Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.13, No.2, 2022

Pollen Study in Two Improved Varieties of Manihot esculenta Crantz

*Adeleke, Martina T. V[1] Musipirinye, Ataisi A.
1. Department of Plant Science and Biotechnology, Rivers State University, Nkpolu, Port Harcourt, Nigeria

Abstract
This study was carried out to investigate the suitability of the pollen grains of two improved vitamin A fortified Cassava varieties, grown in southern Nigeria, as male parents for improving indigenous cultivars. To do this, male flowers from two improved cassava accessions were collected, namely: Umucas 44 and TMS 96/0603. The fertility of the pollen was assessed using acetocarmine stain. The pollen sizes were also measured using a graduated eyepiece and counted. The data were submitted to analysis of variance at 5% probability. Pollen fertility results obtained using the aceto-carmine method shows that TMS 96/0603 has a slightly higher Pollen fertility count with mean value of 97.5% than Umucas 44 with a mean value of 93.9%. Both plant species showed pollen fertility above 90% percent. TMS 96/0603 also showed a higher percentage (37.5%) of large pollen size (130-150µm) than Umucas 44 (17.6%), thus making it a potential male parent for sexual polyploidization in cassava. There was no significant difference in pollen fertility ($p>0.05$) among the replicates.
Keywords: Vitamin A fortified cassava varieties, pollen fertility, pollen size, pollen count.
DOI: 10.7176/JNSR/9-1-01
Publication date: January 31st 2022

Introduction

Cassava (*Manihot esculenta Crantz*) is an important staple food crop for more than 800 million people, and has been evolving as a food crop. This specie presents wide genetic diversity which results from the easy cross-pollination of the species, its high heterozygosity and its abrupt fruit dehiscence (Wania et al., 2002). It is usually represented by native varieties selected naturally or by farmers (Fukuda, 1996).

Progress in cassava breeding for adaptation and quality improvement has taken place over many centuries of selection in the Americas, and in the last 300 years in Africa and Asia, resulting in a wide genetic diversity (Bonierbale et al., 1997) which is concentrated mainly in Latin America and in the Caribbean. Incidences of spontaneous polyploids provide greater genetic variation and offer an opportunity to breed radically new cassava varieties (Hahn et al., 1990)

E orts aimed at breeding cultivars high in protein content, among other important agronomic traits, are fraught with numerous problems, including sexual reproductive barriers.

The formation of (2n) gametes is a common phenomenon in angiosperms (de Wet 1980), which most likely play a major role in the evolution of polyploid series (Harlan and de Wet 1975; Jackson 1976). Until now there have been no cytogenetic or histological reports on the occurrence of megaspores with 2n eggs or bilateral sexual polyploids in cassava.

The objective of this work was to investigate the probable existence of bilateral sexual polyploidization cytogenetically and histologically as an integral breeding strategy in cassava.

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The wild cassava species are essential for a breeding program because they have a high level of variability, can adapt to a broad spectrum of conditions, and therefore they offer many traits for developing more productive plants, such as resistance to pests and disease, as well as a higher tolerance to abiotic stresses (Horsfall & Abia 2003; Nassar et al. 2007 a,b).

Njenga et al. (2014) showed in their study that it is possible to bio-fortify existing cassava varieties for beta-carotene without loss of important agronomic attributes. Breeding for the trait will require careful selection of parents. Potential for bio-fortification of local varieties for beta-carotene will help reduce malnutrition status of the targeted population (Njenga et al., 2014). Quite a number of pro-vitamin A Cassava varieties have been released in Nigeria, amongst which are Umucass 44 and TMS 96/0603.

Cassava hybridizations are normally carried out by two pollination methods, cross pollination and controlled pollination (Wania et al., 2002).

The pollen grains of cassava are relatively large in size and are sticky. Several species of wasp (mainly *Polistes* spp.) and honeybees (*Aphis mellifera*) are considered the main pollinators in Colombia and Africa respectively. Perera et al. (2013) observed that cassava shows pollen tri-morphism in male gametophytes towards the larger or smaller pollen size, as compared with normal size. In some clones, the larger grains are more abundant, whereas in other clones the smaller grains are more common. The capacity for pollen production and the size of the pollen grains produced are important traits that may be factors in the reproductive behavior for the different varieties, directly affecting the genetic flow for each variety (Williams and Rouse 1990; Vieria et al., 2012)

The larger pollen grains have been observed to have better in vitro germination (60% germination after 2 h at 40°C) than the smaller ones, which may have less than 20% germination. Cassava pollen loses fertility rapidly after it is shed. Leyton (1993) found 97% seed set with pollen used immediately after its collection, 56% seed set with pollen stored for 24 h at 25°C, and 0.9% seed set (one seed from 102 pollinations) after 48 h of storage. In practice, breeders take care to perform pollinations within 1 h after collection of pollen to help ensure successful fertilization; pollen fertility seems to decline substantially after this time (Leyton, 1993).

Studies addressing pollen viability and morphology are necessary and important for cassava breeding programs and for supporting future crosses between cultivated and wild Manihot species (Vieria et al., 2012).

In this study, pollen studies were carried out on two vitamin A improved cassava varieties, namely - Umucas 44 and T.M.S 96/0603, cultivated and consumed in Port Harcourt, Nigeria, to see if they could serve as potential male parents for breeders in improving indigenous cassava cultivars.

Materials and methods

The two Cassava varieties used for this experiment TMS 96/0603 and UMUCAS 44 were obtained from the Agricultural Development Program, Port Harcourt, and the experimental site was in the Rivers State University Agricultural farm.

At anthesis, male flowers were collected between 7.30 and 10.30am. Pollen grains were dislodged from the stamen with forceps, spread on a microscope slide and stained with 1% acetocarmine glycerol jelly (Marks, 1954).Counts were made per microscopic field (10) from 2 random samples under a Leitz Diaplan binocular light microscope (x125 magnification). Only completely rounded and deeply stained grains were considered as fertile pollen. Pollen grains from flower buds of these species were analyzed for fertility within 1 hour of collection. Percentage Pollen fertility was calculated with these values.

The diameter of fertile grains were measured with the aid of a graduated eyepiece. Giant pollen grains having diameters greater than or equal to 130µm were classified as 2n pollen, since 2n pollen normally have 1.25 times the diameter of haploid or n pollen (Darlinton, 1937).

Results and Discussion

Pollen fertility results obtained using the aceto-carmine method shows that T.M.S 96/0603 has a slightly higher Pollen fertility count with mean value of 97.5%, and Umucas 44 with a mean value of 93.9% (Table 1). Both species showed pollen fertility above 90% percent. These high values are expected where pollen viability is assessed with stains in species of Manihot (Silva et al., 2001; Vidal et al., 2008). The inefficiency of using these stains in assessing pollen viability has been mentioned by Munhoz et al. (2008). Nevertheless, other factors like bareness, incompatibility between accessions and stigma receptibility can be said to influence the low seed yield per pollination (about three seeds per cross) characteristic of this genus, besides pollen viability (Fregene et al., 1997; Vieria et al., 2012).

Table 1. Pollen fertility test for Umucas 44 and TMS 96/0603

Parameter	Umucas 44	TMS 96/0603
Mean of reps	138.5	70.2
Standard deviation	16.2	24.6
Percentage fertility (%)	93.9%	97.5%

This type of stain-based experiment however, still allows certain important inferences to be made regarding the integrity of the pollen grains, and it is relatively safe for estimating the amount of viable pollen grains, and should therefore not be ignored.

The sizes of pollen grains that were observed in these two cassava varieties varied from 90µm to150µm (Fig. 1). This corresponds with the work of Vieria et al. (2012) on pollen viability, production and morphology for different species in the genus *Manihot*, in which he observed that pollen grains of the accessions from *M. esculenta* Crantz varied between two size classes, <140µm and between 140 and 149µm. Furthermore, the sizes of the pollen grains from the accessions are within the degree of variation that Vidal *et al.* (2008) found, in which the diameter of the pollen grains varied from 128 to 169 µm. There are few studies addressing *Manihot* pollen morphology, and size is a variable that ought to be considered, as it can potentially influence the compatibility between accessions.

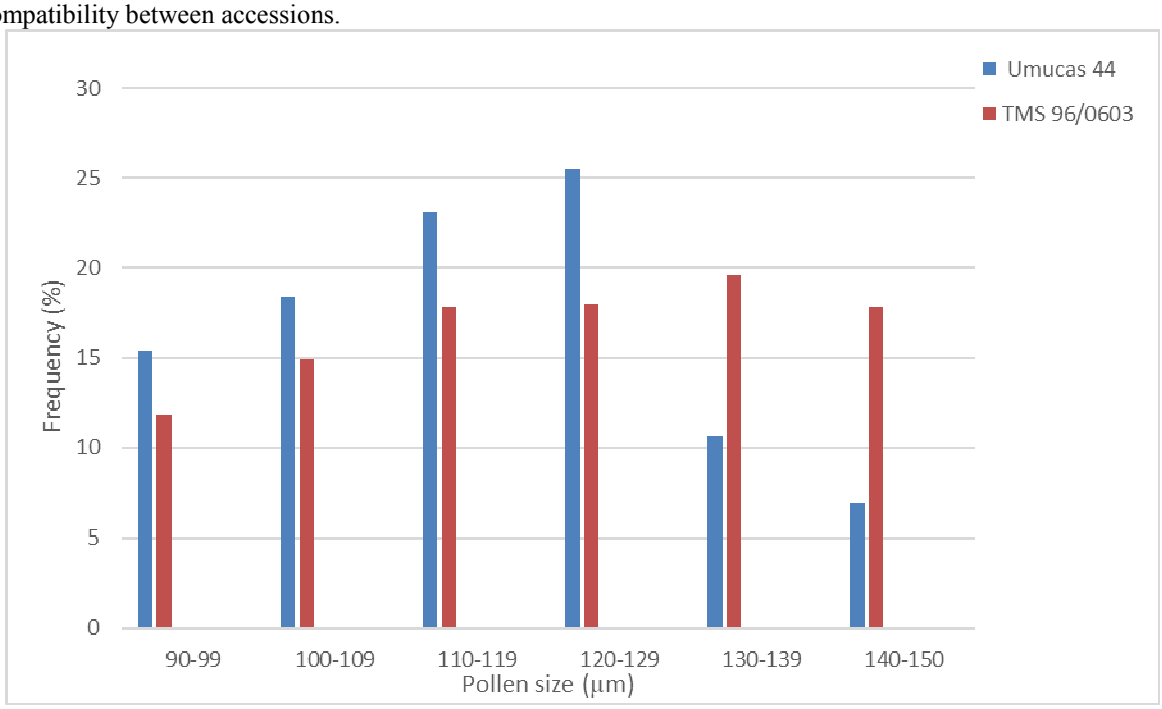

Fig 1. Frequency distribution of pollen diameter in Umucas 44 and TMS 96/0603

Pollen diameter in the two varieties varied significantly ($p < 0.05$). Pollen sizes considered to range from small to normal in this study are those with diameter less than 130µm; while the large are the diameters from 130 to 150µm, as giant pollen grains which are 1.25 times the diameter of haploid pollen are considered as 2n pollen (Darlington, 1937). The results (Fig. 1) show that Umucas 44 had higher percentage of smaller pollen (82.4%) than T.M.S 96/0603 (62.5%); and TMS 96/0603 had more of the larger pollen grains (37.5%) than Umucas 44 (17.6%), thus making TMS 96/0603 a potential 2n gamete or unreduced microspore producer.

The wide range in pollen sizes observed in this study (Fig. 1) are likely products of non-disjunction in meiosis which have resulted in 2n gamete formation or aneuploids of various intermediate sizes, since pollen size is proportional to chromosome number (Chin, 1946). Ogburia et al. (2002) in their cytogenetic study of bilateral sexual polyploidization in Cassava reported that at telophase II, formation of 17-21 micronuclei per pollen cell plate was observed in 16 out of 351 cell plates of microsporogenesis in 'M. mga'. Micronuclei were

observed at low (0.3-2.3%) frequencies at the sporad stage in all clones. Monads, dyads, triads and tetrads, which are established sources of high ploidy levels were observed at low (2.6%) and high (22.2%) frequencies. The observation of different pollen sizes in this work confirms this.

A high frequency of unreduced microspores (pollen mother cells) among cassava clones have been reported (Vasques and Nassar, 1994; Ogburia et al., 2002), and unreduced gametes were responsible for natural polyploidization in Cassava in the past.

There is therefore a possibility that TMS 96/0603 as a vitamin A fortified hybrid, could be used by breeders as a 2n gamete male parent in further improving indigenous cassava varieties. According to Okonwu and Eyaba (2020), TMS 96/0603 is also very low in cyanide content like Umucas 44, and is a good selection in quality, productivity and profitability in crop market value for the local farmer.

In addition, cassava as a highly consumed staple food in Nigeria has cultivars that are adapted to different agro-ecological zones. These are therefore preferences to the local farmers. Improving the indigenous varieties with an already adapted and improved vitamin A fortified hybrid as a male parent would be very much appropriate.

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