Journal of Plant Breeding and Crop Science Vol. 2(1). pp. 012-023, January, 2010
Available online http://www.academicjournals.org/jpbcs
ISSN 2006-9758 © 2010 Academic Journals

Full Length Research Paper

Pollen behaviour and fertililization impairment in Bambara groundnut (*Vigna subterrenea* [L.] Verdc.)

Oyiga Benedict Chijioke*, Uguru Michael Ifeanyi and Aruah Chinenye Blessing

Department of Crop Science, University of Nigeria, Nsukka, Nigeria.

Accepted 1 December, 2009

Two field experiments were conducted in April and August, 2007 cropping season at the Department of Crop Science research farm, University of Nigeria, Nsukka, to evaluate the pollen germination potentials and the pollen tube growth of thirteen bambara groundnut cultivars. The harvested pollen grains were exposed for 0, 5, 10 and 15 min durations to ambient conditions before *in vitro* germination. Pollen germination and pollen tube growth were tested using a medium containing 10 g sucrose, 100 mg/l boric acid and 300 mg/l calcium nitrate made up to 100 ml with deionized water. The results showed that cultivars, had significant effect on the pollen germination only at the late planting. The early and late planting results showed that pollens incubated immediately after harvest had the highest germination percentage, while pollen exposed for five minutes prior to germination showed very poor germination. Pollens exposed beyond five minutes after harvest did not germinate. The cultivars significantly (P < 0.05) affected the pollen tube growth at both early and late planting dates. The pollen tube growth decreased drastically with increase in duration of pollen exposure. The pollen tube had an exponential growth rate at the on set of the pollen tube growth, followed by lag and stationary growth phases in the pollen tube growth curves. The exponential, logistic and Gomperzt growth models were used to estimate the best fit model for the pollen tube growth in bambara groundnut cultivars. In the early planting, the average straightness (R^2) value of these models for growth estimate were 98.4, 98.6 and 98.5%, respectively. During the late planting, the average R^2 values were 91.5, 96.6 and 96.6%, respectively. The three models are therefore considered suitable for the computation of the pollen tube growth rates. The principal component and cluster analyses were used to group the cultivars in relation to the levels of pollen survival under ambient conditions. At early planting, cultivar Bg-01 had moderate surviving pollen grains while Bg-08, Bg-09, Bg-10 and Bg-11 were found to have poor surviving pollens. At late planting, cultivar Bg-04 and Bg-07 had high survival pollens while Bg-01 had poor pollen survival.

Key words: Pollen behaviour, pollination, fertilization, Bambara groundnut, *Vigna subterranea* (L.) Verdc.

INTRODUCTION

Bambara groundnut (*Vigna subterrenea* [L.] Verdc.) is an indigenous African legume cultivated mainly by subsistence farmers under the traditional low input agri-cultural system. It belongs to the family, papilionaceae and is one of the underutilized crops in the sub Saharan Africa. The crop is a major source of dietary protein among rural and urban dwellers in Nigeria. It is a monoecious, self pollinating annual with tremendous pontentials as a food crop and a soil ameliorating agent. The crop has a perfect flower with the stamen and pistil borne in same flower. The staminate and pistillate parts of the flower are covered by a bract or cap-like operculum. This structure offers protection to the male and female parts of the flower and also discourages outcrossing to a large degree. Male fertility depends on the rate of pollen production and viability and, it is strongly influenced by environment (Shivanna et al., 1991; Khatun and Flower,

*Corresponding author. E-mail: ceejaybeecee@yahoo.com. Tel: +2348037317861.

1995). Male infertility can result in hybridization failure, reduced seed set and poor seed production in field crops. A variety of mechanisms have been described to interfere with gene flow between species and therefore, resulting in reproductive isolation. These may be prezygotic mechanism which reduces the frequency at which gametes combine to form a zygote, or *postzygotic* mechanism, which reduces the pollen viability or reproductive potential of the hybrid (Bushell et al., 2003). In flowering plants, prezygotic barriers include different flowering time, attractiveness of pollinators, failure of pollen to adhere to stigmatic surface, pollen viability and abnormal growth of the pollen tube. Postzygotic barriers take effect after successful fertilization and include seed abortion and weakness or sterility of F_1 hybrids and subsequent generations (Rieseberg and Carney, 1998; Tiffin et al., 2001; Bushell et al., 2003). At present, there are no scientific reports that exist on prezygotic and postzygotic mechanisms in bambara groundnut. Attempts to hybridize bambara groundnut using the hybridization methods proposed by Schenkel (2001) and Massawe et al. (2003) have not been successful. Success in artificial hybridization would depend on the clear understanding of the pollen behaviour during and after pollination (Anchirina et al., 2001; Lacroix et al., 2003). The rate of hybrid formation is influenced by the degree of relatedness between species, pollen viability and the presence of suitable pollinizer (Bots and Mariani, 2005). Recently, considerable attention had been given to some of these factors but the influence of pollen viability has received relatively limited research attention. Pollen physiology (germination and viability) is essential in plant breeding and conservation (Kapoor, 1976; Zeng-zu Wang et al., 2004). It plays an important role in risk assessment and biosafety study in plants. A rapid loss in pollen viability would greatly affect the effectiveness of pollination and seed set. Adverse environmental conditions can result in reduced pollen fertility (Tuinstra and Wedel, 2000). Research has shown that variations in temperature, humidity, and cloud cover can also influence pollen production and viability. Cold temperature stress prior to flowering appears to reduce pollen viability during anthesis by disrupting meiosis during the early stages of microsporogenesis (Brooking, 1979). After shedding, pollen grains are exposed to a hostile environment, such as dry conditions and yet they have to reach a receptive stigma while still viable. Pollen longevity after anther dehiscence is very crucial for successful pollination, particularly for outbreeders. The physiological effects of duration of pollen exposure on pollen viability of Bambara groundnut is not yet known. The knowledge of the pre-pollination longevity of pollen grains of Bambara groundnut will be very useful in determining the handling techniques and the possible strategy for artificial hybridization to increase the efficiency of hybrid seed production. This experiment was therefore, initiated to study the bambara groundnut pollen characteristics in terms of its potency and growth rate of the pollen tube after different durations of exposure to ambient conditions.

MATERIALS AND METHODS

Two experiments were conducted in April and August, 2007. Both experiments were carried out in the experimental field of the Department of Crop Science, University of Nigeria, Nsukka (Lat 06° 52'N; Long 07° 24' E and approximately 447.2 m above sea level. Thirteen Bambara groundnut cultivars sourced from bambara groundnut producing areas in Nigeria were used in the present study. The cultivars were selected based on their different seed coat colours (Massawe et al., 2000) and were given accession numbers viz., Bg-01, Bg-02, Bg-03, Bg-04, Bg-05, Bg-06, Bg-07, Bg-08, Bg-09, Bg-10, Bg-11, Bg-12 and Bg-13. The thirteen cultivars were grown in a randomized complete block design (RCBD) with three replications.

Pollen germination and pollen tube growth

Flowers were randomly collected from the thirteen Bambara groundnut cultivars at anthesis between 0900 and 1000 h. Fresh Bambara groundnut flowers were collected from twelve plants per cultivar, and immediately placed in the plastic containers and carried to the laboratory. Pollen germination and tube growth were tested using a medium containing 10 g succrose, 100 mg/l boric acid and 300 mg/l calcium nitrate made up to 100 ml with deionized water, a standard *in vitro* medium for pollen germination in most crop plants (Steer and Steer, 1989; Shavanna and Rangaswamy, 1992; Messerli and Robinson, 1997). The growth medium was prepared according to the procedures outlined by Messerli and Robinson (1997).

The pollen grains that were used for this study were exposed to ambient condition for 0, 5, 10 and 15 min before germination *in vitro*. The growth medium was put on glass slides and pollen grains were placed on the germination medium. The glass slides were then put in Petri dishes with moist whatmann filter paper and incubated in the dark chamber under laboratory condition.

Pollen germination was scored after 80 min of incubation by direct microscopic observation (Nikon Scientific, Kanagawa, Japan) using Grid square micrometer. Pollen grain was considered germinated when pollen tube length was equal to or greater than the grain diameter (Shivanna and Rangasway, 1992; Kakani et al., 2002). Germination percentage was determined as the ratio of germinated pollen grains per field of view to the total number of pollen per field of view and expressed as percentage. The measurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece of the compound microscope. Pollen tube lengths were recorded for 10 randomly selected pollen tubes in each replicate at 20, 40, 60 and 80 min of pollen incubation and the means were used for analysis.

Statistical analyses

The Genstat 7.22 release (2007) data analysis software (Lewis Agricultural Trust, Rothamsted Experimental Station) was used for all statistical analyses. Arcsine root square transformation were made on the percentage data (germination percentage) before the analysis (Snedecor and Cochran, 1967; Sokal and Rohlf, 1995). Data collected were analyzed following the procedures outlined for randomized complete block design (RCBD) (Obi, 2002). Separation of treatment means for significant effect was by Fishers' least

significant difference at 5% probability level.

Curve fitting and analysis

Pollen tube lengths measured at 20, 40, 60 and 80 min of incubation were analysed using linear and nonlinear regression techniques to quantify developmental responses to different pollen exposure durations. Attempts were made to identify the model that best described the pollen tube growth data collected. For this, three nonlinear growth models, viz. exponential, logistics and Gompertz were applied to the data and examined to determine the best-fit model. These models are explained in the equations below. The prediction and goodness of fit of the fitted models were examined by computing the coefficient of determination (R^2). The best model is one with the highest R^2 value (Kakani et al ., 2002). Genstat 7.22 software program was used for the parameter prediction and goodness of fit test.

Exponential growth model

$$y_i = \alpha + \beta \rho^{x}_i + E_i \quad \text{..... ....} \quad (1)$$

Logistic growth model

$$y_i = \alpha + y\,[1 + exp(-\beta(x_i - \mu)]^{-1} + E_i \quad \text{..... .....} \quad (2)$$

Gompertz growth model

$$y_i = \alpha + y\,exp[-exp(-\beta(x_i - \mu)] + E_i \quad \text{..... .....} \quad (3)$$

Where;
y_i = predicted pollen tube length,
α = different asymptote,
β = The maximum steepness (slope) of the curve (at μ),
ρ = nonlinear shape parameter,
y = final asymptotic height,
μ = The time to reach 50% of the final height,
E_i = Experimental error and
x_i = time of incubation.

Principal component analysis (PCA) was applied to the pollen germination and tube growth parameters to identify the parameters that best describe genotypes response to the pollen exposure treatments evaluated. The values of pollen germination (PG) and pollen tube length (PTL) at different pollen exposure durations for the thirteen genotypes were included in the PCA. Eigenvectors generated by PCA were used to identify the parameters that best differentiated the genotypes with respect to pollen longevity when the pollen grains were exposed at different durations. The first two PC scores, PC1 and PC2 that accounted for maximum variability of the parameters tested, were used to group the genotypes as reported by Kakani et al. (2002, 2005). According to Kakani et al. (2002), high positive loadings would indicate that genotypes which had +PC1 and +PC2 scores would be classified as high pollen survival, +PC1 and -PC2 scores as moderate pollen survival, -PC1 and +PC2 scores as low pollen survival and finally -PC1 and -PC2 scores as poor pollen survival. On the other hand, Kakani et al. (2005) reported that high negative loadings would indicate that genotypes with -PC1 and -PC2 scores are classified as high pollen scores as poor pollen survival. Based on the values of the PC1 and PC2 scores, a two dimensional scatter plot using component score survival, -PC1 and +PC2 scores as moderate pollen survival, + PC1 and - PC2 scores as low pollen survival and finally +PC1 and +PC21 as horizontal axis and component score 2 as vertical axis was constructed.

RESULTS

In vitro pollen germination

Table 1 shows the effect of cultivars on the pollen germination at both early and late planting dates. Cultivars had significant effects ($p < 0.05$) on pollen germination only at the late planting date. Although, cultivars did not differ signifficantly in pollen germination at early planting, the cultivar, Bg-13 recorded the highest pollen germination (30.1%) followed by Bg-07 (26.9%), Bg-04 (23.4%), Bg-03 (21.6%), Bg-05 (20.4%) and the least pollen germination was observed on Bg-12 (11.0%). However, during the late planting, the cultivar Bg-12 produced the highest pollen germination (20.3%) which did not differ significantly from the pollen germination in Bg-04 (20.1%) and Bg-08 (19.8%). Pollen germination was not observed in cultivars Bg-02, Bg-05, Bg-09, Bg-10, Bg-11 and Bg-13 in the late planting. Pollen germination of Bambara groundnut was low at both planting dates.

Figures 1a and 1b show the effect of duration of pollen exposure on the pollen germination of thirteen bambara groundnut cultivars after 80 min of incubation. Pollen germination of the thirteen bambara groundnut cultivars were tested after 0, 5, 10 and 15 min of pollen exposure. There was a rapid loss in the pollen germination among the bambara groundnut pollens with the slightest delay in the incubation of the pollen grains at both planting dates. At the early planting, pollen grains incubated immediately after harvest had the highest germination percentage across all the cultivars. However, pollen germination dropped greatly when exposed for five minutes before incubation. The pollens from all the cultivars lost viability and did not germinate at 10 and 15 min of pollen exposure (Figure 1a). In the late planting, the pollen shelf life showed similar trend to that of the early planting, but the decrease in pollen germination was less dramatic at the first 5 min of pollen exposure. About 3% of the pollen grains germinated. There was no pollen germination recorded at 10 and 15 min of pollen exposure (Figure 1b).

Pollen tube growth

Table 2 shows the variation in pollen tube length at 20, 40, 60 and 80 min of incubation among the cultivars during the early and late planting. In the early planting, cultivar differences were observed in the pollen tube length at 20, 40, 60 and 80 min of incubation. At 20 min of incubation, pollen tube length ranged from 10.08 µm in Bg-02 to 40.52 µm in Bg-04. The highest pollen tube length recorded at 40 min of incubation was 57.1 µm in Bg-10. This value did not differ significantly from the 51.0

Table 1. Effect of different Bambara groundnut cultivars on the pollen germination immediately after harvest. Values within the parentheses are transformed data.

Cultivars	Pollen germination (%)	
	Early planting	Late planting
Bg-01	20.9 (27.2)	5.0 (10. 43)
Bg-02	16.1 (23.6)	0.0 (0.00)
Bg-03	21.6 (27.7)	15.5 (23.16)
Bg-04	23.4 (28.9)	20.1 (24.82)
Bg-05	20.4 (26.8)	0.0 (0.00)
Bg-06	18.8 (25.7)	0.3 (1.91)
Bg-07	26.9 (31.1)	6.0 (14.16)
Bg-08	13.2 (21.2)	19.8 (26.10)
Bg-09	12.7 (20.8)	0.0 (0.00)
Bg-10	13.5 (21.4)	0.0 (0.00)
Bg-11	17.6 (24.4)	0.0 (0.00)
Bg-12	11.0 (15.9)	20.3 (26.58)
Bg-13	30.1 (28.0)	0.0 (0.00)
F-LSD$_{0.05}$	NS	(8.67)

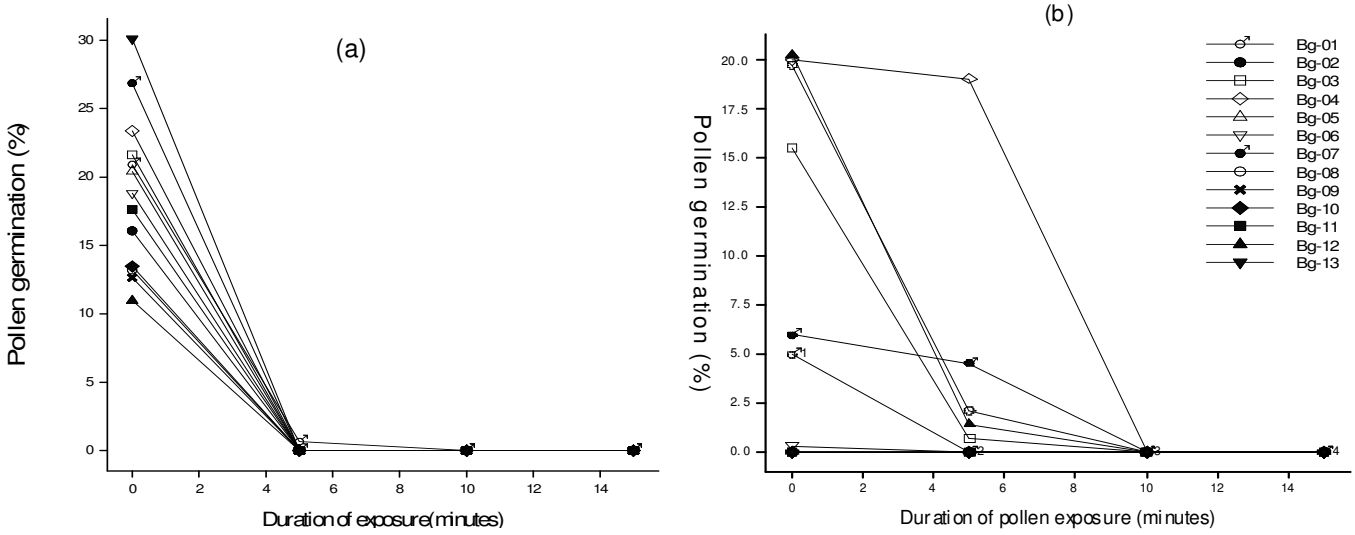

Figure 1. Effect of duration of pollen exposure on the pollen germination of thirteen bambara groundnut cultivars after 80 min of incubation in the (a) early and (b) late planting after 80 min of incubation.

µm in Bg-05, 50.0 µm in Bg-04, 49.7 µm in Bg-01, 49.7 µm in Bg-07 and 42.5 µm in Bg-12. Bg-02 produced the shortest pollen tube length. At 60 min of incubation, Bg-10 produced the longest pollen tube (81.8 µm). This value did not differ statistically from with Bg-05 (75.0 µm), Bg-07 (63.0 µm), Bg-12 (60.7 µm), Bg-04 (56.4 µm) and Bg-01 (54.5 µm). The cultivar, Bg-02 also produced the shortest tube length (13.5 µm). The trend was the same at 80 min of incubation.

Cultivar differences in pollen tube growth were also observed at the late planting at 20, 40, 60 and 80 min of incubation (Table 2). At the first 20 min of pollen incubation. The cultivars differed significantly ($P < 0.05$) in pollen tube length. Pollen tube growth was more rapid in the cultivar, Bg-04 with a pollen tube length of 66.22 µm. This was followed by Bg-12 with a mean length of 41.57 µm. At 40, 60 and 80 min of incubation, Bg-04 also gave a highly significant pollen tube growth increase of

Table 2. Effect of genotype on the pollen tube growth of 13 bambara groundnut genotypes during the early and late planting.

cultivars	Pollen tube length (µm)							
	Period of incubation in minutes (early planting)				Period of incubation in minutes (late planting)			
	20	40	60	80	20	40	60	80
Bg-01	34.52	49.7	54.5	54.5	20.64	58.33	87.30	87.30
Bg-02	10.08	12.7	13.5	13.5	9.52	15.47	23.81	23.81
Bg-03	27.95	37.3	42.1	42.1	18.54	30.16	40.39	40.39
Bg-04	40.52	50.0	56.4	56.4	66.22	239.91	239.91	268.65
Bg-05	29.36	29.0	75.0	75.0	20.64	20.64	20.64	0.00
Bg-06	23.81	49.7	34.5	34.5	0.00	0.00	0.00	0.00
Bg-07	30.55	28.2	63.0	63.0	21.43	21.43	56.26	65.75
Bg-08	15.08	28.2	28.1	28.1	28.17	28.17	36.14	36.14
Bg-09	27.78	34.4	35.0	35.0	0.00	0.00	0.00	0.00
Bg-10	39.47	57.1	81.8	81.8	0.00	0.00	0.00	0.00
Bg-11	23.50	34.5	43.1	43.1	0.00	0.00	0.00	0.00
Bg-12	20.24	42.5	60.7	60.7	41.57	78.97	78.97	78.97
Bg-13	22.22	33.3	39.4	39.4	0.00	0.00	0.00	0.00
F-$LSD_{0.05}$	7.08	15.2	27.94	27.94	2.86	8.15	8.9	9.133

239.91, 239.91 and 268.65, respectively. Five cultivars namely; Bg-06, Bg-09, Bg-10, Bg-11 and Bg-13 did not produce viable pollens in the late planting and therefore no visible pollen tube growth was recorded for them.

Growth models

Table 3 shows the parameter values and the determination coefficients of Exponential, Logistic and Gompertz growth models during the early planting. The determination coefficients of these models among the cultivars were between 94.2% in Bg-08 and 100% in Bg-01 for Exponential model, 94.6% in Bg-10 and 100% in Bg-02, Bg-08 and Bg-09 for Logistic model and 99.4% in Bg-13 to 100% in Bg-02, Bg-08 and Bg-09 for Gompertz model. The results show that the average R^2 value for prediction in the three models tested were similar (Table 3). The highest determination value for prediction was obtained in Logistic growth model (98.6%), followed by Gompertz (98.5%) and Exponential (98.4%) in that order.

Table 4 shows the equation constants for the Exponential, Logistic and Gompertz growth models and coefficient of determination among the cultivars during the late planting. The determination coefficient for Exponential, Logistic, and Gompertz growth model ranged from 84.75 % in Bg-07 to 98.3% in Bg-03, 85.7% in Bg-08 to 100% in Bg-04, Bg-06 and Bg-12 and, 85.7% in Bg-08 to 100% in Bg-04, Bg-06 and Bg-12, respectively. The highest determination value for prediction of the pollen tube length was observed in logistic and Gompertz growth model (96.6%) (Table 4) while the least prediction of 91.5% was observed in exponential model.

Principal component analysis (PCA)

PCA is a multivariate technique for examining relationships among several quantitative variables and is especially a valuable analytical technique in exploratory data analysis (Johnson, 1998). The PCA identified the pollen parameters that best separated the cultivars *via-a-vis* the pollen survival when exposed at four different pollen exposure durations (that is 0, 5, 10 and 15 min). The pollen germination and tube growth paramters at 10 and 15 min duration of pollen exposure were not included in the PCA analysis because no germination occured at both exposure durations; and therefore, did not alter the classification of the cultivars. At early planting, the first three principal component vectors (PC1, PC2 and PC3) accounted for 100% of the total variation (Table 5). The PC1, PC2 and PC3 accounted for 50.72, 26.77 and 22.51% of the total percentage variation, respectively. The PC1 eigenvector had high positive loadings for variables PG % E_{5t} and PTL E_{5t}. The cultivars with higher and lower pollen germination at 5 min pollen exposure were placed on the right and left of the plot respectively (Figure 2). The PC2 had high positive loadings for PG % E_{0t} and PTL $E_{0t.}$ The cultivars were divided into three groups based on the scores of the first two principal components (Table 6 and Figure 2): Group 1 cultivars as moderately pollen survival with positive PC1 and negative

Table 3. Equation constants of exponential, logistic and gomperzt growth models predicted on pollen tube growth of bambara groundnut at early planting.

cultivars	Exponential growth model				Logistic growth model					Gomperzt growth model				
	α	β	ρ	R^2	α	β	y	μ	R^2	α	β	y	μ	R^2
Bg-01	56.72	-56.88	0.952	99.7	-44.37	0.078	99.5	2.78	99.9	69.0	0.075	69.0	6.3	99.9
Bg-02	13.64	-13.64	0.935	100.0	-89.32	0.073	102.9	-26.00	100.0	-68.0	0.071	81.0	-24.0	1000
Bg-03	43.24	-43.23	0.95	99.8	-220.30	0.057	263.3	-28.60	99.6	164.90	0.052	16930	-70.2	99.7
Bg-04	56.87	-56.77	0.942	99.6	-817.90	0.062	874.7	-42.70	99.1	925.390	0.060	925.960	-123.2	99.2
Bg-05	101.3	-102.2	0.981	96.7	-27.80	0.055	108.9	19.20	96.0	-10.5	0.045	93.3	17.3	95.6
Bg-06	34.93	-34.8	0.948	98.8	-363.90	0.055	398.8	-42.50	97.4	-708620	0.053	708970	-143.1	97.6
Bg-07	72.05	-72.49	0.971	98.8	-42.70	0.057	108.6	7.60	98.7	-15.7	0.052	82.0	9.6	98.7
Bg-08	30.43	-30.88	0.958	94.2	-0.01	0.395	28.2	19.63	100.0	0	0.476	28.1	19.0	100.0
Bg-09	35.38	-35.40	0.925	99.9	-15.56	0.13	50.6	6.24	100.0	-2.3	0.128	37.4	8.0	100.0
Bg-10	98.00	-97.90	0.976	97.2	-214.70	0.034	307.4	-25.00	94.6	-126.0	0.032	2190	-19.0	94.5
Bg-11	47.10	-47.16	0.966	99.2	-104.70	0.045	150.3	-18.40	98.5	-51.0	0.044	97.0	-10.3	98.5
Bg-12	86.60	-88.00	0.983	95.7	-9.50	0.072	73.3	25.92	98.0	-1.8	0.057	66.8	22.3	97.5
Bg-13	42.31	-42.44	0.962	99.5	-46.70	0.058	87.3	-2.40	99.4	-19.2	0.056	60.0	2.3	99.4
Mean	-	-	-	98.4	-	-	-	-	98.6	-	-	-	-	98.5

Table 4. Equation constants of exponential, logistic and gomperzt growth models predicted on pollen tube growth of bambara groundnut at late planting.

Cultivars	Exponential growth model				Logistic growth model					Gomperzt growth model				
	α	β	ρ	R2	α	β	y	μ	R2	α	β	y	μ	R2
Bg-01	1530	-157.0	0.988	92.1	-4.22	0.092	94.8	31.9	98.7	-0.01	0.065	92.9	26.8	97.7
Bg-02	32.9	-33.1	0.982	96.1	-11.5	0.048	37.8	16.9	93.7	-5.3	0.039	32.4	15.0	93.6
Bg-03	48.1	-48.4	0.975	98.3	-28.2	0.052	71.2	8.1	97.7	-11.4	0.046	54.9	9.7	97.6
Bg-04	3560	-37.00	0.979	84.7	-3.84	0.159	273.2	26.7	1000	0.01	0.124	2700	22.7	1000
*Bg-05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bg-06	30.1	-31.11	0.994	77.2	0	0.56	9.52	38.8	1000	0.00	0.386	9.5	37.7	1000
Bg-07	85.8	-87.0	0.981	96.6	-12.9	0.072	77.7	22.6	96.8	-2.09	0.062	67.5	20.5	97.3`
Bg-08	35.0	-34.8	0.934	93.3	-124	0.077	158.6	-16.5	85.7	-420.250	0.069	420.600	-103.3	85.7
*Bg-09	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*Bg-10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*Bg-11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bg-12	85.8	87.2	0.959	94.0	-0.03	0.394	79.01	19.7	1000	0.00	0.408	79.0	18.9	1000
*Bg-013	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean				9.15					96.6					96.6

* -No pollen germination was recorded.

Table 5. Principal component analysis eigenvectors PC1, PC2 and PC3 of 13 bambara groundnut cultivars for PG % E_{0t}, PG % E_{5t}, PTL E_{0t} and PTL E_{5t} and the percentage variation accounted for by each eigenvector for early planting.

Parameters	PC1	PC2	PC3
PG% E0t	0.13463	0.68142	0.71940
PG% E5t	0.69799	-0.02034	-0.11136
PTL E0t	-0.08662	0.73132	-0.67651
PTL E5t	0.69799	-0.02034	-0.11136
% Variation	50.72	26.77	22.51

Where; PG % E_{0t} and PG % E_{05} = Pollen germination at 0 and 5 min duration of pollen exposure, respectively. PTL E_{0t} and PTL E_{05} = Pollen tube growth at 0 and 5 min duration of pollen exposure, respectively.

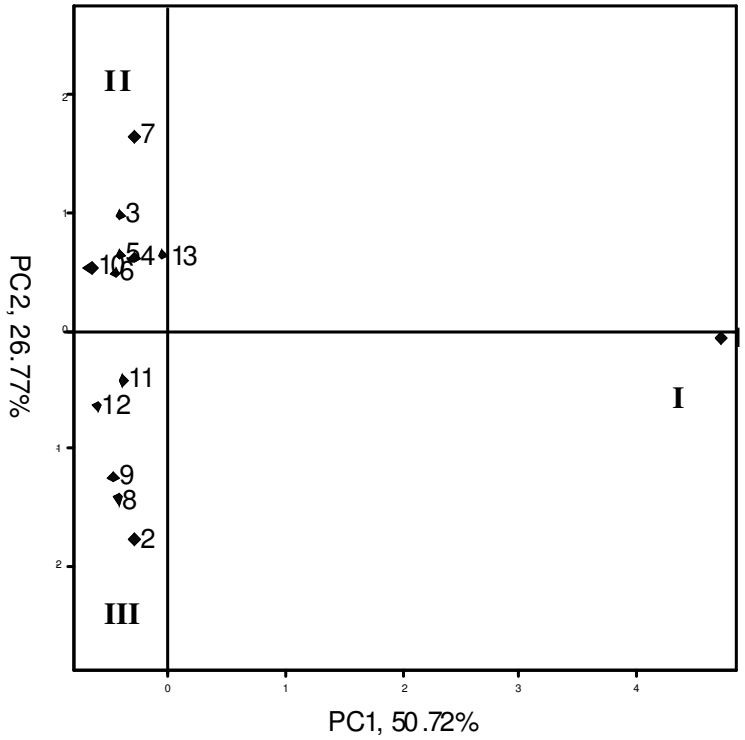

Figure 2. First and second principal component scores (PC1 and PC2) for the identification of bambara groundnut cultivar response to length of pollen exposure. Where the values in the plot represent the Bambara groundnut accession number.

PC2 scores, Group 2 as low pollen survival with negative PC1 and positive PC2 and finally Group 3 as poor pollen survival with negative PC1 and PC2 scores. At late planting, the first three principal component vectors (PC2, PC2, and PC3) accounted for 99.21%of the total variation (Table 7). PC1 accounted for 83.32% of the variation among the cultivars while PC2 and PC3 accounted for only 14.75 and 1.14%, respectively. The PC1 eigenvector contrasted cultivars loaded negatively for all the pollen parameters considered. PC1 loaded highly for PTL E_{0t}, PTL E_{5t} and PG % E_{5t}. The PC2 had high negative loadings for PTL E_{5t}. Cultivars with higher pollen germination and pollen tube growth at 0 and 5 min duration of pollen exposure were placed on the left side of the plot while cultivars with low values were placed on the right flank of the plot (Figure 3). The cultivars were divided into four groups based on the scores of the first two principal components (Table 8 and Figure 3): Group 1 cultivars as

Table 6. Classification of 13 bambara groundnut cultivars based on the scores of first two principal components (PC1 and PC2) during the early planting date.

High pollen survival (+PC1, +PC2)	Moderately pollen survival (+PC1, -PC2)	Low pollen survival (-PC1, +PC2)	Poor pollen survival (-PC1, -PC2)
	Bg-01 (4.71, -0.07)	Bg-02 (-0.28, 1.79)	Bg-08 (-0.43, -1.43)
		Bg-03 (-0.40, 0.98)	Bg-09 (-0.47, -1.23)
		Bg-04 (-0.29, 0.63)	Bg-11 (-0.38, -0.42)
		Bg-05 (-0.41, 0.65)	Bg-12 (-0.62, -0.64)
		Bg-06 (-0.45, 0.50)	
		Bg-07 (-0.28, 1.65)	
		Bg-10 (-0.65, 0.54)	
		Bg-13 (-0.04, 0.63)	

Table 7. Principal component analysis eigenvectors PC1, PC2 and PC3 of 13 bambara groundnut cultivars for PG % E_{0t}, PG % E_{5t}, PTL E_{0t} and PTL E_{5t} and the variation accounted for by each eigenvector for late plant.

Parameters	PC1	PC2	PC3
PG % E_{0t}	-0.41055	0.85793	0.25295
PG % E_{5t}	-0.52243	-0.33338	0.61997
PTL E_{0t}	-0.53920	0.04269	-0.74071
PTL E_{5t}	-0.51747	-0.38858	-0.05478
% Variation	83.32	14.75	1.14

Where; PG % E_{0t} and PG % E_{05} = pollen germination at 0 and 5 min duration of pollen exposure, respectively. PTL E_{0t} and PTL E_{05} = pollen tube growth at 0 and 5 min duration of pollen exposure, respectively.

high pollen survival with negative scores for PC1 and PC2, Group 2 as moderately pollen survival with negative PC1 and positive PC2 scores, Group 3 as low pollen survival with positive PC1 and negative PC2 and finally Group 4 as poor pollen survival with positive PC1 and PC2 scores.

DISCUSSION

Artificial germination of pollen grains is a reliable test of pollen fertility. Stored pollen grains are very much useful to breeding programmes. Pollen longevity is an important factor in fecundity (Fritz and Lukaszewski, 1989; Dafni and Firmage, 2000) and an important physiological attribute for species survival and perenniality. Both pollen germination and pollen tube growth are influenced by the environment. Temperature and other factors like humi-dity, organic solvents appear to be most important factors that affect pollen viability (Visser, 1955).

The results obtained in this study indicate that pollen germination was not only influenced by varietal characteristics but also by environmental conditions. Nikkanen et al. (2000) reported differences in pollen germination among the genotypes of *Picea abies.* Bots and Muriani (2005) also reported that pollen viability is species dependent and that in partially hydrated pollen, dehydration leads to rapid loss of viability. Stanley and Linskens (1974) observed that pollens are more sensitive to adverse environmental conditions than the female reproductive organs, and this has accounted for the impaired fertilization under such conditions. In bambara groundnut, the pollen grains that were incubated immediately after harvest gave the best germination. Pollen germination dropped rapidly with delay in incu-bation. A delay of five minutes before incubation under ambient environmental conditions reduced the pollen viability to less than 3% and beyond this time, there was outright failure in pollen germination. Thus, the viability of pollen is strongly influenced by the duration of pollen exposure to the prevailing weather conditions. The exposure of patato pollen grains for up to 30 min have been reported to have

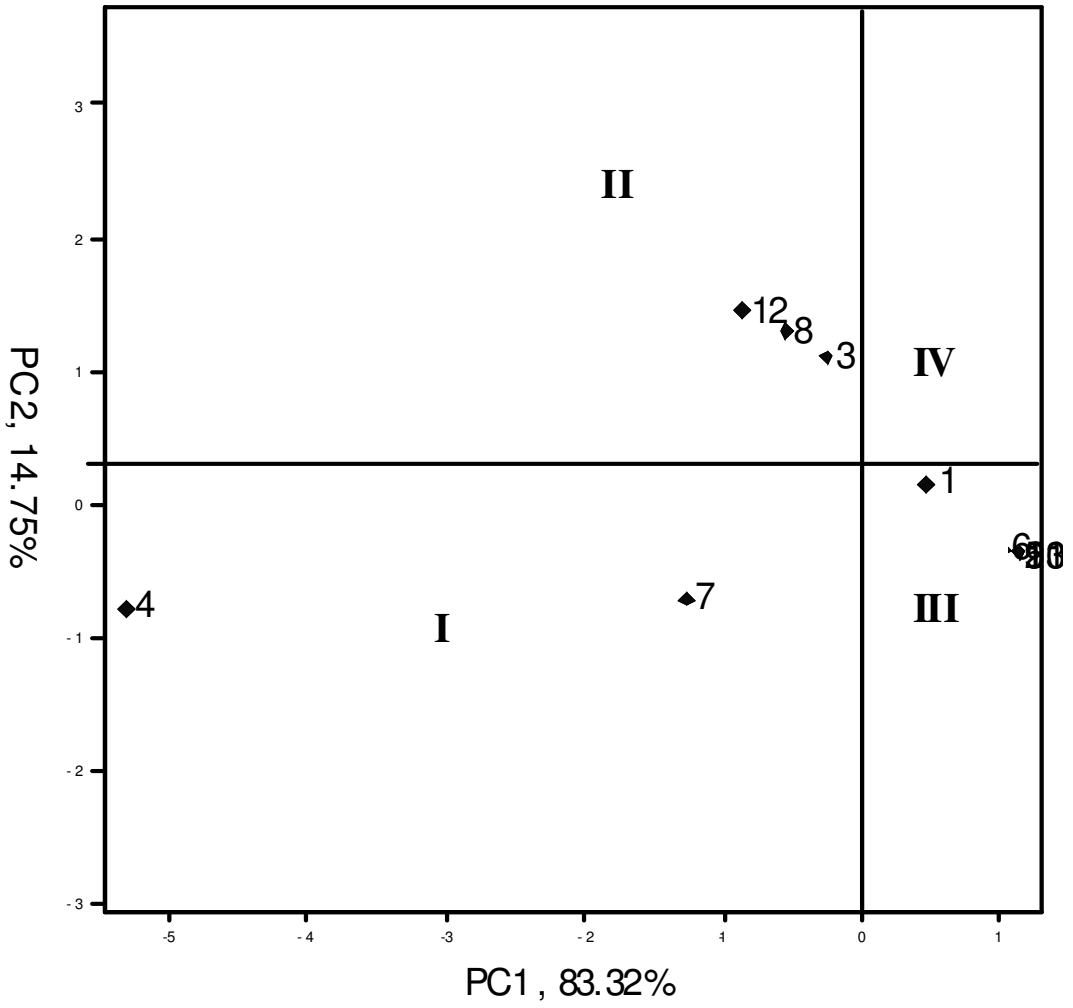

Figure 3. First and second principal copmonent scores (PC1 and PC2) for the identification of bambara groundnut cultivar response to dutarion of exposure. Where the values in the plot represent the bambara groundnut accession numbe

Table 8. Classification of 13 bambara groundnut cultivars based on the scores of first two principal components (PC1 and PC2) during the late planting date.

High pollen survival (-PC1,- PC2)	Moderately pollen survival (-PC1, + PC2)	Low pollen survival (+PC1, -PC2)	Poor pollen survival (+PC1, +PC2)
Bg-04 (-5.32, -0.78)	Bg-03 (-0.24, 1.11)	Bg-02(1.13, -0.37)	Bg-01(0.47, 0.15)
Bg-07 (-1.27, -0.70)	Bg-08 (-0.55, 1.30)	Bg-05(1.13, -0.37)	
	Bg-12 (-0.88, 1.45)	Bg-06 (1.02, -0.33)	
		Bg-09 (1.13, -0.37)	
		Bg-10 (1.13, -0.37)	
		Bg-11 (1.13, -0.37)	
		Bg-13 (1.13, -0.37)	

caused between 30 to 70% decrease in the pollen germination (Pallais et al., 1988). Ayalor (2003 and 2004) reported a continuous loss in pollen germi-nation of *Z. mays* as a result of dehydration, when exposed to ambient

conditions.

There were strong indications that bambara groundnut pollens grains are shed in the form of trinucleate pollens. A trinucleate pollen is short-lived (Fie and Nelson, 2003; Lansac et al.,1994; Leduc et al., 1990) and cannot survive prolonged storage after harvest (Brewbaker, 1967 and Mulcahy and Mulcahy, 1983). Trinucleate pollen grains are also recalcitrant *in vitro (* Kearns and Inouye, 1993). The above characteristics were observed in the Bambara groundnut pollen grains. In cultivars with trinucleate pollens, anthesis occur after generative cell mitosis (Brewbaker, 1957;1959). Trinucleate pollen grains have been reported in crop like *Erythronium grandiflorum* (Kearns and Inouye, 1993); *Oryza rufipogon* and *Oryza sativa* (Song et al., 2001), *Arum italicum* and*Arum maculatum* (Gibernau et al., 2003).

Under tropical conditions, short life span of pollen grains of bambara groundnut could be due to rapid loss of water due to high temperature. The direct effects of temperature on pollen grains of bambara groundnut need to be investigated. The relatively short longevity of Bambara groundnut pollen could also be a direct conesquence of its exceptionally low water content at anthesis. Loss of water leads to irreversible changes in the pollen membranes (Shivanna and Heslop-Harrison, 1981; van Bilsen et al., 1994) and partially hydrated pollens remain viable for a very short period (Bots and Muriani, 2005). The observed differences in pollen tube growth in the present study is a reflection of cultivar variability. Cultivar differences have been implicated in the pollen tube growth of many crops such as; Strawberry (Bots and Muriani, 2005); Cherry (*Prunus avium* L.) (Hedhly et al.,2004) and Cotton (Kakani et al., 2005). Pollen tube lengths similar to those recorded in the present study were reported for muskmelon (Maestro and Alvarez, 1988).

Similar to the pollen reactions during germination, vigorous pollen tube growth was achieved in the pollen grains that were germinated immediately after harvest. Pollen tube growth was very poor when the pollens were delayed for five minutes before incubation. Rapid growth of the pollen tube is a desirable traits, as the tube would reach and transmit the male nucleus into the embrayo sac at a faster rate thereby facilitating fertilization. This results on good pod set with some implications on yield. The vigorous growth rate of pollens incubated at harvest implies that at the point of pollen harvest, bambara groundnut pollen grains still retained its structural integrity. The growth rate decreased significantly with the slightest pollen exposure indicating significant loss in the pollen vigour and the structural intergrity. The implication of this, is that pollen grains of interest must be picked and transfered quickly to an already emasculated female flower in order to achieve rapid pollen tube growth rate and consequently, enhance the pollination and rapid fertilization. In fact, the waiting period of a designated Bambara groundnut pollen for artificial hybridization after shedding should be less than five minutes. This is because pollens exposed for more than five minutes may lose potency thereby leading to germination failure or very poor growth of the pollen tubes. Such pollens would hardly achieve fertilization as the male nucleus may not reach the deeply seated ovule with sufficient vigor.

Pollen tube growth of bambara groundnut is nonlinear. The pollen tube growth models revealled that the logistic equation provided the highest R^2 value for the pollen tube growth prediction. Athough logistic model had performed slightly better than gompertz and exponential models for the prediction of pollen tube growth data set, distinctions among the three models were not significant and so any of the three models could be employed for the computation of the bambara groundnut pollen tube growth. This is because the three models have acceptable coefficient of determination (R^2). The pollen tube growth rate (data not shown) presented sigmoid growth curves with the lag, exponential, linear and stationary phases. The rapid growth phases (exponential and linear phases) occurred during the first 20 min of incubation. The exponential or biosynthetic phase is the period of maximum celular division and greatest growth rate of the pollen, while the linear phase is the period in which the cells grew but with a decrease in cellular division (Scragg and Allan, 1993). The adaptive period (lag phase), in which the pollen tube increased slowly, occured between 20 and 60 min of pollen tube incubation. The stationary phase of pollen tube occurred between the 60 and 80 min. In this phase, the rate of cellular division decreased gradually before attaining a constant status.

The PCA is perhaps the most useful statistical tool for screening multivariate data with significantly high correlations (Johnson, 1998). The first three principal components, PC1, PC2 and PC3, explained 100 and 99.21% of the total cultivar pollen variability in response to the duration of exposure in the early and late plantings, respectively. The PC1 vectors indicated that cultivars with high pollen germination percentage and tube growth at five minutes duration of pollen exposure do not necessarily have high pollen germination or long pollen tubes but longer shelf life. This attribute is very important for plant breeders and could also enhance timely and successful fertilization of the megagametophyte that requires both pollen germination and pollen tube elongation. Therefore, the ability of pollen to germinate and grow well at five minutes pollen exposure could be used as a tool to identify high pollen survival in Bambara groundnut cultivars. The cluster analysis divided the cultivars into three distinct groups in the early planting and four in the late planting. Cultivar Bg-01 was classified as moderately pollen survival cultivars; Bg-02, Bg-03, Bg-04, Bg-05, Bg-06, Bg-07, Bg-10 nd Bg-13 as low pollen survival cultivars and, Bg-08, Bg-09, Bg-11 and Bg-12 as poor pollen survival during early planting. At late planting, Bg-

04 and Bg-07 was classified as high pollen survival cultivars; Bg-03, Bg-08 and bg-12 as moderately survival cultivars; Bg-02, Bg-05, Bg-06, Bg-09, Bg-10, Bg-11 and Bg-13 as low pollen survival cultivars and Bg-01as low pollen survival.

In conclusion, prolonged exposure of pollen grains to ambient weather conditions markedly reduced pollen germination and pollen tube growth *in vitro*. Delay of up to five minutes before incubation resulted in a considerable drop in the pollen germination. Beyond five minutes, the pollen grains of bambara groundnut failed to germinate. Therefore, for artificial hybridization to succeed in bambara groundnut, there is need for germination enhancement by treating the pollens with Indole - 3 - acetic acid. This has practical values in plant breeding and crop improvement.

REFERENCES

Anchirina VM, Yiridoe EK, Bennett-Lartey SO (2001). Enhancing sustainable production and genetic resource conservation of Bambara groundnut. A survey of indigenous agricultural knowledge systems: Outlook Agric. 30(4): 281-288.

Ayalor DE (2003). Rate of dehydration of corn (*Zea mays* L.) pollen in the air. J. Exp. Bot. 54: 2307-2312.

Ayalor DE (2004). Survival of maize (*Zea mays*) pollen exposed in the atmosphere. Agric. For. Meteorol. 123: 125-133.

Bots M, Mariani C (2005). Pollen viability in the field. COGEM, Radbound University Nijmegen 52p.

Brewbaker JL (1957). Pollen cytology and self-incompatibility systems in plants. J. Hered. 48: 271-277.

Brewbaker JL (1959). Biology of the angiosperm pollen grain. Ind. J. Genet. Plant Bred. 19: 121-133.

Brewbaker JL (1967). The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the Angiosperms. – Am. J. Bot. 54: 1060-1083.

Brooking IR (1979). Male sterility in Sorghum bicolor induced by low night temperature. II. Genotypic differences in sensitivity. Austr. J. Plant Physiol. 6: 143-147.

Bushell C, Spielman M, Scott RJ (2003). The basis of natural and artificial postzygotic hybridizzation barriers in *Arabidopsis species*. The plant cell, American Society of plant biologist, Pp.1430-1442.

Dafni A, Firmage D (2000). Pollen viability and longevity: practical,ecological and evolutionary implications. Plant Syst. Evol. 222: 113-132.

Fie S, Nelson E (2003). Estimation of pollen viability, shedding pattern, and longevity of creeping bentgrass on artificial media. Crop Sci. 43: 2177-2181.

Fritz SE, Lukaszewski AJ (1989). Pollen longevity in wheat, rye and triticale. Plant Breed. 102: 31-34.

GenStat Discovery Edition 3 (2007). VSN International Ltd., Hemel Hempstead, UK.

Gibernau M, Macquart D, Diaz A (2003). Pollen Viability and Longevity in Two Species of *Arum*. Aroideana 26: Pp. 58-62.

Hedhly A, Hormaza JI, Herrero M (2004). Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). Am. J. Bot. 91: 558-564.

Johnson DE (1998). Applied multivariate methods for data analysis. New York: Duxbury Press. Pp. 360.

Kakani VG, Reddy KR, Koti S, Wallace TP, Prasad PVV, Reddy VR, Zhao D (2005). Differences in *In vitro* Pollen Germination and Pollen Tube Growth of Cotton Cultivars in Response to High Temperature. Ann. Bot. 96(1): 59-67.

Kakani VG, Prasad PVV, Craufurd PQ, Wheeler TR (2002). Response of *in vitro* pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant, Cell Environ. 25: 165-166.

Kapoor SK (1976). Pollen germination in some Cucurbits. J. Palyn. 12: 87-93.

Kearns CA, Inouye DW (1993). Techniques for pollination Biologist. University press of Colorado 583p.

Khatun ST, Flowers J (1995). The estimation of pollen viability in rice. J. Exp. Bot. 46: 151-154.

Lacroix B, Assoumou Ndong Y, Sangwan RS (2003). Efficient *in vitro* direct shoot regeneration systems in Bambara groundnut (*Vigna subterranea* L. Verdc.). Plant Cell Rep. 21: 1153 -1158.

Lansac AR, Sullivan CY, Johnson BE, Lee KW (1994). Viability and germination of the pollen of sorghum [*Sorghum bicolour* (L.) Moench]. Ann. Bot. 74: 27-33.

Leduc N, Monnier M, Douglas GC (1990). Germination of trinucleated pollen: formulation of a new medium for *Capsella bursa-pastoris*. Sexual Plant Reprod. 3: 228-235.

Maestro MC, Alvarez J (1988). The effects of temperature on pollination and pollen tube growth in muskmelon *Cucumis melo* L. Sci. Hortic. 36: 173–181.

Massawe FJ, Dickinson M, Roberts JA, Azam-Ali SN (2002). Genetic diversity in Bambara groundnut (*Vigna subterranea* (L.) Verdc) landraces revealed by AFLP markers. Genome 45: 1175-1180.

Massawe FJ, Schenkel W, Basu S, Temba EM (2003). Artificial hybridization in bambara groundnut (*Vigna subterranea (L.) VERDC.).* In "proc. The international Bambara groundnut Symposium" Botswana College of Agriculture, Botswana pp. 193-209.

Messerli M, Robinson KR (1997). Tip localized Ca2+ pulses are coincident with peak pulsaltile growth rate in pollen tube of *Lilium longiflorum*. J. Cell. Sci. 110: 1269-1278.

Mulcahy GB, Mulcahy DL (1983). A comparison of pollen tube growth in bi- and trinucleate pollen – In: Pollen. Biology and implications for plant breeding (ed. D.L. Mulcahy and E. Ottaviano),– Elsevier Sci. Publ. Co., Amsterdam pp. 29 - 33.

Nikkanen T, Aronen T, Ha¨ggman H, Vena¨ la¨inen M (2000). Variation in pollen viability among *Picea abies* genotypes – Potential for unequal paternal success. Theor. Appl. Genet. 101: 511-518.

Obi IU (2002). Statistical methods of detecting differences between treatment means. SNAAP press Ltd., Enugu, Nigeria. Pp. 45.

Pallais N, Mulcahy D, Fong N, Falcon R, Schmiediche P (1988). The relationship between potato pollen and true seed: effects of high temperature and pollen size. In: Cresti M., Gori P., Pacini E. (eds.): Sexual Reproduction in Higher Plants. Spinger-Verlag, Berlin Heidelberg pp. 285-290.

Rieseberg LH, Carney SE (1998). Plant hybridization. N. Phytol. 140: 599-624.

Schenkel W (2000). Hybridization of jugo bean and breeding strategies for self pollinating crops. Presentation at UNISWA kwaluseni campus in march 2002 (http:// www. weihenstephanide/pbz/ bambara/html/presentation. Htm).

Scragg AH, Allan EJ (1993). *Picrasma quassioides* Bennet (Japanese quassia tree): *in vitro* culture and production of quassin. In: BAJAJ, Y. P. S. (Ed.). Biotechnology in agriculture and foresty: medicinal and aromatic plants. Berlin: Springer-Verlag 21: 249 - 268.

Shivanna KR, Heslop-Harrison J (1981). Membrane state and pollen viability. Ann. Bot. 47: 759-770.

Shivanna KR, Rangaswamy NS (1992). Pollen biology. A labouratary mannual. *Springer verlag*. New York. NY. 119p.

Snedecor GW, Cochran WG (1967). Statistical Methods. The Iowa State University Press. Iowa, USA. 256p.

Sokal RR, Rohlf FJ (1995). Biometry – The principles and practice of statistics in biological research 3rd edition. New York*:* W.H. Freeman and Company. 887p.

Song ZP, Lu BR, Chen JK (2001). A study of pollen viability and longevity in Oryza rufipogon, O. sativa, and their hybrids. IRRN E-mail: jkchen@fudan.edu.cn. 26: 2.

Stanley RG, Linskens HF (1974). Pollen biology, biochestry and management. Springer, Verlag. Berlin, Heidelberg, New York pp. 119-246.

Steer MW, Steer IM (1989). Pollen tube tip growth. New phytol. 111: 323-358.
Tiffin P, Olson MS, Moyle LC (2001). Asymmetrical crossing barriers in angiosperms. Proc. R. Soc. Lond. B Biol. Sci. 268: 861-867.
Tuinstra MR, Wedel J (2000). Estimation of pollen viability in grain Soghum. Published in Crop Sci. 40: 968-970.
van Bilsen D, Hoekstra FA, Crowe LM, Crowe JH (1994). Altered phase behaviour in membranes of aging dry pollen may cause imbibitional leakage. Plant Physiol. 104: 1193-1199.
Visser T (1955). Germination and storage of pollen. Meded.Landb. Hoogesch. 55: 1-68.
Zeng-Yu Wang, Yaxin GE, Megam S, German S (2004). Viability and longevity of pollen from transgenic and non-transgenic tall fescue (*Festuca arundinaceae*) (Poaceae) plants. Am. J. Bot. 91: 523-530.