Advanced Journal of Microbiology Research Vol. 2004
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Reproductive biology in *Balanites aegyptiaca* (L.) Del., a semi-arid forest tree

Mansor Ndoye[1]*, Ismaïla Diallo[2], Yaye Kène Gassama/Dia[1]

[1]Laboratoire de biotechnologies végétales, Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, B.P. 5005, Dakar, Sénégal.
[2]ISRA-DRPF, Route des Pères Maristes, B.P. 2312, Dakar, Sénégal.

Accepted 30 December 2003

***Balanites aegyptiaca* (L.) is an important tree in the semi-arid ecosystem with beneficial attributes. However, very little information is available in its reproductive biology. In order to better control the reproduction system of *B. aegyptiaca*, three experimental approaches are used: floral morpholgy, fluorochromatic procedure and hand pollination. The floral morphology shows that *B. aegyptiaca* flowers are hermaphrodite and gathered in several types of inflorescence (clusters, fascicles or glomerules). They blossomed asynchronically and nectar is exuded by the flowers. Top ovary holds five anatropous ovules. The second approach consisting in fluorochromatic procedure reveals a pollen with 3 apertures and a viability rate of 92%. This viability decrease down to 50% after a storage at -5°C for 7 days. Finally, the hand pollination proves that *B. aegyptiaca* is a partially auto-compatible plant and the main vectors of pollination are Halictidae (Hymenopterae) and Dipterae.**

Key words: *Balanites aegyptiaca*, fluorochromatic test, pollen, pollination, auto-compatible.

INTRODUCTION

B. aegyptiaca (L.) is a woody and medicinal tree growing in various ecological conditions (from 100 mm to 1000 mm annual rainfall), but mainly distributed in semi-arid and arid zones in tropical Africa (Von Maydell, 1983). This species is one of the most common in Sénégal. It is used for various needs such as fodder, medicines, charcoal and pesticides. The so-called desert date fruit of *B. aegyptiaca* has been the basis of an active trade for many centuries. The almond is rich in saturated fatty acids that is used as cooking oil. It also contains steroids (saponins, sapogenins, diosgenins) used as raw material for industrial production of contraceptive pills, corticoids, anabolisants and other sexual hormones (ONUDI, 1994). Hence, intensive and uncontrolled exploitation of *B. aegyptiaca* fruits, combined with low rate of natural regeneration has led to the drastic depletion of this species.

Recently scientists have shown an increasing interest on the reproductive biology of tropical trees such as *Leucaena* (Brewbaker, 1983), the genus *Acacia* (Tybirk, 1989; Tybirk, 1992; Buitlaar, 1993; Diallo, 1997), *Saba senegalensis* (Traoré, unpubished), and the monospecific genus *Faidherbia albida* (Gassama-Dia, 2003). Regarding *B. aegyptica*, some preliminary studies on seed pregermination treatment and vegetative propagation were mentioned by El Nour et al. (1991). This paper presents results on the flowering, pollination and reproductive system in *B. aegyptiaca.*

*Corresponding author. E-mail: mndoye@ucad.sn. Tel: 5331084 Fax : (221) 824 63 18.

MATERIAL AND METHODS

A sample of trees older than 15 years was used in this study. These are located in the natural protected area of Dakar-Hann (14° 43' N, 17° 26' W) and Dahra (15° 20' N, 15° 26' W) with an annual rainfall ranging from 300 to 600 mm. Temperature and relative humidity vary from 27°C to 37°C and from 65% to 90%, respectively.Trees are unevenly distributed on a grassy carpet, and are often associated with *Acacia* sp., *Anacardium occidentale*, *Neocaria macrofila* and *Zizyphus mauritiana.*

Flower flushing and morphology

Twenty flowers in the late but stage were marked on each tree. Observations on flowers development were recorded every two hours during 48 h. Collected flowers were preserved in 70% alcohol for morphological studies.

Reproductive organs

Pollen: after microscopic observations of pollen morphology, the functionality of the pollen after a one week storage at -5°C and 15°C was studied using fluorochromatic reaction (FCR) test (Heslop-Harrison, 1984) and an *in vitro* germination test on modified Brewbaker and Kwacks (1963) culture medium.

Pistil: the number of ovules per ovary was counted from cross and longitudinal sections of ovary using a stereo microscopy. Stigma receptivity was studied through two methods: the measure of the stylar growth (Diallo, 1997) and the esterasic test using naphtyl acetate reaction (Heslop-Harrison and Shivanna, 1977). Collecting flowers samples and receptivity tests were completed within 3 hours.

Pollen-pistil interactions

Vectors of pollination: Microscope slides wound-up by double sided scotch were placed in various places on tree foliage, permitting the adhesion of the pollen transported by the wind. Insects captured on *B. aegyptiaca* flowers were instantly killed or immediately plugged in ethyl acetate solution; the dry collection were examined and pollen charge counted.

Controlled pollination: To determine *B. aegyptiaca* reproductive system, pollination pockets were placed on 4 trees. The fine mesh pockets prevented insects crossing without modifying climatic conditions inside the pockets. The isolated flowers in the bud stage were marked and 4 treatments were set up :

A: flowers were not bagged. Nearly a hundred flowers were open to natural pollination.
B: flowers were bagged without any handling. This operation was an autopollination, *sensu lato* enabling an estimation of the rate of geitonogamy in absence of pollinators.
C: the isolated flowers individualized in pockets, were manually auto-pollinized before being replaced in the pocket.
D: the isolated flowers individualized in pockets were emasculated in order to avoid any contamination of the stigma through autopollen . Then they were allofertilized with pollen from another tree situated 100 meters away.

The pockets were left on tree until fruits formation began two weeks later. The index of self incompatibility (ISI), the ratio of fruits number obtained by autopollination on fruits number obtained by allopollination (Zapata and Arroyo , 1978), and the outcrossing indice (OCI) i.e. sum of flower caracteristic values (Cruden, 1976), were estimated. Means were compared using Fischer LSD, and statistical analysis (ANOVA) were made using Scheffé and Dunnett tests.

RESULTS

Phenology

The phenological stages in *B. aegyptiaca* were studied during 12 months to determine both the relative length of each phenophase and the adequate time for placing the controlled pollination pockets. The leafing began in May and ended in December. From December to March the whole populations were partially defoliated on all studied sites (Figure 1). The flowering of *B. aegyptiaca* was heterogenous between sites and delined in two distinct periods. The first flowering period lasted from December to March and concerned only 25% of the population. The second period, more important, with 90% of the individuals took place between March and September with a flowering peak in July (Figure 2). Flowering is usually followed by fruiting, but some trees due to their situation and own specificities, failed to produce fruits. The second flowering period leads to the fruiting phase. Flowers pollinated from May to August produced ripe fruits from December to January (Figure 1).

Floral morphology

Flowers are small and gathered in several types of inflorescence (clusters, fascicles or glomerules), carried by lateral branches. They release a specific smell. Green-yellowish flowers are hermaphrodites. They are actinimorph, shortly pedonculated (1, 2 cm). The calyx and the corolla are dialysepals with 5 green pieces. The sepals are pubescent whereas the corolla is glabrous. The androcea is diplostemoneous with 10 stamens arranged on 2 alternate cycles of 5 ; anthers fixation is dorsimedifixe and their dehiscence is longitudinal.

The tomentellous top ovary is thin in a glabrous short stylar (2 to 3 mm) and ends by a papillated stigma. The pistil of *B. aegyptiaca* is glabrous, monofida and presents at maturity 5 papillae able to retain many pollen grains. Above the floral receptacle an intrastaminal green nectariferous disc exude an abondant nectar. The ovary is sundered in 5 carpels and each one contains an anatropous ovule presenting an axial placentation.

Flower flushing

The flowers of a same inflorescence open asynchronally regardless of their position. 70% of the flowers open during the night with an optimum between 3 and 6 am. The two cycles of stamens shed their pollen on two well distinct periods (Figure 3). The first anthesis concerning the

Forest park	Fe 4	Def 1	Def 2	Def 2	Def 2	Def 3	Fe 1	Fe 2	Fe 3	Fe 4	Fe 4	Fe 4	Fe 4
				Fl 4	Fl 5			Fl 3	Fl 4	Fl 5			
	Fr	Fr	Fr	Fr								Fr	Fr
Mbao	Fe 4	Def 1	Def 2	Def 2	Def 2	Def 3	Fe 1	Fe 2	Fe 3	Fe 4	Fe 4	Fe 4	Fe 4
			Fl 4	Fl 5				Fl 3	Fl 4	Fl 5			
	Fr	Fr	Fr									Fr	Fr
Months	N	D	J	F	M	A	M	J	J	A	S	O	N

Legend:

Fe: Leafing
Def : Defoliation

Fl: Flowering

Fr: Fructification

Phenological index
1: initiation
2: 25%
3: 50%
4: 75%
5: + 80%

Figure 1. Phenogram of *B. aegyptiaca* populations in 2 sites (Mbao and the Forest park of Hann) between November 1997 and November 1998.

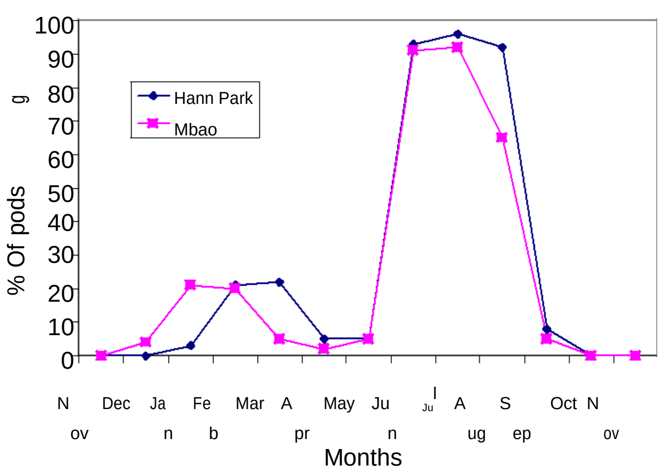

Figure 2. Flowering periods of *B. aegyptiaca* in two sites showing two peaks of flowering.

external cycle of stamens starts at 9 am and lasts between 3 to 5 h; the second anthesis of the internal cycle of anthers begins at 13 pm and last 4 h. The female organs maturity is characterised by a stylar extension, permitting stigma formation under anthers, to be pulled up over anthers: this stylar straining begins at 13 pm and it lasts from 12 to 15 h.

Reproduction

Pollen: The ripe pollen grains (20 to 25 microns of diameter and 25 to 30 microns long) are yellow, oval and present a tri-radial symmetry with three germinative pores (Figure 4). *B. aegyptiaca* shed an important quantity of pollen (22 600 per flower) to ensure fertilization, revealing the importance of male investment during the reproductive period. 91% of shed pollen grain are viable with an optimal germination rate (92%

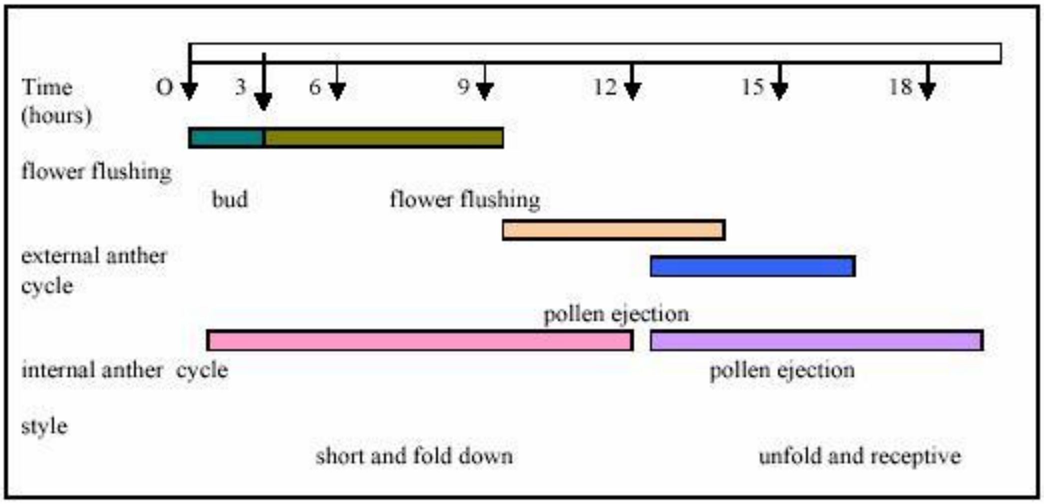

Figure 3. Different development stages of flower's organs in *B. aegyptiaca* from late bud stage to the withered flower.

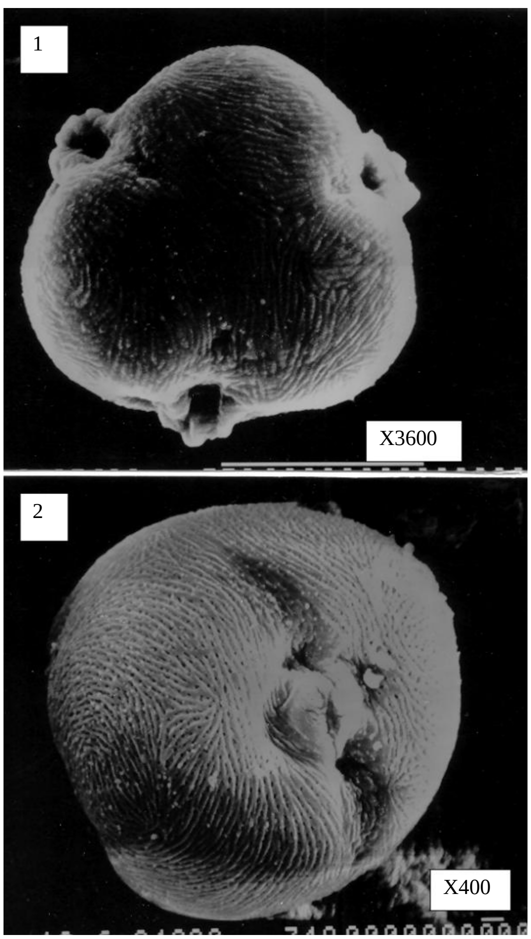

Figure 4. 1: Scanning electron micrograph of pollen of *B. aegyptiaca*. Pollen present a tri-radial symetry with three germinative pores. 2: Scanning electron micrograph showing grain pollen surface with several micropores.

on the BK medium containing 10g/l of sucrose). The whole emission of pollen tube in optimal culture conditions is achieved in nearly 2 min, the pollen tube progressing on a helicoidal movement.

The temperature of pollen storage influences germinative capacity. At 27°C, pollen loose 45 % of its germinative capacity in 24 hours and after 96 hours no germination occurs. When pollen is stored at -5°C or at 15°C it improves and extends the length of its viability for 7 days with 52% of pollen germination at -5 °C and 18 % of germination at 15°C (figure 5).

Pistil: At flower flushing, the stigma is dry and the esterasic test reveals a slight enzymatic activity. One hour after internal cycle pollen shedding, the stigma becomes more receptive. This stage is noticeable by the apparition of digitiform papillae; the esterasic test carried out at that moment, reveals a brown-black coloration.

Pollination vectors

Microscopic observation of double scotched slides, shows many *B. aegyptiaca* pollen grains (49 pollen graines/cm^2), revealing an anemophilous pollination. The most frequent visitors of the flowers are Dipterae, Hymenopterae and Coleopterae. Table I shows that the Dipterae are essentially represented by the *Callophoridae* and the S*yrphidae*. They are very active in the morning from 9 to 11 h (27°C to 37°C and relative humidity about 65%). Among the Callophoridae, *Rhinia apicalis* and *Chrysomia chloropiza* are the most important. They nose around the inflorescences looking for a nectar. Dipterae forage only on nectariferous disc, they rarely fly.

Coleopterae present a great diversity with many families (*Dermestidae, Meloïdae, Curculonidae* and *Scarabeidae*).

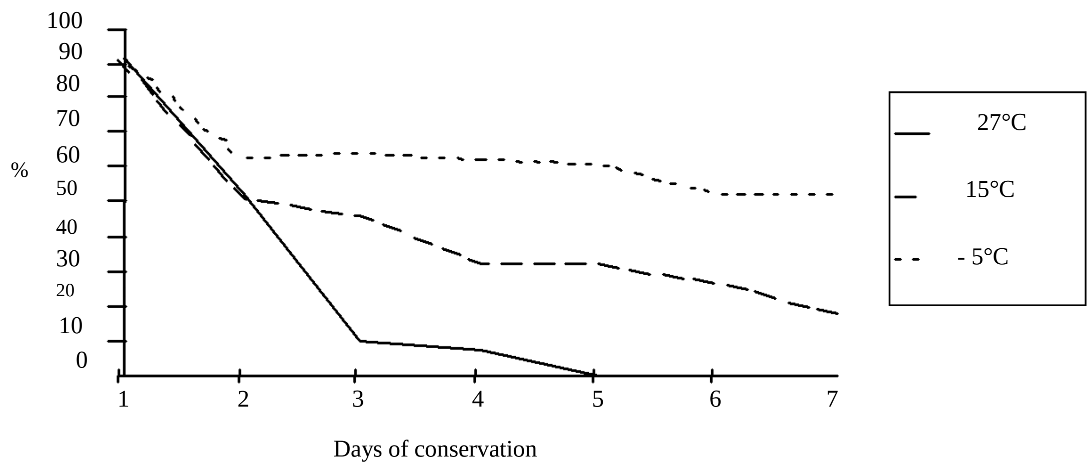

Figure 5. Effect of storage temperature on germination rate of pollen.

Table 1. Pollen loads of individual pollinators caught on *B. aegyptiaca* trees.

Order	Family	Genus	Species	Pollen load
Hymenoptera	Pompilidae	*Batazonellus*	sp	205
	Formicidae	*Camponotus*	*sericeus*	0
	Halictidae	*Nomioïdes*	*rodundiceps*	803
	Halictidae	*Nomioïdes*	*variegata*	1003
	Halictidae	*Nomioïdes*	sp	205
	Halictidae	*Halictus*	*gibber*	1302
Diptera	Calliphoridae	*Rhinia*	*apicalis*	5
	Calliphoridae	*Chrysomyia*	*chloropyza*	51
	Calliphoridae	*Idiopsis*	sp	45
	Syrphidae	*Syritta*	sp	4
Coleoptera	Dermestidae	*Anthrenus*	sp	18
	Dermestidae	*Telopes*	*posticalis*	3
	Meloidae	*Mylabris*	*argentata*	3
	Curculionidae	*Hypotoliscus*	*nubilosus*	7
	Scarabeidae	*Leucocelis*	*nitidula*	8

Among the *Dermestidae*, *Anthrenus sp*. and *Telopes posticalis* are dominant at the bottom of the ovary. They are considered with *Mylabris (Gorrizia) argentata* as great consumers of floral tissues.

Hymenoptera make quick and repeated visits with a rapid collection of pollen. Among these, *Camponotus sericeus* seem to be interested in the nectar, *Batazonellus sp*., *Nomioïdes variegata*, *Halictus gibber* are mainly collectors of pollen.

Pollen load varies considerably quantitatively and qualitatively between groups of vectors (Table I). Hymenoptera are found with a big charge of pollen (more than 1000), Diptera which bear a little charge (5 to 10) are particularly considered as secondary pollinators. However, owing to their population (50% of insects in our study), these Dipterae occupy a very important place in the pollination process. When foraging for nectar, these insects favour pollen transfer on the stigma.

Controlled pollination

The results of the controlled pollination are shown in Table 2. The inflorescences bagged without any handling, and inaccessible to insects give 18% fructification. Autopollination *sensu stricto* by handling between pollen and stigma of the

Table 2. Fructification of *B. aegyptiaca* trees in relation with pollination type.

Treatement	Tree 1		Tree 2		Tree 3		Tree 4		Flower/Fruit ratio
	B	F	B	F	B	F	B	F	%
Self pollination	31	13	32	15	36	18	32	15	46.3b
Auto pollination	30	15	30	18	30	23	30	18	61.6a
Allo pollination	30	6	32	11	36	16	32	16	37.7b
Natural pollination	300	108	200	118	250	195	100	53	61.5a

B = number of floral buds in pockets; F = number of formed fruits.
Values followed by same letter are not significantly different

same flower produce 46% of fructification. Autopollination *sensu lato* (geitonogamy) between pollen and stigma on the same tree gives 61% of fructification. Natural pollination gives the same rate (61%) of fruits as autopollination (*s.l.*). Cross pollination between individuals far from at least 100 meters produces 37% of fructification with an interindividual variation from 20 to 50%. The analysis of variance reveals a significant difference between allopollination and natural pollination (P = 0.047) and also between allopollination and geitonogamy (P = 0.016). There is also a significant difference between autopollination *sensu stricto* and the natural pollination (P = 0,046). Morever, there is no significant difference between geitonogamy and natural pollination.

The index of self incompatibility ISI for the studied population varies from 0.93 to 2 with a mean value of 1.24. The outcrossing index OCI (sum of characteristic values of the flower) is 4. Our results (ISI = 1.24; OCI = 4) show that *B. aegyptiaca* can be considered as a partially auto-compatible species. The significant differences between allopollination and autopollination, show a higher importance of autogamy on allogamy in *B. aegyptiaca.* However, it has been noted as in many other forest trees, inter-individual variability between the studied trees. The seed/ovule ratio is 0.2; on the 5 ovules inside the ovary, only one develops into fruit. Moreover, we found a high ratio of flower abortion from anthesis to maturation of fruits. Of 61% naturally fertilized flowers on one tree, only 4.88 to 7.93% formed ripe fruits.

DISCUSSION

Flowers in *B. aegyptiaca* are arranged in several types of inflorescence (clusters, fascicles or glomerules). Densely massed inflorescences and shorter styles show advantages for insect-pollinated *Balanites* populations. *B. aegyptiaca* provide nectar from the nectariferous disc as a caloric reward together with pollen, that attract a wide range of insect pollinators. This pollination system is characterized by generalistic entomophily, emphasizing hymenoptera and flies. In *B. aegyptiaca,* pollen is released in big quantities (22 600 per flower) with 91% of viability. This viability decreases progressively down to zero within about 5 days of storage at ambient temperature.

This fast lost of viability may be explained by the presence of several micropores on grain pollen surface. These micropores may further an important dehydration and cause the loss of viability of the pollen. Moreover, there is a high correlation between the germination rate of pollen and the percentage of viability of the pollen obtained by FCR. These results show that the medium used in this test is favorable to the germination of pollen grain of *Balanites*.

B. aegyptiaca like *Acacia senegal* (Diallo, 1997) and *A. nilotica* (Tybirk, 1989) shows a synchronization between male (internal staminal cycle) and female phase unlike *Zizyphus jujuba* (Lyrene, 1983). Asynchrony of flowers opening, the quick loss of pollen viability and the high frequency of Diptera, slightly mobile, furthering transport on short distances between flowers of a same inflorescence or of a same tree, all lead towards geitonogamy which is the main strategy developed in *B. aegyptiaca*. However, the ratio of allopollination (37%) can be related to the wind and also to the presence of another population of insects (Hymenopterae) which may be very mobile, carrying substantial charges of pollen on long distances. This strategy helps to maintain a level of heterozygoty (Sedley et al., 1992) in a very changing environment.

This reproductive strategy found in *B. aegyptiaca* is well adapted to the different vectors of pollination observed while the importance of male investment, shows the uncertainty of the transport system (entomophilous and anemophilous) as found in other woody forest species like *Faidherbia albida* (Gassama-Dia, 1996), *A. senegal* (Diallo, 1997) and *A. nilotica* (Tybirk,1989). These results differ from those of most sahelian woody species in which the auto-incompatibility system seems to be the rule (Tybirk, 1992).

The low seed/ovule ratio and the number of ripe fruits show that there is an important rate of abortion in *B. aegyptiaca.* This high ratio of abortion is usual in the sahelian environment where trees produce a great number of flowers to attract pollinators (Tybirk, 1991) and after fertilization, according to ressources allocated (Lloyd, 1980; Bawa and Webb, 1984), select the most competitive fruits for maturation.

B. aegyptiaca has hermaphrodite flowers. The visual attraction is the primary factor in the localization of potential food source (pollen and nectar) for Diptera and Hymenoptera, the main pollinator insects found on *Balanites* inflorescences. Concerning breeding system, the species is partially auto-compatible with a low fruit/flower ratio resulting to high fruit abortions interpreted as an active screening of the progeny best fitted to the environment.

REFERENCES

Bawa KS, Webb CJ (1984). Flower, fruit and seed abortion in tropical forest trees : Implications for the evolution of paternal and maternal reproductive patterns. Am. J. Bot. 71: 731-751.

Brewbaker JL, Kwack BH (1963). The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. 50(9) : 747-858.

Brewbaker JL (1983). Systematics, self-incompatibility, breeding systems and genetic improvement of *Leucaena* species, p. 17 – 22. In: Eds IDRC Ottawa Ontanio <*Leucaena* research in the Asian-Pacific region> Proc. Workshop Singapore 1982.

Buitelaar M (1993). Reproductive biology, phenotypical variability and interspecific hybridation of *A. nilotica* (L.) Willd. ex Del. in Burkina. Stageverslag I.A.H. Larenstein richting Bot. Lab. Tech. 53 p.

Cruden RW (1976). Intraspecific variation in pollen-ovule ratio and nectar secretion – preliminary evidence of ecotypic adaptation. Ann. Bot. Garden 63 : 277 – 289

Diallo I (1997). Biologie florale et pollinisation chez *Acacia senegal* (L.) Willd. Acta bot. Gallica 144 (1). 73-82.

El Nour M, El Khalifa, Massimo K, El Hassen B (1991). Preliminary study on seed pregermination treatement and vegetative propagation of *Balanites aegyptiaca* (L.) Del. In: Physiology des Arbres et Arbustes en zones arides et semi-arides. Groupe d'Etude de l'arbre – Paris, France, pp. 413 – 415.

Free JB (1993). Insect pollination of crops. Academic press limited.684 p.

Gassama-Dia YK, Sané D, Ndoye M (2003). Reproductive biology of *Faidherbia albida* (Del.) A. Chev. Silva Fennica 37 (4): 429-436.

Heslop-Harrison J, Heslop-Harrison Y, Shivanna KR (1984). The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. Theor. Appl. Genet. 67: 367-373

Heslop-Harrison Y, Shivanna KR (1977). The receptive surface of the Angiosperm stigma. Ann. Bot. 41: 1233 – 1258

Lloyd DG (1980). The distributions of gender in four angiosperm species illustrating two evolutionary pathways to deoecy. Evolution 34: 123 - 134.

Lyrene PM (1983). Flowering and fruiting of chinese jujubes in Florida. Hortsciences 18: 208-209.

Nikles DG (1981). Future directions for research development and traiting in breeding technologies for tropical Acacia, p. 9-11. In ACIAR Proceedings No. 37.

ONUDI (1984). *Balanites aegyptiaca* : possibilités offertes par une matière première inutilisée dans l'agro-industrie, 99 p.

Sedgley M, Harbard J, Smith R-M (1992). Hybridation techniques for Acacia ACIAR technical reports 20 pp. 3-11.

Traore A (2000). Etude de la reproduction et du développement de *Saba senegalensis* (A. DC.) Pichon au Sénégal.). Thèse de Doctorat de 3[e] cycle, Université Cheikh Anta Diop, Dakar, Sénégal, 100 p.

Tybirk K (1991). Planting trees in Sahel – doomed to failure ? – Pp. 22 – 28 in : Poulsen, E. And Lawesson, J. E. (eds.), Dryland degradation: causes and consequences. *Aarhus* University Press, Aarhus.

Tybirk K (1989). *Acacia nilotica* in Kenya : aspects of flowering, pollination, seed production and regeneration. Special reports Botanisk Institute, 75 p.

Tybirk K (1992). Pollination, breeding system and seed abortion in some african *Acacia*. Institute of Biol. Sci., Dpt. of Syst. Botany, Aarhus University, Nordlandsvej, DK-8240 Risskov, Danmark, pp.107-137.

Von-Maydell H-J (1983). Arbres et arbustes du Sahel: leurs caractéristiques et leurs utilisations. Eschborn : GTZ, 531 p.

Webb CJ (1979). Breeding system and the seed set in *Euonymus europaeus* (Celastraceae). Plant Syst. Evol. 132: 299-303.

Zappata TR, Arroyo MTK (1978). Plante productive ecology of a secondary deciduous tropical forest in venezuela. Biotropica 10 : 221-230