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Reproductive Biology in *Ziziphus mauritiana var Gola*, and Controlled Hybridization Test with Senegalese Local Jujube

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Abstract: ***Reproductive biology of Ziziphus mauritiana var Gola: floral morpholgy, fluorochromatic procedure and hand pollination. Flowers are hermaphrodite and gathered in several types of inflorescence (clusters, fascicles or glomerules). They blossomed asynchronically exuding nectar and nectar is exuded by the flowers. Top ovary holds two ovules. The second approach consisting in fluorochromatic procedure reveals pollen with 3 apertures and a viability rate of 85%. This viability decrease down to 50% after a storage at +5°C for 2 days. Finally, the hand pollination proves that Ziziphus mauritiana var Gola is a partially auto-incompatible plant and the main vectors of pollination are Diptera (Calliphoridae), Coleoptera (Lycidae and Dermostidae) and Hymenopterae: Sceliphon spirifer (Splecidae), Belenogaster junecus (Vespidae) and Apismellifica (Apidae). We studied total period of different phenological phases (flowering days, blooming, fruit set, fruit setting) and the peak of each phenophase of Ziziphus mauritiana var Gola in Senegal. Flower morphology, pollen pistil interactions, viability of pollen, pollinators and controlled pollination were performed on gola varieties in order to study parameters for selection and variety improvement.***

Keywords: *Ziziphus mauritiana*, Gola, fluorochromatic test, pollen, pollination, auto-compatible, hybridation

1. Introduction

Ziziphus mauritiana Lamk is one of the ancient and indigenous fruits of India. It belongs to family Rhamnaceae and order Rhamnales (2n=48) (Adhikary, 2019).

Ziziphus mauritiana Lamk is a woody and medicinal tree growing (Kerharo & Adam, 1974; Von-Maydell, 1990).Very resistant species to high temperatures, periods of drought 6 to 12 months per year with an annual rainfall of 200 to 600 mm (Munier, 1973, FAO, 1983; Depommier 1988). *Ziziphus mauritiana* Lamk prefers well-drained, neutral or slightly alkaline pH deep sandy soils (FAO, 1983). It has a resistance to salinity (Vashishta, 1997). However, the best yields are obtained under conditions of full sunlight (Morton, 1987), with optimum 22 to 30°C (Mathur *et al*. 1993) and a relative humidity greater than 50% (Vashishta, 1997), but mainly distributed in semi-arid and arid zones in tropical Africa (Von-Maydell, 1990). This species is one of the most common in Senegal. It is used for various needs such as fodder, medicines, (diarrhea, high blood pressure, fevers, sleepiness, and ulcers) charcoal and in agroforestry technologies (hedges, fodder banks...). The fruit is rich in vitamins A and C, phosphorus and protein (Von-Maydell, 1990). Hence, intensive and uncontrolled exploitation of *Ziziphus mauritiana* Lamk fruits, combined with low rate of natural regeneration has led to the drastic depletion of this species.

Gola introduced in Senegal for fruits characteristics usually grafted on local varieties;

Gola variety jujube, introduced by ISRA in Senegal in 1999, adapts well to soil and climatic conditions of the country. It presents large fruit is characterized by a volume of the fruit 10 to 20 times higher than that of wild-type fruits (Danthu *et al*. 2000). But the fruits of this variety have the disadvantage of not being able to be dried for long term storage as local jujubes.

Such an operation aimed to improve fruit quality, requires, a good knowledge of the biology of the species, their population dynamics and the genetic potential of existing equipment on the entire range of the species.

Indeed, the control of the reproductive biology of a species can create powerful intraspecific hybrids for selected traits (Sedgley *et al.*, 1992).

In India, the majority of varieties of West Bengal was previously studied by Kumar et al., (2017), but this study is based on vegetative characters and fruit quality attributes.

This paper presents results on the flowering, pollination, reproductive system of jujube (*Ziziphus mauritiana var Gola*).

2. Material and methods

A sample of trees older than 6 years was used in this study. These are located in the natural protected area in the Dakar region (14° 40' N and 17° 25' W);300 mm rainfall and an average annual temperature of 25°C of are noticed.

Phenology

The phenological stages in *Ziziphus mauritiana var Gola* were studied during 17 months to determine the relative length, intensity and peak of each phenophase.

Flower flushing and morphology

Thirty flowers in the late but stage were marked on each tree. Observations on flowers developments 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 one week storage at -15°C, +5°C and +35°C, was studied using fluorochromatic reaction (FCR) test (Heslop-Harrison et al., 1984) and an *in vitro* germination test on modified culture medium (Brewbaker and Kwacks, 1963).

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 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

Insects captured on *Ziziphus mauritiana var Gola* flowers were instantly killed or immediately plugged in ethyl acetate solution; the dry collection was examined under microscope and pollen charge counted.

Controlled pollination

To determine *Ziziphus mauritiana var Gola* reproductive system, pollination pockets were placed on 4 trees. The fine mesh pockets used, 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:

- Flowers were not bagged. Nearly a hundred flowers were open to natural pollination.
- Flowers were bagged without any handling. This operation was an autopollination, *sensulato* enabling an estimation of the rate of geitonogamy in absence of pollinators.
- The isolated flowers individualized in pockets were manually autopollinized before being replaced in the pocket.
- 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.

The pockets were left on trees until fruits formation began two weeks after manipulation. The index of self incompatibility, the ratio of fruits number obtained by auto pollination on fruits number obtained by allopollination (Zappata and Arroyo, 1978), and the outcrossing index i.e. sum of flower characteristic values (Cruden, 1976), were estimated.

Controlled hybridization

It is a cross-pollination between flowers feet of local jujube and Golavariety jujube. The experimental device consists at the first time to pollinate stigmas of 130 flowers in 4 local feet (31 flowers for the first tree, 15 flowers for the second, 43 flowers for the third and40flowerstothe fourth) with pollen from different Gola trees. The second time we pollinate stigmas of 100 flowers in 4 different Gola trees with pollen from local trees.

3. Results

Phenology

The leafing began in June and ended in January. From February to May the whole populations were partially defoliated on all studied sites (Figure 1).

The flowering of *Ziziphus mauritiana var Gola* is heterogenous between sites and declined in two distinct periods. The first flowering period (in CFPH site) began in June and concerned only 50% of the population. The more important flowering, with 90% of the individuals took place between July and September with a flowering peak in August. The second flowering period (in CNRF site) began in July and ended in October. Flowering is usually followed by fruiting beginning in July and ended in October with a fruiting peak in September. The ripe fruits are produced from December to January (Figure 1).

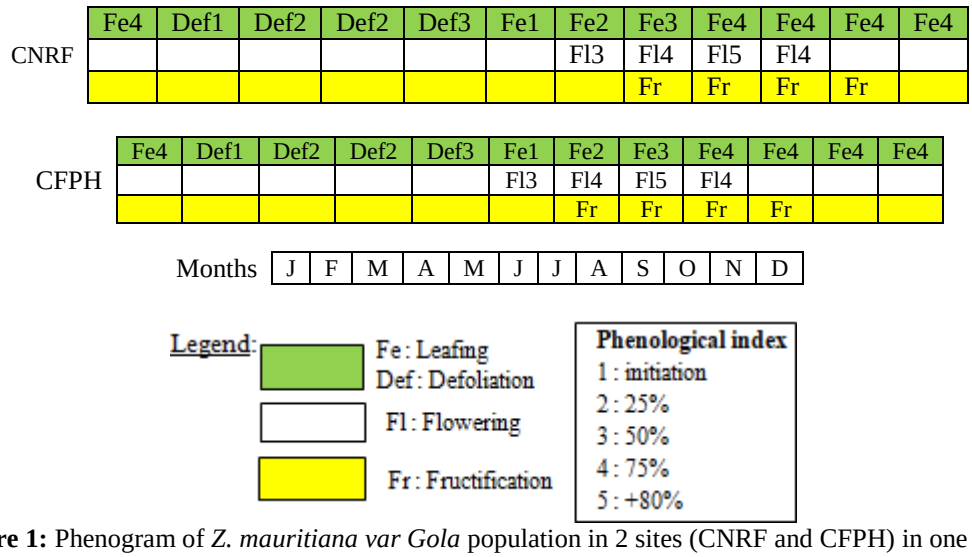

Figure 1: Phenogram of *Z. mauritiana var Gola* population in 2 sites (CNRF and CFPH) in one year

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 actinomorphe, shortly pedonculated (8 mm). The calyx and the corolla are dialysepals with 5 green pieces. The sepals are pubescent whereas the corolla is glabrous. The androcea is monostemoneous with 5 stamens at one cycle; anthers fixation is dorsimedifixe and their dehiscence is longitudinal.

The pistil of *Ziziphus mauritiana var Gola (*L.) is white withan ovary surmounted by a short glabrous stylar (2 mm) and ends by abifid stigma presents at maturity papillae able to retain many pollen grains. Above the floral receptacle a white intrastaminal nectariferous disc exude an abondant nectar between 9:00 and 11:00 am until the next day opening floral. The ovary is sundered in 2 carpels and each one contains an anatropous ovule presenting an axial placentation.

Flower flushing

The flowers of a same inflorescence open asynchronally (on average 3 flowers per inflorescence per day) regardless of their position on the inflorescence axis. However, the basal flowers open the first. The flowers open during the morning between 9 am and 13 pm with an optimum between 11 am to 13 pm. The anthesis starts at 9 am and lasts at 15 pm. The maximum pollen is shed between 11 am and 14 pm. 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 15 pm and it lasts from 12 am to 15 pm (Figure 2).

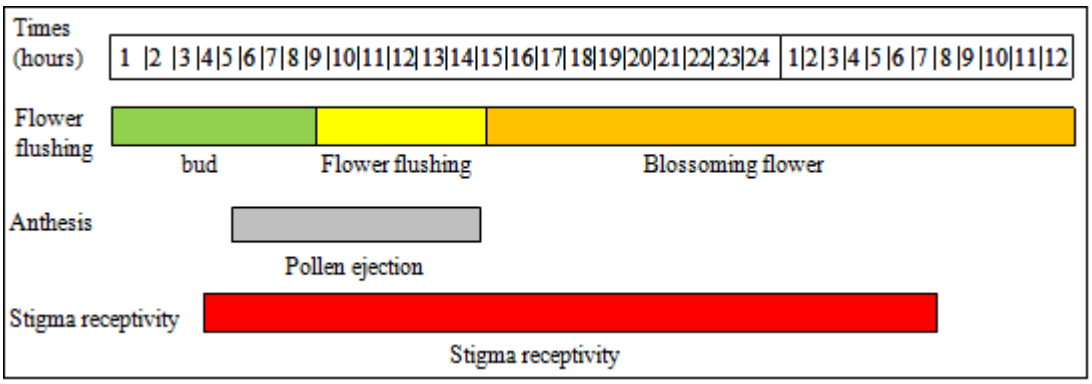

Figure 2: Different developmental stages of flower's organs in *Z. mauritiana var Gola* from bud stage to the withered flower

Reproduction

Pollen

The ripe pollen grains are yellow, oval and present triradial symmetry with three germinative pores (Figure 3). *Ziziphus mauritiana var Gola* shed an important quantity of pollen (6175 per flower) to ensure fertilization, revealing the importance of male investment during the reproductive period. 86% of shed pollen grain are viable (Figure 4) with an optimal germination rate of 67% on the BK medium containing 10g/l of sucrose. The whole emission of pollen tube in optimal culture conditions is achieved in nearly 5 min.

The temperature of pollen storage influences germinative capacity. At 35°C, pollen loose 58 % 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 4 days with 3 times (figure 5):

- After 24 hours: 53% of pollen germination at +5 °C and 27 % of germination at -15°C;
- After 48 hours: 16% of germination at +5 °C and at -15°C;
- After 72 hours: 2% of germination at +5°C and after 96 hours no germination occurs. But at -15°C: 5% of germination and after 96 hours 3% of germination.

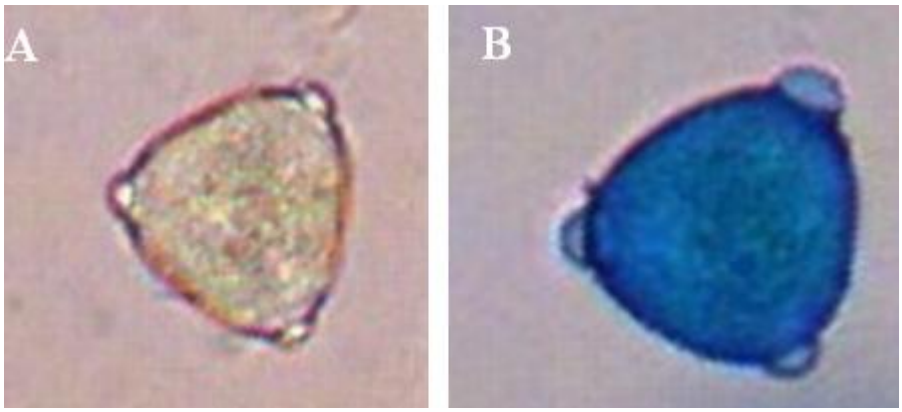

Figure 3. Pollen of Z. *mauritiana* var gola with three germinatives pores in optical microscope (x400)

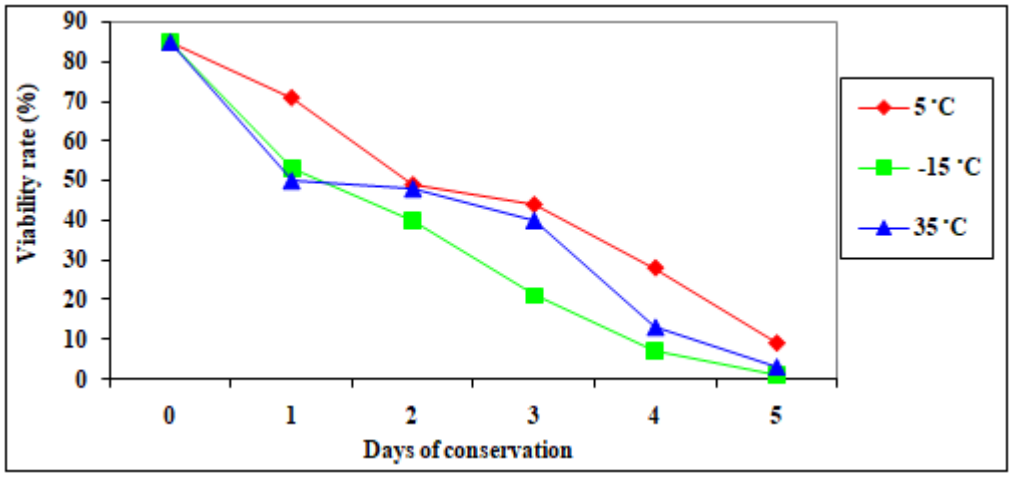

Figure 4: Effect of storage temperature on viability rate of pollen of Z. *mauritiana* var gola

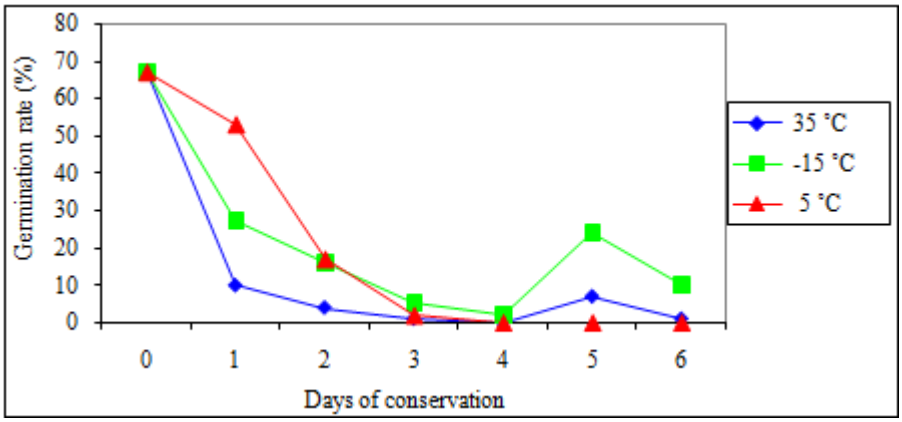

Figure 5: Effect of storage temperature on germination rate of pollen of *Z. mauritiana* var gola

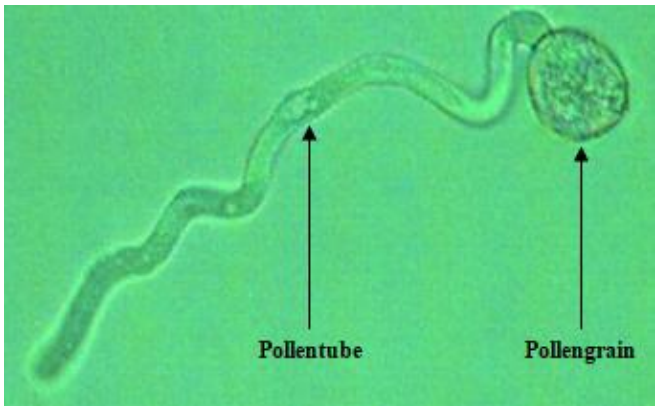

Figure 6: Pollen of *Z. mauritiana* var gola on germination in BKM medium (x400)

Pistil

At flower flushing, the stigma is dry and the esterasic test reveals a slight enzymatic activity. One hour after maximum pollen shedding, the stigma becomes more receptive (from 15 pm). This stage is noticeable by the flower blossomed apparition; the esterasic test carried out at that moment, reveals a hard red coloration of stigma and the upper part of the pistil (Table 1).

Table 1: Esterasic test of stigma flower *Ziziphus mauritiana var Gola* at different stages of development

Floral stage	Floral bouton	Start opening flower	Blossoming flower	24H after opening flower	Wilting flower	Wilted flower
Intensity of the stigma colouring	No stigma colouring	Low stigma colouring	Strong colouring of the stigma and the upper part of the pistil	Coloring stigma alone	No stigma colouring	No stigma colouring

Pollination vectors

The most frequent visitors of the flowers are *Hymenopterae*, *Coleopterae*, *Dipterae* and *Lepidoptera*. Table 2 shows that:

- The *Dipterae* are essentially represented by the *Callophoridae* and the Syrphidae. 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*, *Sarcophaga hemorroidalis*, *Thynchomyia sp* and *Isomyia sp* are the most important. They nose around the inflorescences looking for a nectar. Dipterae forage only on nectariferous disc, they rarely fly.
- The *Coleopterae* are essentially represented by two families: the *Lycidae* and the *Dermestidae*. They are virtually on the flowers of the same tree and rarely change tree suggesting they disseminate a high proportion of self-pollen. Among the *Lycidae*, *Lycus*

(*Chlamydolycus*) *trobeatus*, *Lycus semiamplexus* and *Lycus sp* are dominant at the bottom of the ovary. They are considered with *Trogoderma inclusum* (*Dermestidae*) as great consumers of floral tissues.

- The Hymenoptera are essentially represented by three families: the *Vespidae*, the *Splecidae* and the *Apidae*. They make quick and repeated visits with a rapid collection of pollen. Among these, Apismellifica and *Sceliphon spirifer* (*Splecidae*) are bodies and legs very hairy favourable of pollen carrying contrary to *Belenogaster junecus* of *Vespidae* family.
- The Lepidoptera essentially represented by the *Lampidinae* with *Tarucus sp*.
- Pollen load varies considerably quantitatively and qualitatively between groups of vectors (Table 2).

Hymenoptera are found with a big charge of pollen. *Diptera* which bear a little charge are particularly considered as secondary pollinators. However, owing to their population, these *Dipterae* occupy a very important place in the pollination process. When foraging for nectar, these insects favour pollen transfer on the stigma.

Table 2: Pollen loads of pollinators caught on *Z. mauritiana var Gola*

Order	Family	Genus - Species	Insects number	Pollen load
Coleoptera	*Lycidae*	*Lycus trobeatus*	2	64
		Lycus semiamplexus	7	120
		Lycus sp	2	72
	Dermostidae	*Trogoderma inclusum*	9	35
Hymenoptera	*Splecidae*	*Sceliphon spirifer*	1	183
	Vespidae	*Belenogaster junecus*	1	128
	Apidae	*Apis mellifica*	2	161
Diptera	*Calliphoridae*	*Sarcophaga hemorroidalis*	3	84
		Isomyia sp	3	46
		Thynchomyia sp	2	63
Lepidoptera	*Lampidinae*	*Tarucus sp*	1	6

Controlled pollination

The results obtained from pollination tests on four trees show that no fruit is obtained both for autopollination sensustric to, for autopollination sensula to for allopollinisation. The same results are obtained after evaluation of the percentage of flowers tied (four days after laying pockets). By cons, fruits are obtained for natural pollination whose fruiting rate for all four trees are shown in Figure 6. There is no significant difference between all four trees for the fruit production.

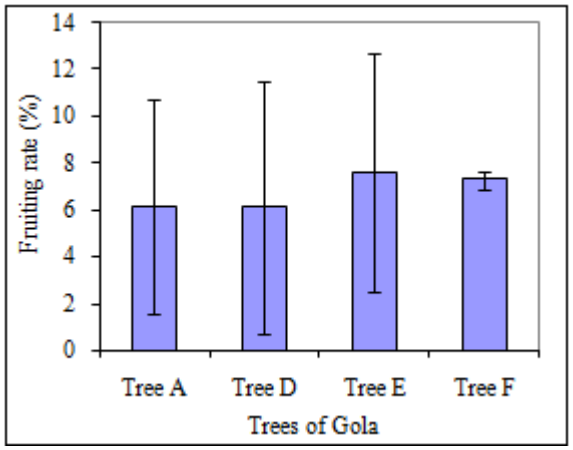

Figure 6: Fruiting rate for 4 trees of *Z. mauritiana var Gola* in natural pollination

The absence of fertilization in controlled pollinations show that flowers pollinate of *Ziziphus mauritiana var Gola* can be considered as essentially entomophilous. So in addition to their role as carrier of pollen, the insects carry promoting action flowers fertilization of *Ziziphus mauritiana var Gola*.

The Pollen/Ovule ratio (3087.5) class *Ziziphus mauritiana var Gola* from allogamy species.

These results show that *Ziziphus mauritiana var Gola* can be considered as a self-incompatible species as was reported for most forest woody where the self-incompatibility system seems to be the rule. Is the same of *Acacia nilotica* (Tybirk, 1992) of *Acacia Senegal* (Diallo, 1994), *Acacia albida* (Gassama Dia-1996), *Balanites aegyptiaca* (Ndoye, 1999), *Tamarindus indica* (Diallo, 2001), *Anacardium occidental* (Niang, 2002) and *Ziziphus mauritiana* (Diallo, 2002) where the existence of autocompatible system is only partial.

The low rate of fruit compared to the high production of flowers shows a significant loss of flowers as was reported for most forest woody.

The abortion rate (4.7%), reflecting the difference between the rate of flowers tied (7.3%) and the rate of fruiting flowers (2.6%) was measured at tree F (Figure 7).

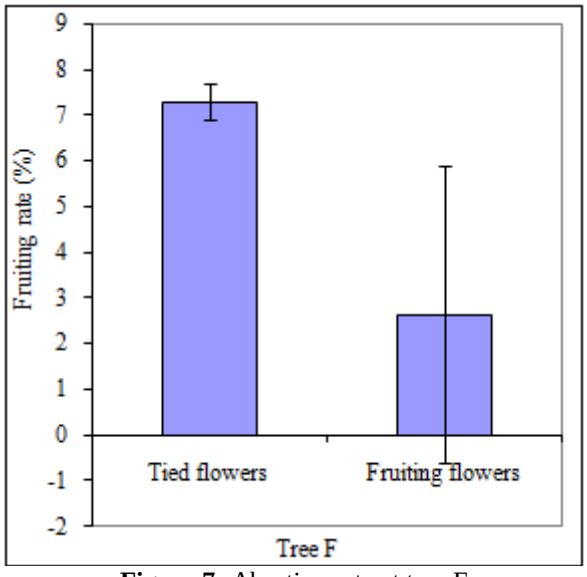

Figure 7: Abortion rate at tree F

Controlled hybridization

For the pollination of flowers Gola variety of jujube by pollen of jujube local no fruit is obtained. By pollinating flowers against local jujube by pollen from the Gola variety of jujube fruits is obtained in three trees for a sample of four (Figure 8).

Based on these results and those obtained previously, we can say that *Ziziphus mauritiana var Gola* is an out crossing species mainly insect pollination.

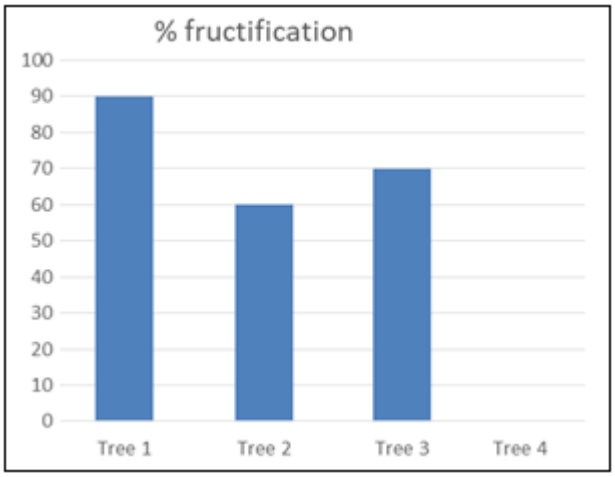

Figure 8: Fruiting rate of four local trees pollinated by pollen of Gola

4. Discussion

In this study we provide useful information on reproductive biology of *Ziziphus mauritiana* var Gola recently introduced in Senegal, giving to breeders, information on proper times of flowering, fruit setting and pollinators interaction.

In our studies, the leafing began in June and ended in January. From February to May the whole populations were partially defoliated on all studied sites (figure1).

Kulkarni (2016) distinguished five seasonal growth stages : 1) winter dormancy, 2) vegetative growth, 3) early flowering, 4) full bloom, 5) fruiting. Phenology varies slightly between Z. mauritiana and Z. spina-christi but it was distinct in Z. jujube.

We showed that the flowering is usually followed by fruiting beginning in July and ended in October with a fruiting peak in September.

According to Kulkarni (2016), fruiting period for Z. jujube is from Mid August to December depending on genotype. In general, Z. jujube had earlier flowering and fruiting stages as compared to the Z. mauritiana and Z. spina christi genotypes. In the latter two species, peak flowering period was between July to November, fruiting period between October to January, and winter dormancy between February to May.

Densely massed inflorescences and short styles show advantages for insect-pollinated *Ziziphus* populations. *Ziziphus mauritiana var Gola* 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 *Ziziphus mauritiana var Gola*, pollen is released in big quantities (6175 per flower) with 85% of viability. This viability decreases progressively down to zero within about 5 days of storage at ambient temperature.

This decrease is related to pollen size and the storage nutrients that particularly affect the pollen tube germination (Roulston, T. 2000)

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 *Ziziphus*.

Kulkarni (2016) showed that the percentage viable seed set following open pollination varies significantly among the three species. It is highest in Z. spina-christi (84.2%) as compared to Z. mauritiana (19.7%) and Z. jujube (17.5%). Within species [between genotype] variation for seed set ranged between 11.0 to 22.1% in Z. *mauritiana* and 4 to 36% in Z. jujube.

Ziziphus mauritiana var Gola like *Zizyphusjujuba* (Lyrene, 1983) shows a synchronization between male (internal staminal cycle) and female phase unlike *Acacia Senegal* (Diallo, 1997), *A. nilotica* (Tybirk, 1989) and *B. aegyptiaca* (Ndoye, 2004).

The lack of fruit during controlled pollination unlike natural pollination suggests that *Z. mauritiana*var Gola develops a reproductive strategy well adapted to the different vectors of pollination which indicates a transport system entomophilous.

A large percentage of fruit dropped before reaching the maturity stage. However, the heaviest drop was recorded in the variety Banarasi Karaka and minimum in Umran. In all the cultivars the fruit drop was maximum up to 30 days after fruit set, declining later on (T. Adhikary, 2019).

Our results are the same that those of most sahelian woody species which the auto-incompatibility system seems to be the rule (Tybirk, 1992). Unlike as found in other woody forest species like *Faidherbiaalbida* (Gassama-Dia, 1996), *A. Senegal* (Diallo, 1997) and *A. nilotica* (Tybirk, 1989) which we note the uncertainty of the transport system (entomophilous and anemophilous).

Ziziphus mauritiana var Gola 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 *Ziziphus* inflorescences. Concerning breeding system, the species is auto-incompatibility with a low fruit/flower ratio.

The low rate of fruit compared to the high production of flowers shows a significant loss of flowers as was reported for most forest timber.

The results show that the mean of fruit set was 55% at the end of our experiences.

However, T. Adhikary (2019) showed that the higher fruit set was also recorded in the jujube varieties (BAU Kul-1 (26.5 %), Umran (26.1%), Banarasi Karaka (24.8%), Dandan (23.3%), Topa (23.1%), Illaichi (21.4%), Madhavpur (20.8%) and Jogia (20.4%)).

5. Conclusion

In this study were we studied total period of different phenological phases (flowering days, blooming, fruit set, fruit setting) and the peak of each phenophase of *Ziziphus mauritiana var Gola*, is a very important contribution on the *Ziziphus* reproductive biology. This work opens interesting perspectives for the genetic improvement of the local species.

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