AT WHAT PHENOLOGICAL PHASE IS THE STIGMA OF ARGAN (*ARGANIA SPINOSA* (L.) SKEELS) FLOWER RECEPTIVE TO POLLEN ADHESION AND GERMINATION?

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ABSTRACT

Because argan (*Argania spinosa* (L.) Skeels) reproductive biology is not yet fully understood, this study aimed to determine the flower phase when stigma is the most receptive to pollen adhesion and germination under natural pollination. An average of 9.9 from 10 stigmas were bearing pollen at the blooming flower phase (FE), whereas 6.5 stigmas were covered with pollen at the flower bud with an emerging style phase (BFS). Although the number of deposited or adhered pollen grains did not differ between both phenological phases, only an average of 1.6 pollen grains would germinate on BFS stigmas as compared to 6.4 on FE. More over, highly significant differences were observed between phenological phases for stigma height, pollen tube number per stigma, maximum pollen tube length and maximum pollen tube length to stigma height ratio, FE being the phase showing the highest values for these variables. No pollen germination was observed on the flower bud (BF) stigmas, and pollen tubes, although present on BFS, never attained the base of its style reaching the ovule. However pollen tubes reached the base of the style in 25 % of FE. Therefor, FE seems to be the phase, when, most likely, stigma is to be receptive to pollen. To avoid any possible contamination during artificial pollination, however, emasculation should be carried out at early BF phase as a precaution, because pollen may germinate on BFS stigma as well. Tree and phenological phase × tree interaction were significant for all variables. Therefore, the variability of pollen adhering and germination among individual trees may indicate a limitation of allele exchange among genotypes because of differential stigma receptivity and male gametophytes viability adding to the previously encountered time diversity of FE number and pollen fertility.

Key words: *Argania spinosa*, flower, phenological phases, pollen tube, pollination, variability

INTRODUCTION

Argan tree (*Argania spinosa* (L.) Skeels) is adapted to aridity in the south west of Morocco (EMBERGER 1939; PRENDERGAST & WALKER 1992; LE HOUÉROU 1989; FERRADOUS *et al.* 1996; ZAHIDI & BANI-AAMEUR 1999a and b). It flowers and fruits with as little as 100 mm rainfall (PERROT 1907; METRO 1952; FERRADOUS *et al.* 1996; BANI-AAMEUR *et al.* 1998; BENLAHBIL & BANI-AAMEUR 1999; BANI-AAMEUR 2000; BANI-AAMEUR 2002a). However large variability of flowering intensity was observed among climatic years, sites, tree genotypes and shoot types. In any case, the peak of flowering occurs in spring

Argan inflorescence is a glomerule of up to 15 pentamerous hermaphroditic flowers. They are grouped in the axils of the leaves or on the nodes of the shoots. The glomerule may include all phenological phases, i.e. flower bud – BF (1 to 2 mm), flower bud with an emerging style – BFS (1 to 2 mm), blooming flower FE (2 to 3.15 mm), dry flower with corolla – FSCP (2 to 3.15 mm) and finally, dry flower without corolla – FSC (1 to 2 mm), as well as young fruit. Anthesis, which may occur at BF, is complete at BFS phase, while the anthers are still covered with the perianth (BELMOUDEN & BANI-AAMEUR 1995, DEROIN & BANI-AAMEUR 1999). Pollen fertility, using acetocarmine staining, varied from 100 to 49.1 % (BANI-AAMEUR 2002b). Its size varied from 17 to 32 µm and germination pore number varied from two to six. Tested *in vitro*, pollen is not completely ripe for germination before FE phase (BENLAHBIL & BANI-AAMEUR 2002).

Pollination includes male/female interactive processes where pollen germination on the stigma is a succession of stages from pollen adhesion to reaching the ovule by the pollen tube for fecundation (ULRICH 1952). However, argan pollination behaviour is not understood yet. Especially, flower stigma receptivity is not clearly established, what is a limitation for setting up a method of artificial pollination to facilitate the crossing of selected genotypes. Our overall goal in this study is to determine the flower phase when stigma is

the most receptive to pollen adhesion and germination. In the course of three kinds of observation, we aimed to establish at which flower phenological phase the stigma receptivity is the most favourable to pollination.

MATERIALS AND METHODS

Plant material

The site of investigations was located at Ait Melloul, South west of Morocco. Sampling took place at the peak of flowering in spring 1997 for the first two kinds of observation and in spring 2001 for the last one. Flowers were collected at random when the phenological phases were at their full development, i.e. BF was collected just before reaching BFS, this phase was observed just before blooming and FE was collected at full bloom.

In situ observation of pollen deposition

To examine the degree of exposure of phenological phases to pollen reception, we examined in the field the presence or the absence of pollen on the stigma. Four replications of each of 10 BFS and 10 FE flowers of three random trees were observed using a field magnifier (G × 20).

Light microscope observation of pollen adhesion

Pistils of ten BFS flowers and ten FE flowers from three random trees were excised using a laboratory magnifier (G × 20). The stigmas were agitated in a drop of acetocarmine on a slide to separate pollen covering the stigma but not adhering to it. The pistils were cut longitudinally with a razor blade and the stylar canal was stained with acetocarmine under a light microscope (G × 40).Then it was possible to count both adhered and germinating pollen grains (ASCHER & PELOQUIN 1968; RAMSEY &VAUGHTON 2000).

Fluorescent microscope observation of pollen tube growth

To determine in vivo pollen growth, pistils from ten BF, ten BFS flowers and ten FE flowers collected from six random trees were *in situ* excised using a field head magnifier (G × 20). They were immediately fixed with a mixture of formaldehyde, ethanol 80 % and glacial acetic acid (ratio 1: 8: 1) and stored for 24 hours at 4 °C (MARTIN 1958; TANGMITCHAROEN & OWENS 1997; KALINGANIRE *et al.* 2000). Thereafter the pistils were rinsed with distilled water for five minutes and cleared in 8 M NaOH for 96 hours at room temperature until most of the tissues became transparent. They were then rinsed again in distilled water for five minutes, mounted on a slide and stained with 0.1 % aniline blue in 0.1 M K_3PO_4. They were observed using an Olympus BH2-RFC photomicroscope with UVFL objectives and barrier filter L-435, fluorescence with 405 nm excitation and 490 nm barrier filters at G × 100. Style height, maximum pollen tube length and pollen tube number per stigma were measured.

Data analysis

ANOVA was a two-factors design of trees and phenological phases (SOKAL & ROHLF 1995) In the first kind of observation, it was performed on the number of stigmas covered with pollen for four replications of ten flowers. In the second case, it concerned the number of pollen grains on the stigmas, adhering to their surface or germinating on it using ten flowers as replications. Finally, in the third kind of investigation, it was performed on stigma height, maximum pollen tube length, pollen tube number per stigma as well as on maximum pollen tube length to stigma height ratio using ten flowers as replications. In this case, maximum pollen tube length to stigma height ratio was subject to a $\sqrt{(x+0.5)}$ transformation. The Least Significant Difference test (LSD, $\alpha = 5$ %) of equality of means was used to compare differences between means. Statistix (Analytical Software) software was used for computation.

RESULTS

In situ observation of pollen deposition

Phenological phase was a highly significant main factor for the number of stigmas bearing pollen, whereas neither tree nor interaction was significant (Table 1). Mean number of stigmas bearing pollen was 9.9 for FE

Table 1. Analysis of variance of the number of stigmas with the deposit pollen for three argan trees and two phenological phases under natural conditions.

Source of variation	DF	Mean square
Tree	2	1.17 ns
Phase	1	70.04 **
Tree × phase	2	2.17 ns
Error	18	0.95

DF: degrees of freedom; **: significant at 0.01 level; ns: non significant

contrasting with 6.5 stigmas for BFS. Note that argan stigma is dry.

Light microscope observation of pollen adhesion

Phenological phase was a highly significant main factor for the number of pollen grains germinating on the stigmas, whereas the factor tree was significant for the number of deposited pollen grains (Figure 1A, Table 2). Interaction was not significant for anyone of the observed variables. Both BFS and FE received similar amounts of deposited pollen, although these numbers varied between 16 and 70 grains depending on the tree (Table 3). But an average of 6.4 grains would germinate on FE stigmas as compared to 1.6 for BFS.

Fluorescent microscope observation of pollen tube growth

Phenological phase was a highly significant main factor for stigma height, pollen tube number per stigma, maximum pollen tube length as well as the maximum pollen tube length to stigma height ratio (Table 4). Tree was significant for maximum pollen tube length and for maximum pollen tube length to stigma height ratio. The interaction phenological phase × tree was highly significant for pollen tube number per stigma and maximum pollen tube length. Mean style height was 3875.5 µm for FE and 3470 µm for BFS where it significantly varied among trees from 3250 µm to 3625 µm (Table 5). On average, 10 pollen grains formed pollen tubes on FE stigmas, varying from 2.4 to 21.3 among trees (Table 5). It contrasts with 1.5 pollen tubes developed in BFS, where trees did not differ significantly. Maximum pollen tube length involved on average, 68 % of FE style length (between 55 % and 85 % depending on the trees), what contrasted with 6 % in BFS. Pollen tubes never exceeded the maximum of 42.9 % of BFS style length and only 5 % of cases exceeded 25 % (thus never attaining the ovule) whereas 42 % of the exam-

Table 2. Analysis of variance of the number of pollen grains deposited on the stigma, adhered to it and germinating for three argan trees and two flower phenological phases under natural conditions.

Source of variation	DF	Mean square of the number of pollen grains		
		Deposited	Adhered	Germinating
Tree	2	18933 *	11139 ns	27.6 ns
Phase	1	13440 ns	2996.3 ns	345.6 **
Tree × phase	2	9830.2 ns	19456 ns	0.9 ns
Error	54	5131	9159	43.8

DF: degrees of freedom; ** : significant at 0.01 level; * : significant at 0.05 level; ns: non significant

Table 3. Mean, maximum and minimum deposited, adhered and germinated pollen grain number for three argan trees at two flower phenological phases BFS and FE as described in the text.

Number of pollen grains	Phase	Tree			Mean	Maximum	Minimum
		1	2	3			
Deposited	BFS	9.8	27.4	71.2	36.1	263	0
	FE	21.4	107.9	68.9	66.1	302	1
	Mean	15.6 b	67.5 a	70.05 a	51.1		
Adhered	BFS	7.2	26.1	110	47.7	56	0
	FE	36.7	39.8	24.4	33.6	88	5
	Mean	21.9	32.9	67.2	40.7		
Germinating	BFS	0	2.3	2.5	1.6 b	11	0
	FE	5.3	6.8	7.1	6.4 a	48	0
	Mean	2.7	4.6	4.8	4		

Different letters note significant differences (LSD at 0.05 level) as comparisons are made among tree means or between phenological phase means.

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REFERENCES

ASCHER, D. P. & PELOQUIN, J. S. 1968: Pollen tube length and incompatibility following intra- and inter-specific pollinations in *Lilium longiflorum*. *Amer. J. Bot.* **55**: 1230–1234.

BANI-AAMEUR, F. 2000: Phenological phases of *Argania spinosa* (L.) Skeels flower. *Forest Genetics* **7**: 329–334.

BANI-AAMEUR, F. 2002a: *Argania spinosa* (L.) Skeels flowering phenology. *Genetic Resources and Crop Evolution* **49**: 11–19.

BANI-AAMEUR, F. 2002b: Variation of pollen grain fertility, dimension and aperture number in *Argania spinosa* L. (Skeels) (Sapotaceae). *Forest Genetics* **9**: 115–118.

BANI-AAMEUR, F., LOUALI, L. & DUPUIS, P. 1998: Maturation et chute des fruits de l'arganier. *Actes de l'Institut Agronomique et Vétérinaire (Maroc)* **18**: 137–144.

BELMOUDEN S. & BANI-AAMEUR, F. 1995: Contribution à l'étude de l'anthèse et de la viabilité du grain de pollen. *In*: Colloque International: la Forêt face à la Désertification "cas des Arganeraies". pp. 57–62. Université Ibn Zohr, Faculté des sciences. 26–28 octobre, Agadir.

BENLAHBIL S. & BANI-AAMEUR, F. 1999: La pollinisation de l'arganier est surtout entomophile. *In*: F. Bani-Aameur (ed.), Colloque International sur les Ressources Végétales: L'arganier et les plantes des zones arides et semi-arides. pp. 193–194. Faculté des Sciences d'Agadir 23, 24 et 25 Avril 1998.

BENLAHBIL, S. & BANI-AAMEUR, F. 2002: *In vitro* germination pollen of argan (*Argania spinosa* (L.) Skeels. *Acta Botanica Galica* (in press).

DEROIN, T. & BANI-AAMEUR, F. 1999: Les singularités de l'anatomie florale de l'arganier (*Argania spinosa* (L.) Skeels, *Sapotaceae*), réponses aux contraintes de l'aridité. *In*: F. Bani-Aameur (Ed), Colloque International sur les Ressources Végétales: L'arganier et les plantes des zones arides et semi-arides. pp. 202–206. Faculté des Sciences d'Agadir 23, 24 et 25 Avril 1998.

EMBERGER, L. 1939: Aperçu général sur la végétation du Maroc. *Bulletin de la Société des Sciences Naturelles du Maroc*: 40–157.

FERRADOUS, A., BANI-AAMEUR, F. & DEPUIS, P. 1996: Climat stationnel, phénologie et fructification de l'arganier (*Argania spinosa* L. Skeels). *Actes de l'Institut Agronomique et Vétérinaire (Maroc)* **17**: 50–60.

KALINGANIRE, A., HARWOOD C. E., SLEE M. U. & SIMONS, A. J. 2000: Floral structure, stigma receptivity and pollen viability in relation to protandry and self-incompatibility in silky oak (*Grevillea robusta* (A.) Cunn.). *Annals of Botany* **86**: 133–148.

LE HOUÉROU, H. N. 1989: Classification éco-climatique des zones arides (s.l.) de l'Afrique du Nord. *Ecologia Mediterranea* **15**: 95–144.

MARTIN, F. W. 1958: Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technology*: 125–128.

METRO, A. 1952: bservation préliminaire faites sur l'arganier à l'Oued Cherrate et à Dar Askraoui en vue de sélections généalogiques. *Annales de la Recherche Forestière du Maroc (Rapport Annuel 1952)*: 201–215.

NIKKANEN, T., ARONEN, T. & HAGGMAN, H. 2000: Variation in pollen viability among *Picea abies* genotypes potential for unequal paternal success. *Theor. Appl. Genet.* **101**: 511–518.

PERROT, E. M. 1907 : Les végétaux utiles de l'Afrique tropicale. Fascicule II: 127–158.

PRENDERGAST, H. D. V. & WALKER, C. C. 1992: The argan multipurpose tree of Morocco. *The Kew Magazine* **9**: 76–85.

RAMSEY, M. & VAUGHTON, G. 2000: Pollen quality limits seed set in *Burchardia umbellata* (Colchicaceae). *Am. J. Bot.* **87**: 845–852.

SOKAL, R. R. & ROHLF, F. J. 1995: Biometry. The Principles and Practice of Statistics in Biological Research. 3rd edn. W. H. Freeman and Company, New York.

TANGMITCHAROEN, S. & OWENS, J. N. 1997: Pollen viability and pollen-tube length following controlled pollination and their relation to low fruit production in teak (*Tectona grandis* (Linn.) F.). *Annals of Botany* **80**: 401–410.

ULRICH, R. 1952: Nouaison consécutive à une fécondation. *In*: Comment naissent les fruits. 13–32.

ZAHIDI A. & BANI-AAMEUR, F. 1999a: Phénologie de la foliation de l'arganier. *In*: F. BANI-AAMEUR (ed.), Colloque International sur les Ressources Végétales: L'arganier et les plantes des zones arides et semi-arides. pp. 173–176. Faculté des Sciences d'Agadir 23, 24 et 25 Avril 1998.

ZAHIDI A. & BANI-AAMEUR, F. 1999b: Phénologie de la ramification de l'arganier. *In*: F. BANI-AAMEUR (ed.), Colloque International sur les Ressources Végétales: L'arganier et les plantes des zones arides et semi-arides. pp. 77–179. Faculté des Sciences d'Agadir 23, 24 et 25 Avril 1998.

ZINKL, G. .M., ZWIEBEL, B. I., GRIER, D. G. & PREUSS, D. 1999: Pollen-stigma adhesion in *Arabidopsis*: a specific interaction mediated lipophilic molecules in the pollen exine. *Development* **126**: 5431–5440.

ZINKL, G. M. & PREUSS, D. 2000: Dissecting *Arabidopsis* pollen-stigma interactions reveals novel mechanisms that confer mating specificity. *Annals of Botany* **85**: 15–21.