SHORT COMMUNICATION

VARIATION OF POLLEN GRAIN SIZE, FERTILITY AND PORE NUMBER IN *ARGANIA SPINOSA* (L.) SKEELS (SAPOTACEAE)

Fouzia Bani-Aameur

Laboratoire de Recherche sur la Variabilité Génétique, Université Ibn Zohr, Faculté des Sciences, Département de Biologie, BP: 28/S 80 000 Agadir, Morocco; Phone: (212) 48 22 09 57; Fax: (212) 48 22 01 00, e-mail: baniaameur@hotmail.com

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ABSTRACT

Pollen grain size, fertility, and pore number were observed in argan (*Argania spinosa* (L.) Skeels). Fertility varied between 100 and 49.1 %. In a given sample, pollen grain size varied from 17 to 32 µm and five classes of germination pores (two, three, four, five and six) were described revealing a polymorphism that is common to Sapotaceae family. In average, pollen grains with two-pores were the most frequent (74.7 %), whereas only one tree showed traces of grains with three pores. Pollen grains with four or five pores had intermediate frequencies (11.9 and 12.2 %) as compared to grains having six pores, which were rare (1.1 %). Grains between 22 and 27 µm were significantly the most frequent (80.2 %), followed by both of 17 to 22 µm (10.4 %) and 27–32 µm (9.4 %) size classes. Size and pore number were not independent, grains with four, five or six pores being larger than 22 µm. Highly significant variability was observed between trees for fertility, pore-number and pollen grain size class frequencies.

Key words: *Argania spinosa*, pollen fertility, pollen pore number, pollen size.

INTRODUCTION

Argania spinosa (L.) Skeels, a perennial tree endemic to Morocco, is the unique species of the genus *Argania* that belongs to Sapotaceae family, Syderoxyloideae sub-family (AUBERVILLE 1964; PRENDERGAST & WALKER 1992). Argan peak of flowering under natural conditions is attained in spring (BANI-AAMEUR, 2002; BANI-AAMEUR, 2000). Flowers are grouped in glomerules on the axils of the leaves of shoots or the nodes of mature wood. Pollen grains are shed at flower bud stage while the anthers are still contained within the closed corolla (BELMOUDEN & BANI-AAMEUR 1995; DEROIN & BANI-AAMEUR 1999). This study dealt with pollen size and pore number distribution in nine trees at each of three locations. It is a contribution to enhance basic and practical information on floral biology to facilitate controlled hybridisation, an emerging need of research on argan genetics, diversity and breeding (MSANDA *et al.* 1994; BANI-AAMEUR & HILU 1996; EL MOUSADIK & PETIT 1996; ZAHIDI, FERRADOUS & BANI-AAMEUR 1996; ZAHIDI 1997; BANI-AAMEUR & FERRADOUS 2001).

MATERIALS AND METHODS

The experiment concerned trees at Ait Melloul at 35 m altitude in the Souss plain, Argana at 620 m altitude on southern slopes of High Atlas Mountains and Ait Baha (AB) at 50 km from the Atlantic ocean at 550 m altitude on the northern slopes of Anti Atlas mountains south west of Morocco (FERRADOUS, BANI-AAMEUR & DUPUIS 1996; BANI-AAMEUR & FERRADOUS 2001). At anthesis, in March 1996, two flowers of nine random trees at each of the three locations were sampled, each flower serving as a replicate. Duplicate samples of pollen were collected by splitting the flower on a glass slide. Thus four samples of 200 pollen grains, coloured with aceto-carmine stain (JANSSEN & HERMSEN 1976), were screened for fertility, pore number and size using an ocular micrometer under light microscope (40 ×).

Analysis of variance was performed on the number of fertile pollen grains, on the number of grains from a size class and on the number of pollen grains in each of the five pore number combinations. A global ANOVA was a mixed model using a three-factor design of three sites, nine random trees per site and four pore-classes or three size-classes. Individual ANOVA that were a mixed model using a two-factor design of three sites

Table 1. Analysis of variance of count of fertile pollen per size and pore-number classes.

Source of variation	Df	Mean squares							
		Fertility	2 pores	4 pores	5 pores	6 pores	17-22µm	22-27µm	27-32µm
Sites	2	1961.7ns	1606.8**	492.1**	329.2**	3.5 ns	133.4**	3648.5**	2680.6**
Tree/site	24	5639.2**	596.7**	266.4**	142.6**	53.4**	924.2**	600.4**	712**
Error	81	40.4	41.5	26	16.6	2.8	17.0	40.9	16.0

DF: degrees of freedom, ns: non significant, **: significant at $\alpha = 0.01$

and nine random trees per site were used for each pore or size class. The factor tree was hierarchical to the factor site because the trees were not repeated between sites (STEEL & TORRIE 1960; SOKAL & ROHLF 1995). The Least Significant Difference test (LSD) (a = 5%) of equality of means was used to compare differences between means. Log-linear model was used to test whether, given site and tree, pollen diameter and pore number are independent (SOKAL & ROHLF 1995). Statistix (Analytical Software) software was used for computation.

RESULTS AND DISCUSSIONS

Argan fertile pollen grains coloured with aceto-carmine were uniformly red and round. Pollen fertility of individual trees varied significantly from 100 % to 49.1 % (Table 1 and 2). As it is, trees with low fertility may be expected to show a lesser potential of alleles trans-

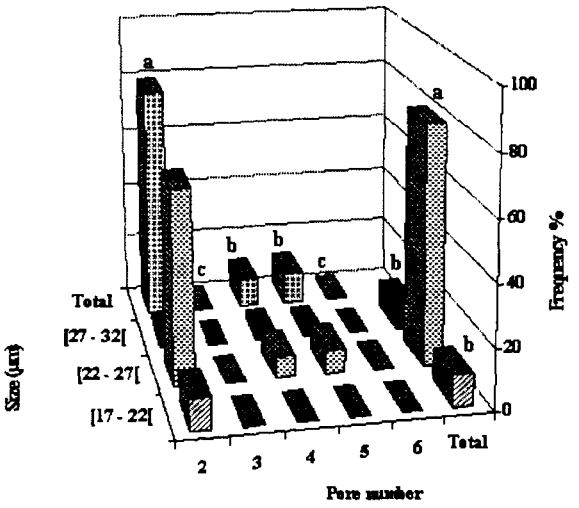

Figure 1. Distribution of *Argania spinosa* pollen grains per diameter and pore number. Values are the mean percentage of four samples, 200 pollen grains, nine trees and three sites (Ait Melloul, Ait Baha and Argana). Different letters note significant differences (LSD, $\alpha = .05$) as comparisons were made between pollen grain pore-number or size classes.

Table 2. Mean percentage of fertile pollen grains of sites of *Argania spinosa* from Ait Melloul, Argana and Ait Baha. Values are the mean percentages using four samples of 200 pollen grains.

Tree	Site		
	Ait Melloul	Argana	Ait Baha
1	99.9 a	99.9 a	100.0 a
2	99.8 a	99.9 a	99.9 a
3	98.8 ab	99.8 a	99.9 a
4	97.2 b	98.7 a	99.0 a
5	98.3 ab	98.3 a	98.9 a
6	98.0 ab	98.2 a	98.9 a
7	88.5 c	74.5 b	89.5 b
8	57.9 d	56.2 c	50.3 c
9	97.5 b	51.2 d	49.1 c
Mean	92.9	86.3	87.3

Different letters note significant differences (LSD, $\alpha = .05$) as comparisons were made between trees of a site.

mission to further generations, although further investigations are needed to establish the dynamic of gene flow in argan populations.

Two-pores pollen grains were significantly the most frequent (74.7 %), whereas only traces of three-pores grains were observed within one tree (Figure 1). Pollen grains with four or five pores were equally less frequent (11.9 and 12.2 %), whereas grains with six pores were rare (1.1 %). Grains between 22 and 27 µm were significantly the most frequent (80.2 %), followed by both of 17 to 22 µm (10.4 %) and 27–32 µm (9.4 %) size classes. Given sites and trees, pollen size and pore number were not independent (a << 0.05). Indeed, pollen grains with four, five or six pores were larger than 22 µm, whereas most of grains having two pores were of intermediate size (22 to 27 µm) (Figure 2). Locations and trees of a location differed significantly for the frequency of size classes or pore number class frequencies (Table 1). Although argan individual trees showed pollen size and pollen pore number polymor-

Table 3.Tree mean, minimum (Min) and maximum (Max) percentage of pollen grains per size-class of *Argania spinosa* from Ait Melloul, Argana and Ait Baha. Values are the mean percentages of four samples of 200 pollen grains.

Size (µm)		Site		
		Ait Melloul	Argana	Ait Baha
17–22	Min	3.0	3.3	4.8
	Max	28.4	28.0	23.5
	Mean	10.8 a	10.9 a	9.3 b
22–27	Min	69.9	70.9	75.3
	Max	83.4	91.5	92.8
	Mean	75.5 c	79.6 b	85.5 a
27–32	Min	0.0	0.5	1.4
	Max	20.9	19.8	11.4
	Mean	13.7 a	9.5 b	5.2 c

Different letters note significant differences (LSD, $\alpha = .05$) as comparisons were made between trees of a site.

Table 4. Tree mean, minimum (Min) and maximum (Max) percentage of pollen grains per pore-number class of *Argania spinosa* from Ait Melloul, Argana and Ait Baha. Values are the mean percentages using four samples of 200 pollen grains.

Pore number		Site		
		Ait Melloul	Argana	Ait Baha
2	Min	63.5	63.5	79.9
	Max	78.0	90.5	89.0
	Mean	76.6 b	76.8 b	82.6 a
4	Min	4.8	5.5	3.1
	Max	23.5	20.6	11
	Mean	11.1 a	10.5 a	7.6 b
5	Min	5.5	6.7	5.5
	Max	16.3	16.6	10.5
	Mean	11.1 a	11.8 a	8.9 b
6	Min	0.0	0.0	0.0
	Max	7.5	5.5	3.0
	Mean	1.2	0.9	0.9

Different letters note significant differences (LSD, $\alpha = .05$) as comparisons were made between trees of a site.

phism as it is often the case for Sapotaceae family (BHATNAGAR & GUPA 1970; AYENSU 1972; HARLEY 1986), the large variability observed between trees may suggest some cytogenetic basis to the phenomenon that may be worth investigating (Table 3 and 4). More specifically, pore number polymorphism associated to pollen size polymorphism may suggest meiotic irregularities causing formation of restitution nuclei leading to of variation of chromosome number as it is the case in some other plant species (QUINN, MOK & PELOQUIN 1974; HENNY 1979; CAVALCANTÉ, SCHIFINO, & DORNELLES, 2000). However further study is needed to determine the cause of pollen low fertility and polymorphism in argan. In that prospect this study has contributed to the knowledge of argan pollen descriptors which are now available to enhance argan breeding, conservation and exchange database.

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