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Effect of pollen load, source and mixture on reproduction success of four cultivars of *Citrullus lanatus* (Thunb.) Matsumara and Nakai (cucurbitaceae)

Adjoumani Koffi*, Bony Beket Séverin, Koffi Kouamé Guillaume, Yao Koffi Bertin, Kouonon Léonie Clémence, Yao Konan Sylvère, Djè Yao

Université d'Abobo-Adjamé, Laboratoire de Biologie et Amélioration des Productions Végétales, 02 BP 801 Abidjan 02, Cote d'Ivoire

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ABSTRACT

Citrullus lanatus is cultivated in Côte d'Ivoire for its edible kernels which are used as a soup thickener. There are four distinct cultivars (one *Bebu* and three *Wlêwlê*). A better understanding of the reproduction system for those annual monoecious cultivars may improve its production. We studied the impact of pollen load, pollen source and diversity on the yield of fruits and seeds.

Various pollination treatments were applied during four growing seasons to assess pollen load, pollen source and diversity (cross pollination with single source, cross pollination with multiple sources, self pollination and natural pollination) and pollen mixture. Pollination and reproductive success were determined by a fertilization index and by assessing fruits and seeds production. The self-compatibility and inbreeding depression index were also evaluated.

Higher or lower pollen quantity decreases fertilization success of fruits and seeds production while medium pollen load leads to optimal yield. All cultivars were self compatible and higher level of inbreeding depression had been observed. When cross pollinated, yield was higher than natural pollination and self pollination yield. With manual pollination, cross pollination with multiple sources produces more fruits and seeds resulting in increased yield. Pollen diversity also improves fruits and seeds production. Pollen mixture will increase yield if the proportion of cross pollen in the mixture is higher than self pollen proportion.

In conclusion, fruits and seeds production of those local self compatible cultivars were influenced by the amount of pollen load, pollen source and diversity, and also by proportion of pollen type in the pollen mixture.

These results are therefore necessary in order to predict the evolutionary trajectory of mating systems and to make plant breeding program.

1. Introduction

Citrullus lanatus is an important cucurbit crop accounting up for 7% of the worldwide area devoted to vegetable production. Two types have been described: watermelon type from *C. lanatus* var. *lanatus* widely studied in America and Europe (Gusmini and Wehner, 2007) and oleaginous (preserving melon or Africa melon) type called "*egusi*" from *C. lanatus* var. *citroides* frequently used in Africa (Minsart and Bertin, 2008). Watermelon is cultivated for its commercial fruit (consumed fresh fruits) (Gusmini and Raleigh, 2003) while Africa melon is produced for its economical seeds (edible seeds) (Lévi et al., 2001; Zoro Bi et al., 2003).

C. lanatus should be grown at a wide spacing because of their long and trailing vines. It is the only economically important cucurbit with pinnatifid (lobed) leaves; all of the other species have whole (non-lobed) leaves. Flowering begins four weeks after seeding. The number of staminate flowers is usually greater than pistillate flowers on the same plant with the ratio ranging from 4:1 to 15:1, depending on cultivar. Each staminate flower contains three fused anthers which release approximately 850 grains of pollen (Zaman, 2006). The pistillate flowers have an inferior ovary, and the size and shape of the ovary are correlated with final fruit size and shape (Wehner, 2008). These flowers are generally pollinated by insects mainly by honey bee (*Apis mellifera* L.) (Bayoumi, 2009). After pollination, fruits and seeds are developed. Various fruit and seed shapes and colors are reported in *C. lanatus* (Gusmini et al., 2004). Large edible fruits contribute to the diets of consumers throughout the world. Although it is mainly

* Corresponding author. Tel.: +225 07101610.
E-mail address: adjoumani.koffi@yahoo.fr (A. Koffi).

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consisted of water (often over 90%), it also contains important nutritional compounds, including sugars, lycopene and cardiovascular health-promoting amino acids, such as citrulline, arginine and glutathione (Perkins-Veazie et al., 2006). Lycopene has been classified as a useful component of human diet for prevention of heart-attacks and certain types of cancer (Perkins-Veazie et al., 2006).

In sub-Saharan Africa oleaginous types of *C. lanatus* are prized for their seeds reported to be rich in nutrients (60% lipids and 30% proteins) (Loukou et al., 2007; Gichimu et al., 2009). These oleaginous seeds are consumed as thickeners of a traditional soup called "*pistachio soup*" in Côte d'Ivoire. Furthermore, they represent an important source of income for farmers, mainly for women, in West and Central Africa (Zoro Bi et al., 2003).

Despite the economic, social and nutritional role played by this oleaginous cucurbit, it is classified as a secondary crop. In Côte d'Ivoire, these species comprises two groups according to their fruits and seeds morphology. The first group (locally called *Wlêlwlê*), contains three cultivars all characterized by smooth seeds which have tap at their point of connection. The second group (locally called *Bebu*) contains only one cultivar characterized byovoid and flattened seeds, with a thick and rough margin (Zoro Bi et al., 2006).

Although these cultivars are well adapted to extremely divergent agro-ecosystems and various cropping systems characterized by minimal inputs, their production is so low that it cannot guarantee food security and sustainable incomes for peasants (IPGRI, 2002; Loukou et al., 2007). Efforts are being made to improve cropping systems. Improvement of local germplasm of *C. lanatus* has been one of the key research projects of practical interest since it provides comprehensive and useful data for breeding scheme implementation and management decisions. The knowledge of the reproduction mechanisms constitute an important factor that must be known before starting a breeding program (Ainsworth, 2000).

However, many plants have developed barriers that operate in the pistil either before fertilization to prevent the "wrong" cross by inhibition of pollen tube growth, either after fertilization, causing abortion of the illegitimate embryo (Ainsworth, 2000; Roselino et al., 2009). In this incompatibility case, the stigma is able to reject its own pollen from the same plant (self incompatibility) or from a foreign plant (cross incompatibility) (Goring and Walker, 2004).

Plant self-incompatibility (SI) systems are genetic systems which prevent self-fertilization through recognition and rejection of pollen expressing the same allelic specificity as that expressed in the pistils (Castric and Vekemans, 2004). Two classes of SI are known, namely heteromorphic and homomorphic systems. Heteromorphic systems are characterized by morphological differences between the two or three genotype classes associated with different incompatibility specificities, particularly differences in the length or shape of the styles (heterostyly). In contrast, in homomorphic systems, the incompatibility genotypes cannot be distinguished morphologically, and the incompatibility response relies entirely on physiological mechanisms (Castric and Vekemans, 2004).

For compatible species, the transferred pollen to stigma adheres, hydrates, germinates, and tube growth occurs before reaching the ovaries (Sanzol et al., 2003). Each ovary which is fertilized a viable pollen grain can yield seed. This means that the quantity and the genetic composition of the pollen load are determinant for reproduction success and affect the number and size of seeds produced per fruit. For example, when mixtures of pollen from its own (self) plant or a differing (cross pollen) are deposited onto a stigma, the self pollen is less likely to achieve fertilization in species with partial or complete self-incompatibility systems (Stone et al., 2006). This may lead to reduce seed production if there is insufficient cross pollen to fertilize all the ovules.

The ability to prevent self-fertilization is an essential feature of many plants mating systems that probably evolved as a means to avoid the deleterious effects of inbreeding. Inbreeding depression, defined as the reduction in fitness of self progeny relative to outbred progeny, is a major factor influencing the evolution of plant mating systems: most models of mating system evolution predict a threshold level of inbreeding depression (0.5 in the simplest cases) below which the transmission advantage of selfing favors alleles that increase the selfing rate and above which the reduced fitness of inbred offspring favors alleles that promote outcrossing (Ercan and Kurum, 2003; Kariyat et al., 2011). Lack of severe inbreeding indicates that the frequency of natural self pollination is high or that species evolved as small populations in nature, thus having a high level of inbreeding due to intercrossing of related individuals (Grisales et al., 2009). Inbreeding increases homozygosity and reduces the proportion of heterozygosity in the population thus reducing the vigor of plants (Ercan and Kurum, 2003; Grisales et al., 2009). Inbreeding depression is not notable in Cucurbitaceae (Allard, 1978) although it has been registered in advanced lines of cucumber *Cucumis sativus*, *Cucurbita moschata*, *Cucumis melo* and *C. lanatus* (Hallauer, 1999; Robinson, 1999; Hayes et al., 2005a,b).

The partially allogamous system of reproduction observed in *C. lanatus* suggests that self pollen and cross pollen could be compatible on the same stigma. For this kind of reproduction, stigma reacts differently during pre-zygotic and post-zygotic phases (Wang and Cruzan, 1998). Although floral structure, viability and germination pollen, the stigma receptivity, pollen tube growth in the style and ovary, pollinators are widely examined (Zaman, 2006; Sanz and Serrano, 2008; Bayoumi, 2009), the source and pollen mixture in the reproduction success in *C. lanatus* are unknown. The purpose of this paper is to evaluate pollen size, self and cross-pollen, pollen diversity and the mixture performance of the four Ivorian local cultivars of *C. lanatus* cultivated for seed consumption.

2. Materials and methods

2.1. Study area and plant material

This study was carried out from 2007 to 2008 at the experimental station of Abobo-Adjame University (Abidjan, Côte d'Ivoire). Abidjan (48410 N, 48000 W) is located in southern region the country. This zone is characterized by abundant rainfalls (annual mean > 2000 mm) and by annual mean temperature (28 °C). Tropical rain forest constitutes main vegetation, with mangrove on the coastal side (Zoro Bi et al., 2006). The sandy and clayey soils are rich in organic matter (organic carbon and organic nitrogen) with alkaline pH (7.94 < pH < 8.7). Metal such as lead (Pb) zinc (Zn), iron (Fe) and copper (Cu) were retained by the organic matter (Kouamé et al., 2010). Soil was relatively deeper (more than 10 m deep). The climate of the region is characterized by two rainy seasons (from April to July and from October to November) and two dry seasons (August to September and December to March) (Kouonon et al., 2009).

Each year (2007 and 2008), two series of experiments were performed during the rainy seasons (from March to June and from September to December) on *C. lanatus* cultivars. Seeds were collected from the cucurbits seeds' collection of the University of Abobo-Adjamé. Large, medium and small seeds of *Wlêwlê* cultivars resulted respectively from accessions numbers NI 211, NI 206 and NI113 while *Bebu* cultivar was represented by accession number NI 383. All these oleaginous cucurbits seeds' accessions are from