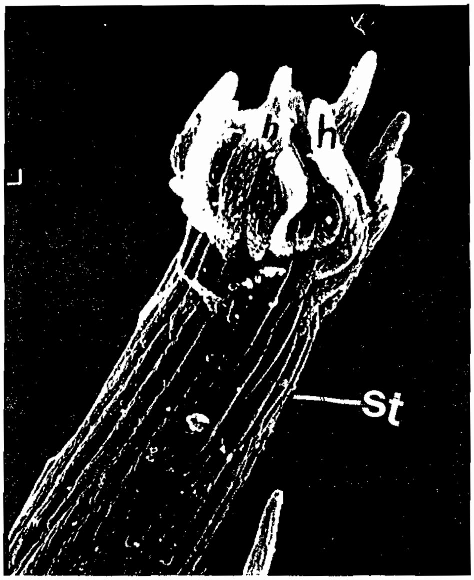

Fig. 1c. The Type C stigma typical of species with the perennial growth cycle represented here by the stigma of *A. villosa* 22585 (x 450). Note the presence of prominent hairs that have over-arched and covered the stigmatic surface.
h = hair, pa = papillae, s= stigma, st = style

Fig. 1a-c. Representatives of scanning electron micrograph of the three types of stigma found in the *Arachis* species that were studied.

observations were similar to the types observed by Lu *et al.* (1990). Table 1 shows a summary of the stigma type, growth cycle type, and prominent features observed in the species/variety studied.

The Type A stigmas were larger and more elongated than the other two types (B and C), and were composed of many papillae. The Type A stigmas had no guard hairs, the styles were covered with short, dense hairs, and were found in annual species. All the perennial species examined, including the accession that belonged to section *Rhizomatosae* (PI 262090), had the Type C stigma. They had small stigma surfaces that were surrounded by prominent guard hairs. The correlation reported by Lu *et al.* (1990) between stigma and growth cycle types was also noticed in nearly all of the species studied. Two of the newly described annual species, *A. cruziana* and *A. decora,* and the D genome species *A. glandulifera* had the Type B stigma, which was intermediate in size compared to the types that were typical of annuals and perennials, with barely any guard hairs.

TABLE 1

Summary of the Stigma Types, Growth Cycles and Prominent Features Observed in the Species/Variety Studied

Species/variety	*Stigma type*	*Growth cycle*	*Extent of stylar hair growth*
A. benensis 35005	A	Annual	Short and sparse
A. batizocoi 9484	A	Annual	Short and sparse
A. duranensis 10038	A	Annual	Short and sparse
A. 'ipaensis' 30075	A	Annual	Short and sparse
A. magna 30093	A	Annual	Short and sparse
A. valida 30011	A	Annual	Short and sparse
A. hypogaea spp. *fastigiata* var. *peruviana*	A	Annual	Short and sparse
A. cruziana 36024	B	Annual	Short and profuse
A. decora 029882	B	Annual	Short and profuse
A. glandulifera	B	Annual	Short and profuse
A. correntina 7830	C	Perennial	Prominent and over-arching
A. kuhlmannii 30035	C	Perennial	Prominent and over-arching
A. villosa 22585	C	Perennial	Prominent and over-arching
PI 262292 (section *Rhizomatosae*)	C	Perennial	Prominent and over-arching

Discussion

The morphological investigations into the new *Arachis* entries in the collections at the University of Reading, United Kingdom, were conducted for additional taxonomic information on the species, and also to provide guidance on their use in future interspecific crosses. In general, there is a sharp distinction between morphological features of stigmas of annuals and those of perennial species in *Arachis*. The three species that were classified as 'intermediate' by stigma size and extent of hair growth around the stigma have been reported by Krapovickas & Gregory (1994) to have the annual life cycle. They were, therefore, observed to be exceptions in stigma morphology of annuals.

The similarities in the stigmas of the perennial species in section *Arachis* and those of the perennial species in section *Rhizomatosae* seem to indicate that section *Arachis* and the latter share the same source of inheritance for stigma characteristics. Cytological data indicate that two chromosome series, $2n = 2x = 20$ and $2n = 2x = 40$, are present in the genus *Arachis* (Husted, 1933, 1936; Singh, 1986, 1988), though most species are diploid with $2n = 2x = 20$. Polyploidy is thought to have arisen twice in the genus, independently, once in section *Rhizomatosae* through autotetraploidization, and again in section *Arachis* through amphidiploidization. Singh & Simpson (1994) have indicated that the autotetraploid genome of section *Rhizomatosae* has some homology with the A genome of section *Arachis*, and this may be the source of the similarities observed in stigma morphology. However, the genetical basis of inheritance of stigma characteristics remains to be investigated.

Probable implications of the differences in stigma morphology in pollination

A comprehensive genome analysis in section *Arachis*, by Singh & Moss (1984), has shown that hybrids from the diploid species they studied, regardless of their life cycle, had near normal bivalent frequency (9.1-9.8) with moderate to high pollen fertility (60-91 per cent), except those with *A. batizocoi*. This means that there is no rejection reaction to suggest incompatible pollination in *Arachis*. The critical factors, therefore, seem to be those that determine how pollen gets to the surface of the target stigma. The absence of exudate on the surfaces of the stigmas agrees with earlier observations by Lakshmi & Shivanna (1985) and Lu *et al.* (1990) that *Arachis* stigmas are of the 'dry' type without copious fluid secretion. Lu *et al.* (1990) observed a hydrated overlaying pellicle which produced exudate on tripping by external agents, or when pollen landed on the surface. The pellicle layer is invisible in the figures presented in this study.

The large stigmatic surfaces in the annual species will provide larger room for pollen to land, and the absence of the guard hairs will facilitate free access of pollen grains to the stigma surface. Pollen-stigma interactions are cell by cell (Heslop-Harrison, 1975), often between one alighting pollen grain and one stigma papilla. Therefore, the annual species that have many papillae are probably more successful with pollen germination, as many pollen grains will have these reactions. In the perennial species with small stigmatic surfaces which are also over-arched by long hairs, pollen may have to be deposited by external agents (most probably insects in the wild). Stalker *et al.* (1994) observed low fruit set in these species in glasshouses in North Carolina. Singh (1986) also observed high pollination failures in perennial species used in autopolyploid production. Presumably, natural pollinators required to deposit pollen on the stigmas were lacking under the glasshouse conditions in the two independent studies.

The enhancement of fruit set in crosses involving the perennial species under glasshouse conditions (that is, in environments outside *Arachis'* native habitat in South America) may require experimentation to develop efficient pollination techniques. Additionally, field studies of the perennial species in their natural habitats could lead to identification of the particular insect pollinators which could be reared *ex-situ* for pollination. The morphological differences in stigmas of annuals and perennials seem to imply that in interspecific hybridizations involving the two types, it may be more successful to select annuals as the maternal parents and perennials as pollen donors.

To develop autotetraploids of the perennials may require that pollen be deposited artificially onto the small stigmatic surfaces for hydration and pollen tube growth. Lu *et al.* (1990) observed that it required manipulations to ensure that self-pollen were deposited on the Type C stigmas. The perennials with the Type C stigma are, therefore, believed to be efficient out-crossers, and were reported to benefit much from the high level of bee activity on peanut nurseries (Stalker *et al.*, 1994).

The observations made in this study as well as those of previous investigators (Lakshmi & Shivanna, 1986; Lu *et al.* 1990) suggest that stigma characteristics in *Arachis* are important factors in pollen-pistil interactions which must be considered for successful hybridization in interspecific crosses.

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