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Pollen fertility of *Myrianthus arboreus* (P. Beauv) in five localities in Côte d'Ivoire

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Abstract: Pollen fertility could have a significant effect on fruit quality and seed production in most plants. This study was carried out to evaluate the pollen fertility of *Myrianthus arboreus* in the localities of Abengourou, Adzopé, Daloa, Diabo and Zouan Hounien. The vegetation of these localities, consisting largely of forest, has disappeared in favor of fallow land and orchards. Rainfall varies between 1000 and 2200 mm of water per year with an average monthly temperature of 27 °C. The soil is generally ferralitic. One hundred and fifty plants, aged at least 5 years, were evaluated in 2018. Pollen viability was assessed by staining with 1.5 % acetocarmine. Germination was tested *in vitro*, on two different culture media. The results revealed that the viability rates of pollen grains of plants from Abengourou, Adzopé, Daloa, Diabo and Zouan Hounien were 94.35 %, 93.52 %, 93.59 %, 65.35 % and 93.33 %, respectively. The germination rates with medium 1 were 93.29 % for Abengourou plants, 92.12 % for Adzopé, 92.95 % for Daloa, 63.29 % for Diabo and 92.14 % for Zouan Hounien. Concerning medium 2, the rates were 91.73 %, 90.56 %, 90.51 %, 61.87 % and 90.36 %, respectively, for the plants of Abengourou, Adzopé, Daloa, Diabo and Zouan Hounien. The correlation test carried out on the two fertility parameters revealed the existence of a strong positive correlation between the viability rate and the pollen germination rate.

Keywords: *Myrianthus arboreus*, pollen, viability, germination, Côte d'Ivoire.

Introduction

Myrianthus arboreus is a plant native to Africa. It is known by various names in local languages: "oujoujou" among the Igbo of Nigeria; "bokekou" in Douala in Cameroon and "tikliti" in Gagnoa in Côte d'Ivoire (Eyog et al., 2006; Djaha et Gnahoua, 2014). The distribution area of this plant extends from West Africa to East Africa, including Central Africa (Amata, 2010).

In Côte d'Ivoire, *M. arboreus* is present throughout the territory except for the northern regions (N'dri, 2021). It is a dioecious, uncultivated plant, highly prized in Côte d'Ivoire for its numerous nutritional (Eyog et al., 2006) and medicinal (N'dri et al., 2008) properties. Indeed, biochemical analysis of young leaves has shown that they are rich in iron, mineral salts and proteins (Amata, 2010). In addition, work on the bark and roots has shown that the latter contain antioxidants that are very effective against diabetes (Pierre et al., 2015). Furthermore, the work of Agyare et al. (2014) and Memvanga et al. (2015) in pharmacology, have respectively highlighted the healing and anti-plasmodial properties of this plant.

Most of the scientific work on *M. arboreus* has concerned the ethnobotanical, pharmacological and biochemical aspects (Zoro Bi et al., 2014; Olonode et al., 2015). Very few works have dealt with the domestication of this plant. In addition, due to its abusive exploitation and the effects of climate change, access to *M. arboreus* in our forests is becoming more and more difficult.

To continue to sustainably exploit this wild resource, important both nutritionally and medicinally, it is necessary to find strategies to multiply and domesticate it. To do this, Akaffou et al. (2018) vegetatively propagated *M. arboreus*. Their work showed that the success rate of *M. arboreus* multiplication by cuttings is low. To remedy this, it is advisable to attempt sexual multiplication. For this, it is important to understand pollen fertility in this species. Thus, the present study aims to evaluate the pollen fertility of *M. arboreus* in Côte d'Ivoire. To achieve this objective, the viability and germination of *M. arboreus* pollen were determined in five localities in Côte d'Ivoire.

Results

Pollen viability of Myrianthus arboreus

Pollen viability did not vary within each locality ($p > 0.05$). However, significant differences were observed between localities for viable pollen counts ($F = 761.5$; $p < 0.001$).

The viability rates of pollen grains of plants from Abengourou, Adzopé, Daloa, Diabo and Zouan Hounien are respectively 94.35 %; 93.52 %; 93.59 %; 65.35 % and 93.33 %. The highest pollen viability rates were observed in the localities of Abengourou, Adzopé, Daloa and Zouan Hounien. These rates have been above 90 % and similar in these localities. The lowest levels of viable pollen were recorded in Diabo plants (Figure 1 and Figure 2).

Pollen germination of Myrianthus arboreus

For all collection localities, the germination rates of pollen grains were approximately identical on the two germinative media used. Within each locality, the germination rates of pollen grains were not significantly different ($p > 0.05$). On the other hand, between localities, significant differences were observed for pollen germination rates on culture media 1 ($F = 850.4$; $p < 0.001$) and 2 ($F = 672.2$; $p < 0.001$).

The germination rates with medium 1 are 93.29 % for Abengourou plants; 92.12 % for Adzopé; 92.95 % for Daloa ; 63.29 % for Diabo and 92.14 % for Zouan Hounien. Concerning medium 2, the rates are 91.73 %; 90.56 %; 90.51 %; 61.87 % and 90.36 % respectively for the plants of Abengourou, Adzopé, Daloa, Diabo and Zouan Hounien. The germination rates of pollen grains

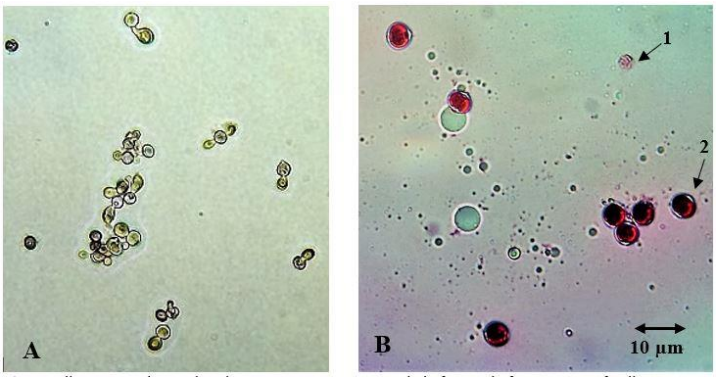

Fig 1. Pollen grains observed under a microscope respectively before and after staining of pollen grains with 1.5 % acetocarmine (Gx40). A: Pollen grain before coloring; B: Pollen grain after staining; 1: non-viable pollen; 2: viable pollen

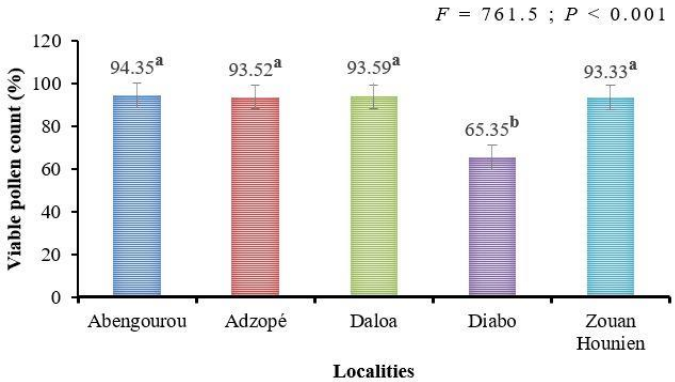

Fig 2. Pollen viability rate of *Myrianthus arboreus* plants studied in various collection areas. *F*: value of the statistic associated with the Fisher test; *p*: probability value associated with the Fisher test. The means indexed by the same letter are statistically identical according to the test of the smallest significant difference at the 5 % probability threshold.

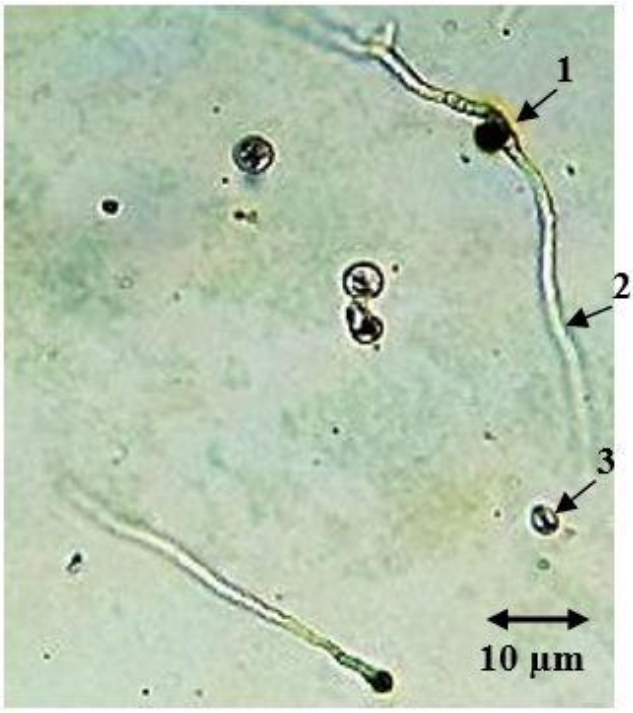

Fig 3. Pollen germination, observed under a microscope, on culture medium. 1: germinated pollen grain; 2: pollen tube; 3: ungerminated pollen grain.

collected in Abengourou, Adzopé, Daloa and Zouan Hounien were similar and greater than 90 % for the two germinative medium. Pollen grains collected at Diabo had lower pollen germination rates (Figure 3 and Figure 4).

Correlation between viability and pollen germination of Myrianthus arboreus

The correlation test between viability rates and pollen germination rates of media 1 and 2 revealed highly significant positive correlations ($p < 0.001$). The correlation coefficient r was 0.9630 and 0.96427 for the correlations between pollen viability and pollen germination respectively on media 1 and 2 (Fig 5 and Fig 6).

Discussion

Pollen fertility of Myrianthus arboreus

The study of pollen grains provides a clue to hybridization and selection systems (Alotaibi et al., 2020). Knowledge of pollen viability and germination is crucial for the genetic improvement of many species (Souza et al., 2015). Pollen viability and vigor then determine pollen quality (Veluru et al., 2021). These two parameters provide valuable information for the success of plant fertilization (Shivanna, 2009). Thus, pollen tube viability and growth are necessary for any rational approach to increase productivity (Alcaraz et al., 2011).

A pollen viability test was carried out on *M. arboreus*. The results obtained showed more than 90 % viable pollen in individuals from Abengourou, Adzopé, Daloa and Zouan Hounien compared to 65 % in individuals from Diabo. However, acetocarmine staining, although simple and rapid, is not a viability test per se as observed by Boughediri et Carbonnier (1993) since carmine attaches to the cytoplasm and pollen. which degenerate also stain, since the cytoplasm is still present there. However, according to this author, it is useful for estimating pollen quality. As it is likely that acetocarmine staining overestimates pollen viability, *in vitro* germination tests were done to confirm pollen viability. *In vitro* germination and pollen tube length are very important parameters for testing pollen viability (Pham et al., 2011). The *in vitro* germination test makes it possible to detect the differences between the various types of pollen. Franchi et al. (2007) showed that the *in vitro* germination test advantageously complements determinations limited to pollen viability by cytological staining techniques.

The pollen germination rates obtained were the same on both medium 1 (solid) and medium 2 (liquid). These results could be explained by the composition of the environments. Indeed, although one is solid and the other liquid, the two environments have the same nutrient composition. This indicates that pollen germination is much more influenced by the composition of the medium than by its solid or liquid state. On two germination media, the germination rates were greater than 90 % for the four zones of Abengourou, Adzopé, Daloa and Zouan Hounien, while this rate was around 60 % for the Diabo zone. This highlights the existence of variability in pollen fertility of this species in the collection areas. Environmental effects could strongly be responsible for the variations in pollen fertility during this study. These results mirror those obtained by Sivaraman et al. (2016). Indeed, according to these authors, pollen is like any living cell, its behavior and survival are influenced by the environment and the genotype.

Our results obtained by the germination test therefore made it possible to confirm the estimation of pollen viability by acetocarmine staining in *M. arboreus*. The pollen viability and germination rates observed in all the areas studied are greater than 50 %. These results reveal that the pollen grains from *M. arboreus* would be of good quality and the reproduction of this plant would be easy. Certainly, the pollen fertility rates observed are greater than 50 % but those observed in the forest areas, in Abengourou, Adzopé, Daloa and Zouan Hounien, are greater

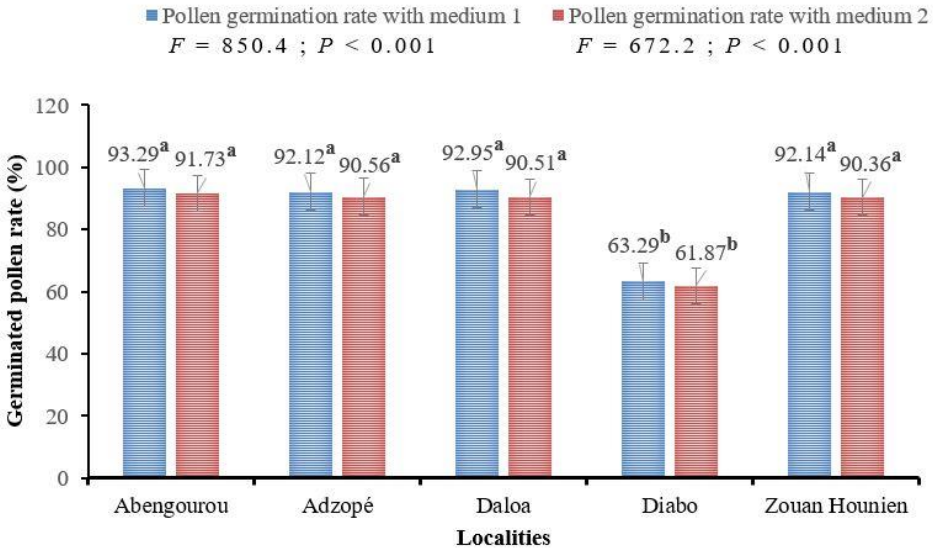

Fig 4. Pollen germination rates of *Myrianthus arboreus* plants studied in various collection areas. *F* : value of the statistic associated with the Fisher test ; *p* : probability value associated with the Fisher test. The means indexed by the same letter are statistically identical according to the test of the smallest significant difference at the 5 % probability threshold.

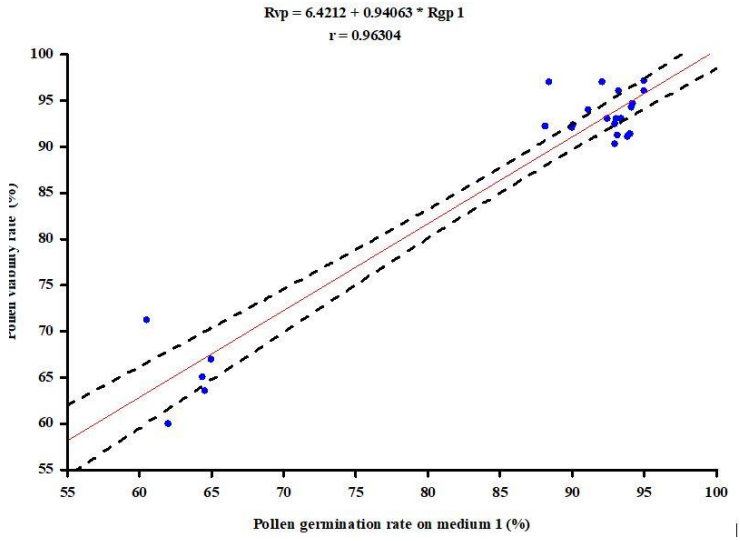

Fig 5. Relationship between viability rate and germination rate of medium 1. Rpv : pollen viability rate ; Rgp 1 : pollen germination rate on medium 1 ; r : correlation coefficient.

than 90 % compared to those of Diabo which is an area of savannah.

Correlation between viability and pollen germination of Myrianthus arboreus

In-depth knowledge of pollen biology, including viability and germination, is necessary for any rational approach to increasing productivity (Bilgin, 2022). Pollen grains are therefore essential for the sexual reproduction of plants and their viability, as well as their longevity and storage are essential to plant physiology, ecology and plant breeding (Althiab et al., 2023). Our results showed that the viability rate increases with the pollen germination rate. This could be explained by the fact that high pollen viability leads to good development of the pollen tube. Our results confirm those of Badii et al. (2013). Indeed, their work has revealed that there is, in general, a linear relationship between pollen viability and germination capacity in many fruit species.
However, our work contrasts with that of Sudha et al. (2022) who worked on coconut pollen grains (*Cocos nucifera* L.). The latter found a difference between the pollen viability and germination rates which varied from 92.35 to 96.5 % and 16.05 to 31.59 % respectively. These results could be explained by the fact that pollen germination depends on the concentration and incubation time of the culture medium as shown by Verma et al. (2017).

Material and Methods

Collection areas and plant material

The collection areas were chosen because of the high use of *Myrianthus arboreus* in the different localities of Côte d'Ivoire. These are Abengourou, Adzopé, Daloa, Diabo and Zouan Hounien (Figure 7).
The chosen plants were found in forests, fallows, as well as cocoa and cashew orchards in these five localities of Côte d'Ivoire (N'dri et al., 2008; Olonode et al., 2015; Ehilé et al., 2019).
The study focused on 75 adult wild male plants of *M. arboreus*, distributed among the sampled localities at a rate of 15 plants per locality.

Pollen collection

Mature yellow inflorescences were collected from 15 individuals per locality and five inflorescences were collected per individual. These inflorescences were collected at different times of the day, namely: 8 a.m., 10 a.m. and 12 p.m. in order to determine the time of massive availability of pollen grains. For this purpose, the inflorescences which made it possible to assess pollen viability were collected at 10 a.m., in each chosen locality and placed in aluminum foil. Then, they were transported to the laboratory

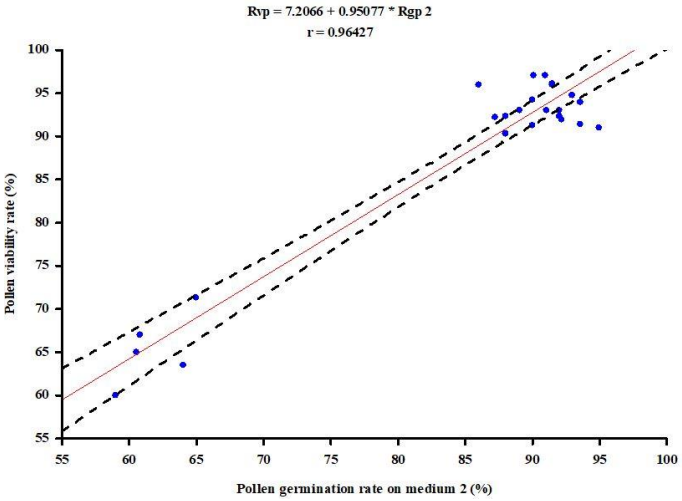

Fig 6. Relationship between viability rate and germination rate of medium 2. Rpv: pollen viability rate; Rgp 2: pollen germination rate on medium 2; r: correlation coefficient.

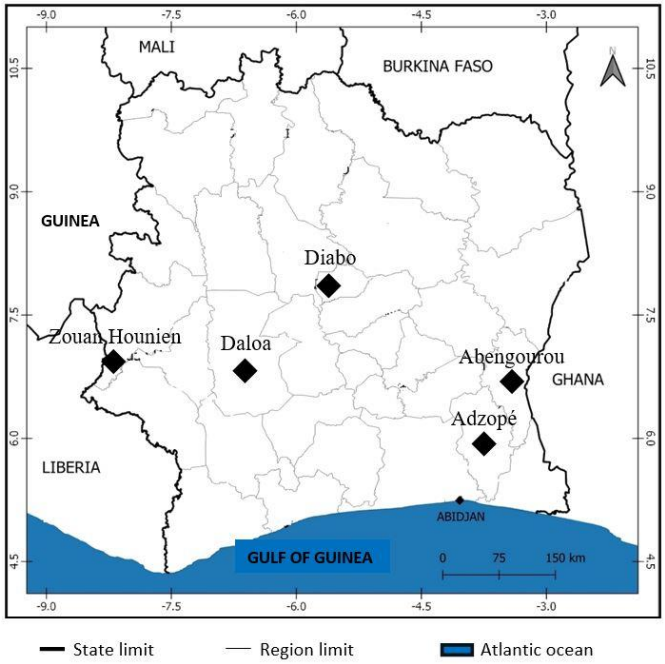

Fig 7. Collection areas. Abengourou (6°49'47'' Nord ; 3°29'47'' Ouest) ; Adzopé (6°06'25'' Nord ; 3°51'36'' Ouest) ; Daloa (6°54'28'' Nord ; 6°26'25'' Ouest) ; Diabo (7°47'00'' Nord ; 5°11'00'' Ouest) et Zouan Hounien (6°55'00'' Nord ; 8°13'00'' Ouest) (Yao et al., 2012).

and put in self-fertilization envelopes, then stored in the freezer at -10 °C (Tisserat et De Mason, 1980).

Assessment of pollen viability

Three days after storage, pollen viability was tested by staining with 1.5 % acetocarmine ; prepared according to the method of Akaffou et al. (2014). The test first consists of placing a drop of acetocarmine on a microscope slide (Fig 8 A). Subsequently the inflorescence is tapped above the blade to collect the pollen in the drop (Fig 8 B). The drop is then covered by a coverslip. Thirty minutes after staying in acetocarmine, pollen grains were observed.

Pink-colored pollen grains of uniform size were considered viable (Konan et al., 2007). Five ZEISS optical microscope fields (magnification 40) were scanned per slide and 100 pollen grains were observed per field. The viability rate was estimated (as a percentage) using the following formula :

Fig 8. Implementation of pollen viability testing. **A**: deposition of acetocarmine and **B**: collection of pollen grains.

Fig 9. Seeding a petri dish with pollen grains by tapping the inflorescence.

$$\text{Viability rate} = 100 \times \frac{Number\ of\ viable\ pollen}{Total\ number\ of\ pollen\ observed} \quad (1)$$

Assessment of pollen germination

Pollen germination was tested *in vitro*, on two culture media, one of which is solid (Medium 1) and the other liquid (Medium 2). The first was composed of 10 g of sucrose (0.5 M), 20 mg of boric acid (2.43 mM), 30 mg of calcium nitrate (2.12 mM), 1 g of agar (1 %) and 100 mL of distilled water (Tuinstra et Wedel, 2000). The mixture was brought to a boil in a beaker on a hot plate, then distributed into 90 mm diameter Petri dishes at a rate of 20 mL per dish. The medium was cooled and solidified at room temperature (25°C). The second medium was composed of 500 µL of sucrose (0.5 M) and 20 mg of boric acid (2.43 mM) contained in 2 mL Eppendorf tubes (Chloé, 2010).

In the first medium, pollen was inoculated under a hood (Fig 9) and left to incubate for 12 hours at room temperature (25°C). In the second medium, after 2 hours of incubation at 32°C in a water bath, the pollen was inoculated under a hood in each tube and 2 drops of 1.5 % acetic carmine were added. After 10 min of staining, 20 µL of solution was taken and mounted between slide and coverslip.

The proportion of pollen germinated in each of the media was evaluated using a ZEISS brand optical microscope at Magnification 40. The pollen has germinated when the length of the pollen tube is greater than or equal to its diameter (Tuinstra et Wedel, 2000; Acar et Kakani, 2010).

For each of the two environments, five microscope fields were scanned per slide and 100 pollen grains were observed per field. The germination rate was determined (as a percentage) by the following formula:

$$\text{Germination rate} = 100 \times \frac{Number\ of\ pollen\ germinated}{Total\ number\ of\ pollen\ observed} \quad (2)$$

Statistical analysis of data

First, the rates of viable and germinated pollen grains were respectively subjected to a one-way analysis of variance (ANOVA). The source of variation was the locality of collection. Then, the means of the different collection areas were compared using the Least Significant Difference (PPDS) test at the 5 % threshold. Finally, the Pearson correlation test was used to study the relationships between viability and germination rates. All statistical analyzes were carried out using Statistica version 7.1 software (StatSoft, 2005).

Conclusion

Knowledge of pollen fertility is of paramount importance in hybridization programs and crucial for the genetic improvement of many species. At the end of this study, we note that *M. arboreus* has good pollen fertility but this good pollen fertility is much higher in forest areas. Viability and pollen germination are directly proportional. As pollen viability and longevity are essential factors for food security and adaptation to climate change, we highlight the need to further develop fundamental research to better understand the complex molecular mechanisms that modulate pollen fertility and research applied to develop new methods to maintain this fertility. It would also be wise to carry out *in vivo* tests to observe the overall effects on the pollen grains of these plants.

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