Global Scientific JOURNALS

GSJ: Volume 8, Issue 5, May 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

Evaluation of pollen viability and in vitro pollen germination in relation to different maturity stages of flowers inkola (*Cola nitida*).

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Abstract

Successful fruit set and production depend largely on the ability of a flower to produce functional and fertile pollen grains. Pollen viability test and germination capacity were among the procedures used in determining pollen quality. Flowers at different maturity stages (anthesis, pre-anthesis and post-anthesis) were collected in the morning between 8:30 – 9:30 am, after which pollen grains were squeezed out for viability test and in vitro germination. Aniline blue and pollen germination medium were used to determine viability test and in vitro pollen germination. Pollen grains from freshly open flowers (anthesis) were superior in terms of viable pollen grains (33.00), percentage pollen viability (35.40), pollen germination (4.67) and percentage pollen germination (9.15) among other flowers types. Low pollen germination (3.08) was recorded at post-anthesis, while no pollen germination (0.00) occurs at pre-anthesis. The implication of this is that freshly open flowers were ideal for evaluating pollen quality and in vitro pollen germination for successful hybridization program.

Keywords: C. nitida, pollen viability, in vitro germination, flowers.

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INTRODUCTION

Kola trees are the tropical crop that belongs to the genus Cola and family Sterculiaceae but broadly incorporated into Malvecae (Akogninous et al., 2006). Cola nitida is native to West Africa, from where it was spread to other African countries including Nigeria. According to Onomo *etal*., (2006), the genus comprises of 140 species and out of these, 50 species were known to be cultivated in West Africa (Adebola, 2003). The demand of *C. nitida* nut is due to alkaloids, caffeine and theobromine content used by many industries like pharmaceutical companies as sources of raw (Okeke, 1992), coca-cola, tea and coffee (Irvine, 1956) industries.

C. nitida yield appear to vary low in many plantations not only in Nigeria but even in West Africa the centre of its diversity (Adebola, 2003).This could be attributed to a higher degree of incompatibility/sterility, high drop of female flowers, long gestation period coupled with inefficient natural pollination, which accounts for low pollination success that translated into low yields (Jacob 1971).Cola flower has a penetrating aroma that attracts insects, for Pollination of the female flower (Bodard 1962: Russel, 1955). Winds also account for a small percentage of natural pollination. Increase fruit set up to 50 per cent in kola had been reported by Van Eijnatten, (1969 b) through controlled pollination. Artificial pollination has been used to produce various progenies for yield trials in Nigeria (Joseph, 1978).

Flushes occur immediately at the onset of rainfall, which is followed by flowering. (Russel 1955). Flowering in *C. nitida* occurs between July and August flowed by sporadic flowers which continue throughout the year unless drought set in (Voelcker, 1935).Both male and hermaphrodite flowers are found on inflorescences (Anonymous, 2002), with great variation in the proportion of male to female flowers between the trees and from trees to trees. The male flower which contains pollen grains is usually smaller than the female flowers, but similar in colour. The anthers of male flowers are fused into a single column, while that of female (hermaphrodite) flowers have two or more anthers at the base of the superior ovary.

The anther of male flowers contains pollen grains that are yellowish and slightly sticky (Keay *et al*., 1960), that makes the pollen grains to be anemophilous. Anthers of hermaphrodite flowers do not dehisce, however, the report indicated that the pollens are viable (Okpeke, 1982), but there are not functional as pollens used in cross-pollination set no fruits (Bodard 1962: Okpeke, 1982).

Proper understanding of male gametes (pollens) quality is a prerequisite for adequate fertilization in kola plants. Fruit production through sexual means depends on viable pollen grains (Asma,

2008), thereby making pollen quality determinant an important for hybrid production (Nautiyal et al., 2009).Hence estimation of pollen quality such as viability test and germination capacity is important for successful pollen –base program. During pollen collection, it is essential to collect the pollen grains at the right stage of flower maturity, to maintain viability and ability to germinate when the hybridization is performed. Prasad *et al*., (2011), reported highest percentages of germination that occur at anthesis, while lowest germination percentage occurred during post-anthesis and worst germination occurred 20 hrs after anthesis (Kumari *et al*., 2009).

Pollen grains, being single cell structure provide a unique system for *in vitro* studies (Katara, 2013).Non-fluorescent dyes and in vitro pollen germination is used to determine pollen quality (Rodriguez and Dafni, 2000). These two basic different approaches used to estimate pollen quality can provide reliable information about pollen quality character. In vitro germination is the process of determining the actual germination ability of pollen under suitable media. Thus make it to be reliable than a staining technique that do overestimate pollen viability.

Improve on fruit set in kola trees through assessment of the pollen quality in a breeding program, is what informed conduct of this research. The aim was to evaluate the viability of pollen grains and the germination capacity of isolated pollen grains. Also determine the best flower maturity stage of pollen collection for hybridization program in *C. nitida* genotypes. The study will provide a basis for enhancing the developing of new *C. nitida* genotypes through hybridization and to increase fruit set.

Materials and Methods

Cola nitida trees genotypes were selected and tagged among the population of trees growing together. Flowers buds at three stages of maturity (Anthesis, Pre- Anthesis and Post- Anthesis) were collected from different selected trees in the morning following the natural flower opening times between 8:00 am to 9:30 am, and immediately placed in Petri dishes and taken to the laboratory for evaluation of Pollen germination and pollen viability test . Six flowers buds were collected from different plants of the same cultivar to avoid variations in pollen viability test and in vitro germination process that might be due to plant as suggested by (Sari-Gorla *et al*., 1994). Pollen viability test using dye is the rapid method to determine pollen quality after 1-2 hrs. Staining techniques aim to determine pollen enzymatic activity, membrane integrity, and the staining ability of the nucleus. Pollens were collected. Aniline blue (Cotton blue) test: detects the cellulose in the pollen walls (Hauser and Morrison, 1964). Pollen collected from six anthers per

tree species were distributed on glass slides, after squash (squeezing) the anthers to release the pollen grains. One to two drops of aniline blue dye was added to pollen grains and left for 30 minutes to allow pollens to pick enough of the stain before observation. A clean dry cover slip was gently lowered on each slide to avoid air trap. Pollen viability observations were done under a light microscope, equipped with a camera. The viable pollen stained dark blue colour, while nonviable pollens are unstained or light blue. Field of view was carried out to count the numbers of both viable and nonviable pollen grains, which gave the total number of pollen grains counts.

The per cent pollen viability was calculated using the formula below

$$\text{Percentage Pollen viability \%} = \frac{\text{Number stained pollen grains}}{\text{Total number of pollen grains count}} \times 100$$

Fresh flowers were collected at for in vitro germination test. The flowers used for viability test and germination test were taken from the same trees. Flowers were taken at three different stages of maturity anthesis (freshly open), pre-anthesis (about to open) and post-anthesis (about to wither). Pollen germination medium was prepared using Brewbaker and Kwack protocol of 1964. Pollen grains from three different flowers were immersed in an aqueous concentration of pollen germination medium (PGM), in a cavity slide. Pollens collected were cultured based on the method described by (Singh *et al*., 1992). The slides were incubating under natural environment during which periodic observations on slides were carried out to determine the progress of germination. Field of view of Pollen germination counting was done using a light microscope, equipped with a camera. A pollen grain was considered germinated when pollen tube length was at least equal to or greater than the pollen grain diameter (Kakani et al., 2002: Tuinstra and Wedel, 2000). Germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen per field of view and expressed as a percentage. The values obtained from pollen germination and pollen viability were subjected to ANOVA using Minitab 2017 software. Means were separated using Fisher's least significant difference ($P \leq 0.05$)

The percentage pollen germination was calculated using the formula below

$$\text{Pollen germination \%} = \frac{\text{Number germinate pollen grains}}{\text{Total number of pollen grains count}} \times 100$$

Result and Discussion

From the analysis of the result as shown inTable 1: Histochemical analysis of pollen grain viability tested, revealed significant differences ($P \leq 0.05$) among the three stages of flower maturity. The significant difference was observed among the different maturity stages of flowers, with freshly open flowers proven to have highest numbers of viable pollen grains (33.00) that was significantly different from other flowers. This was immediately followed by pollens grains from post-anthesis flowers (25.75) and pre-anthesis flowers recorded the lowest viable pollen grains (22.75). There was significantly different ($P \leq 0.05$) to percentage pollen viability of flowers collected at different stages of development. Freshly open flowers had higher numbers of percentage pollen grains viability (35.40) as compared with the pollen grains collected from post-anthesis flowers (27.49) and pre-anthesis flowers (24.59), Table 1., and Figure 2: The result was able to establish a higher percentage of pollen viability at flower anthesis (freshly open). Similar observations were made by Nepi *et al*., 2003: Prasad *et al*., 2011, concerning the highest percentages of pollen viability during flower anthesis. Despite the high percentage of viable pollen, fruit set appears to be low due to the sticky nature of pollen grain, suggesting insect and hand pollination could increase and fruit set as reported by (Van Ejnatten, 1969b).

Table 1: Pollen viability determinant in different stages of flower maturity in *C. nitida*

Flower buds stages	Stained pollens	Unstained pollens	Total Number of pollens count	% pollen viability
Fresh open flower	33.00a	93.50a	126.50a	35.40a
Post-Anthesis	25.75ab	93.50a	121.50a	27.49ab
Pre -Anthesis	22.75b	93.25a	116.25a	24.59b

Means with the same letters were significantly not different at ($P \leq 0.05$)

There was an effect for in vitro pollen germination ($P \leq 0.05$) with regard to stages of flower maturity, (Table 2). Freshly open flowers showed significant superiority of numbers of non-germinated pollen grains count (49.67) than other flower developmental stages. Post-anthesis flowers pollen grains as revealed shows low non-germinated pollen grains count (35.67). Pre-anthesis flower had least non-germinated pollens (26.67) that were less significant than other flower types. Numbers of pollen germination (4.67) and percentage pollen germination (9.15) were significantly higher at anthesis than the pollens collected from both pre-anthesis and post-anthesis flowers which were statistically not different (Table 2). Low Pollen germination occurs at post-anthesis (3.08) and no pollen germination occurs at pre-anthesis (0.00) flowers. The result gave an insight into using pollen grains from fresh open flowers for hybridization program could enhance pollen germination on stigma and eventually increase fruit set. Low pollen germination observed in post-anthesis flowers revealed lost of viability as a result of a long period of a flower opening. The implication of this indicates dehydrated pollens lost their ability to germinate as reported by (Kumari *et al*., 2009: Temnet et al., 2011). The ability of pollen to germinate must contain some certain quantity of moisture. Non germination record at pre-

anthesis presumed immature pollen grains does not germinate. The implication of this finding suggested low fruit production by *C. nitida* trees could be due to sporophytic nature of flowers rather than a heteromorphic system. Percentage pollen viability was higher (35.40) than percentage pollen germination (9.15), indicating dye couldn't discriminate between viable pollen and non-viable pollen as well as non-functional pollen (Okpeke, 1982).

Table 2: In vitro germination evaluation in different stages of flowers maturity in *C. nitida*

Flower buds stages	Non germinated pollens	germinate pollen	Total pollen count	% pollen germination
Anthesis	49.67a	4.67a	54.33a	9.15a
Post-Anthesis	35.67ab	0.67b	35.67a	3.08b
Pre-Anthesis	26.67b	0.00b	26.67a	0.00b

Means with the same letters were significantly not different at ($P \leq 0.05$)

Figure 1: Showing graphical representation of pollen germination percentage at different levels of flower maturity. Anthesis flowers had shown superior among other flowers as it revealed a better germination percentage at 9%, post-anthesis at 3% and pre-Anthesis at 0%.

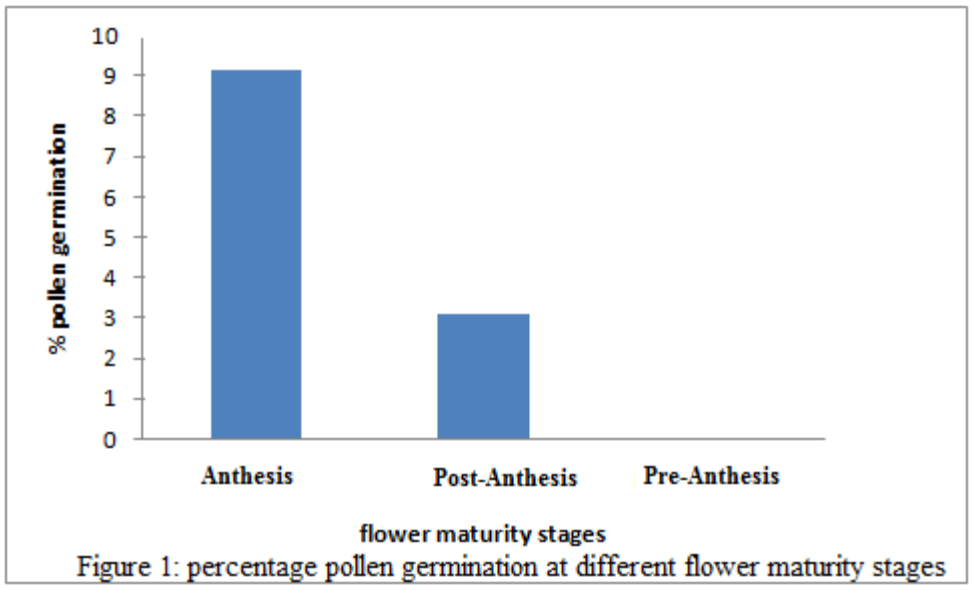

Figure 1: percentage pollen germination at different flower maturity stages

Figure 2: Showing graphical representation of percentage pollen viability at different stages of flower maturity. Anthesis flower had a higher percentage of pollen viability at 35%, post-Anthesis at 27% and pre-Anthesis at 24%.

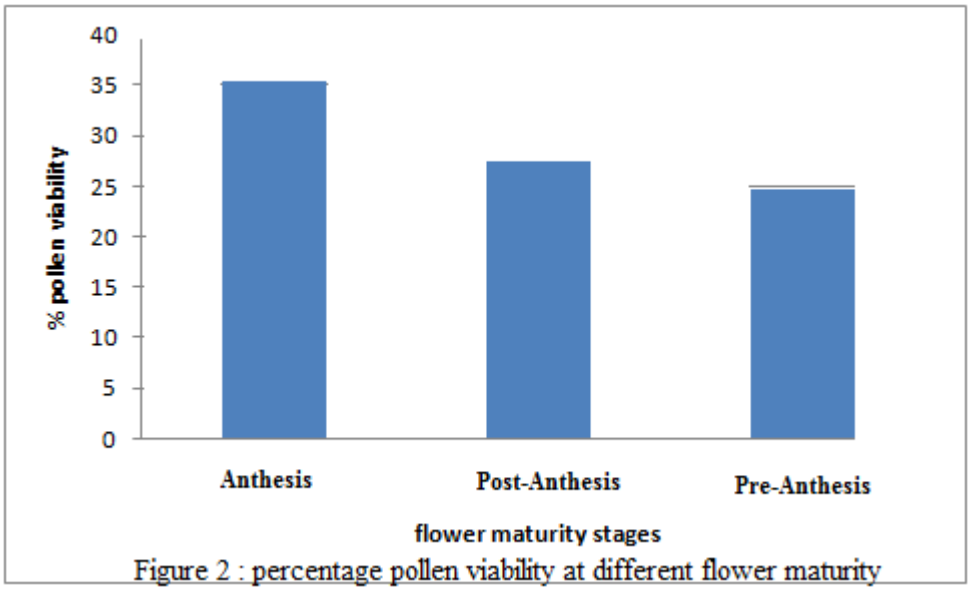

Figure 2 : percentage pollen viability at different flower maturity

Figure: 4and 5 shown *C.nitida* pollen grains that were stained with aniline blue. Viable pollens stained deep blue, while nonviable pollen grains stained light blue or unstained. Figure 6 and 7 showing germinated pollen grains. Figure 3: showing *C. nitida* pollen from Anthesis flower.

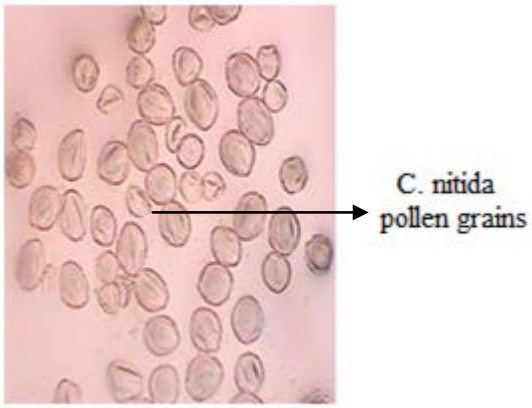

Figure 3: C. nitida pollen grains

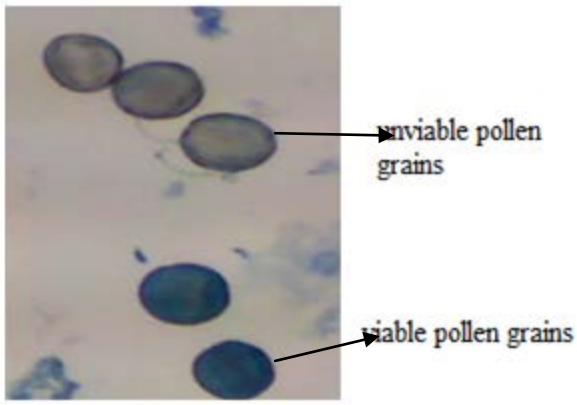

Figure 4: Stained pollen grains

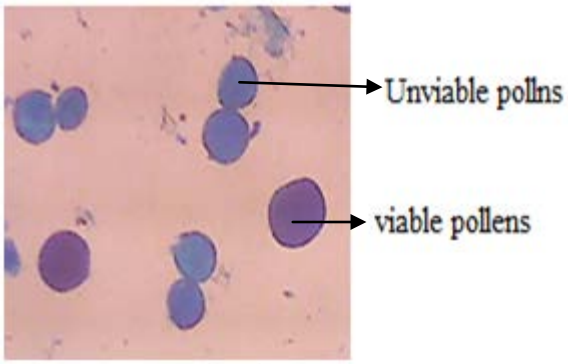

Figure 5 : Stained pollen grains

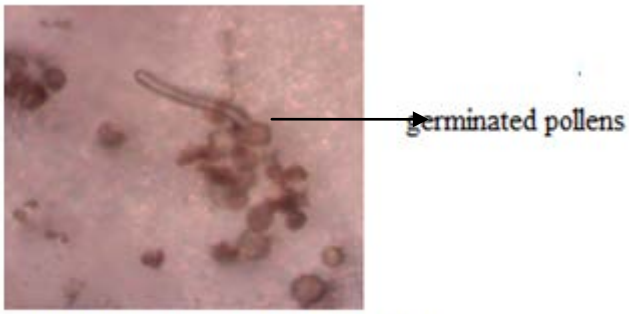

Figure 6: In vitro pollen germination

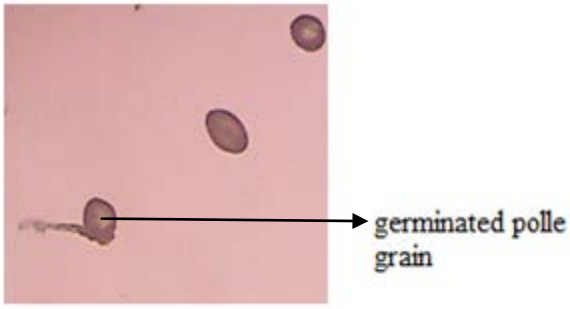

Figure 7 : In vitro pollen germination

Conclusion

There is little information on pollen quality evaluation on C. nitida, which is an orphan crop, but of high economic important. Pollen evaluation procedures, both staining and in vitro germination had proven to be reliable to an extent in determining pollen quality that invariable could be used for future hybridization program. The result of pollen viability and in vitro pollen germination obtained in this study showed the significant importance of flower collection for breeding program at anthesis since that is the appropriate time of pollen being at their full physiological maturity. This has biological implication as flowers collected before anthesis contains immature pollen grains and could not germinate as revealed by the results.

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