STUDIES ON ASPECTS OF REPRODUCTIVE BIOLOGY AND POD YIELD IN BAMBARA GROUNDNUT (*Vigna subterrenea* (L.) *Verdc)*

BY

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PG/M.Sc./05/40282

DEPARTMENT OF CROP SCIENCE
UNIVERSITY OF NIGERIA, NSUKKA

NOVEMBER, 2009

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A DESSERTATION SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE, UNIVERSITY OF NIGERIA, NSUKKA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE (PLANT BREEDING AND GENETICS)

NOVEMBER, 2009

CERTIFICATION

OYIGA, Benedict Chijioke, a postgraduate student in the Department of Crop Science and with the registration number PG/M.Sc./05/40282 has satisfactorily completed the requirements for course and research work for the Degree of Master of Science in Crop Science (Plant Breeding and Genetics).

The work embodied in this dissertation is original and has not been submitted in part or full for any other diploma or degree of this or any other University.

.....	.....
Prof. M. I. Uguru	Prof. K. P. Baiyeri
Supervisor	Head of Department

DEDICATION

Dedicated to my late elder brother, Mr. Collins Chukwuemeka Oyiga. May his gentle soul rest in peace. Amen.

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ABSTRACT

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, rate of pollen tube growth, floral, agronomic and yield attributes of thirteen bambara groundnut cultivars. The first experiment (early planting) was in April and the second (late planting) was in August 2007. The results obtained showed that

genotypes had significant effect on the pollen germination only at the late planting. In the early and late planting, pollen grains incubated immediately after harvest had the highest germination percentage, while pollen grains exposed for five minutes prior to *in vitro* germination showed very poor germination. Pollens exposed beyond five minutes after harvest did not germinate. Genotypes 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 curve fitting analysis results showed that exponential, logistic and gomperzt growth models can be used for the computation of the pollen tube growth rates. The PCA and cluster analyses were used to group the genotypes in relation to the levels of pollen survival under ambient conditions. During the early planting, the genotype, Bg-01 had moderate surviving pollen grains while Bg-08, Bg-09, Bg-11 and Bg-10 were found to have poor surviving pollens. At late planting, the genotype, Bg-04 and Bg-07 had high pollen survival while Bg-01 had poor pollen survival. The planting dates had significant effects ($p<0.05$) on all the floral and agronomic traits measured except for stigma diameter. Significant genotype and genotype x planting date interaction effects were observed for pistil length, stamen length, stigma-anther separation and days to 50% flowering. The principal component analysis of the floral and agronomic traits showed that the first three components accounted for 70.54% and 72.96% of the total variation in the early and late plantings, respectively. The traits representing the genotypes along the first principal axis were anther diameter, number of pods per plant, stigma-anther separation and seed weight per plant for the early planting and number of flowers per plant, number of leaves per plant, number of pods per plant, plant height, stamen length, stigma-anther separation and seed weight per plant in the late planting. Genotypes were differentiated on the basis of anther length and days to 50% flowering in the early planting and anther diameter, anther length and pistil length during the late planting along the second principal axis. The cluster plot revealed

that the 13 bambara groundnut genotypes were grouped into three and two clusters during the early and late plantings, respectively. In the early planting, the genotypes in cluster I were associated with large anther diameter, very marginal stigma-anther separation and high potentials for production flowers, pods and high seed yield while cluster II genotypes flowered earlier and had smaller anther diameter, wide stigma-anther separation, good vegetative growth and low seed yield. The cluster III are early flowering genotypes with long pistil and stamen. During the late planting, the cluster I comprised of genotypes with large anthers, very marginal stigma-anther separation, high vegetative growth and high seed yield attributes while cluster II comprised genotypes with long pistil and stamen but performed poorly in pod production and seed yield. The correlation coefficient for seed weight per plant was highly significant and positive with number of leaves per plant, plant height, number of flowers per plant, number of pods per plant and anther diameter indicating that increase in these traits will ultimately increase seed weight per plant. However, stigma-anther separation was negatively correlated with seed weight per plant ($r = -0.61^{**}$) and number of pods per plant ($r = -0.45^{*}$) implying that the two yield traits decreased with increase in stigma-anther separation.

INTRODUCTION

In Africa, bambara groundnut (*Vigna subterrenea* (L.) Verdc) is the third most important legume after groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Howell, 1994) and a major source of vegetable protein (Amarteifio and Moholo, 1998; Uguru and Akubuo, 1999; Essien and Akaninwor, 2000; Basu *et al.*, 2003). It has several production advantages

in that it can yield on soils of low fertility and with little rainfall. It is nutritionally superior to other legumes, and is the preferred food crop of many local people (Brough and Azam-Ali, 1992). Bambara groundnut is primarily grown for its seeds. The seeds command a high market price, with demand far outweighing the supply in many areas (Coudert, 1982). Currently, there is a growing awareness on its potential importance as food and a major source of dietary protein among rural and urban dwellers. Despite there potentials, bambara groundnut is mainly cultivated by poor resource farmers at subsistence level. To wean bambara groundnut from the subsistence production and integrate it fully into a commercial/full scale production would require some level of improvements aimed at generating genotypes with good agronomic potentials.

Attempts to improve bambara groundnut through the conventional breeding methods have not been successful. Hybridization has been largely constrained by the failure of the crop to set seeds after artificial crosses. Thus, the available genotypes are selections from the aboriginal landraces. Hybridization and selection of new forms are important crop improvement strategies. Hybridization of selected parental lines allows for creation of new forms through genetic recombinations. The reasons for hybridization failures have not been clearly understood. The cytological status of Bambara groundnut lines have been studied (Uguru and Agwatu, 2006) with no discernable cytological impediments to fertilization and seed set. The architecture of the reproductive structures and longevity of the pollen grains after shedding may provide a clue to the possible causes of hybridization failures. After shedding, pollen grains are exposed to the prevailing environmental conditions and they have to reach a receptive stigma while still viable. The duration of pollen viability after anther dehiscence is very crucial for successful pollination. Pollen viability and longevity are also important physiological attributes that enable the breeder understand species reproductive

performance and therefore, enhance the successful implementation of breeding programmes. Therefore, the knowledge of the duration of pollen viability of bambara groundnut will be very useful in developing strategies to manage pollen transfer and increase the chances of successful pollination and fertilization. There is also lack of research result on the relationships among the agronomic, floral and the yield components in bambara groundnut. This has created an information gap on the yield related traits that will be relevant in the improvement of the crop. This experiment was therefore, designed to study;

- the reproductive structures of bambara groundnut lines as a prelude to ascertaining the causes of hybridization failures in breeding programmes.

- the agronomic and reproductive biology of bambara groundnut.

- the yield potentials of bambara groundnut lines in relation to the reproductive biology, floral and agronomic traits.

LITERATURE REVIEW

Origin

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an indigenous African legume, and one of the most important food crops in Africa and other parts of the world. The common English name appears to be derived from a tribe of agriculturalists, the Bambara, who nowadays live mainly in Mali (Linnemann, 1993). For many centuries, bambara groundnut has been cultivated in the tropical regions south of the Sahara where it is indigenous. Major

producers are Nigeria, Niger, Ghana, Haute Volta, and Côte d'Ivoire, but it is also widely grown in Eastern Africa and Madagascar (Linnemann, 1993) and is even found in parts of South and Central America. The crop was taken to Asia, particularly India, Indonesia, Malaysia, Phillipines and Sri Lanka and, is also found in parts of Northern Australia (Linnemann and Azam-Ali, 1993).

Importance of bambara groundnut

Bambara groundnut is a major source of vegetable protein in sub-Saharan Africa. It is well adapted to harsher conditions and constitutes an important part of the local diet, culture and economy (Goli, 1997; Heller *et al.,* 1997). The literature reports protein values between 12-26% (Basu *et al.*, 2003; Essien and Akaninwor, 2000; Amarteifio and Moholo, 1998; Glew *et al*., 1997). In addition, Rowland (1993) reported that bambara groundnut has high concentration of edible protein and hence high lysine content.

The seed is regarded as a completely balanced food because it is rich in iron 4.9-48 mg/100 g, compared to a range of 2.0-10.0 mg/100 g for most food legumes, protein 18.0-24.0% with high lysine and methionine contents (Rowland, 1993), ash 3.0-5.0%, fat 5.0-7.0%, fibre 5.0-12.0%, potassium 1144-1935 mg/100g, sodium 2.9-12.0 mg/100g, calcium 95.8-99 mg/100g, carbohydrate 51-70%, oil 6-12%, and energy 367-414 kal/100 mg (Rowland, 1993). The gross energy value of bambara groundnut seed is greater than that of several other pulses (Anchirina *et al.,* 2001; Rowland, 1993; Lacroix, 2003; Amarteifio *et al.,* 2002).

In many countries in West Africa, fresh pods are boiled with salt and pepper and eaten as a snack. In East Africa, the beans are roasted, pulverized and used in preparing soup. According to Linnemann (1990), bambara groundnut flour is used to make bread. Ripe/dry seeds are hard and therefore difficult to grind. Usually they are either pounded to flour and boiled to a stiff porridge or soaked and then boiled. The porridge keeps well and is traditionally used on journeys. Bambara milk is preferred to that prepared from other pulses because of its flavour and colour (Goli, 1997). Bambara seed and haulm have been used to feed livestock and poultry (Anchirina *et al.,* 2001).

Bambara groundnut fixes atmospheric nitrogen in symbiosis with Bradyrhizobium strains through a nodulation process (Gueye *et al.,* 1988). The crop requires relatively low inputs and contributes to the sustainability of the cropping systems in West Africa. The ability of

bambara to survive and grow under harsh conditions is due to specific adaptive traits which include production of subterranea pods and a combination of physiological regulations: osmotic adjustment, leaf area index reduction, increase leaf reflectivity and changes in leaf orientation and stomatal regulation (Collinson *et al.,*1999 and 1997; Chapman *et al.,* 1993).

Morphology

Bambara groundnut is an intermediate, annual herb of up to 30 cm in height with creeping, multi-branched, leafy lateral stems just above ground level. The plant has a well developed tap root with lateral roots on the lower part. In association with *Rhizobium*, the roots form rounded and sometimes lobed nodules (Linnemann and Azam-Ali, 1993). Bambara groundnut landraces differ in many aspects from each other, with a wide variety of seed and pod colours, and growth habits varying from bunch type, to semi bunch and spreading.

Bambara groundnut has small yellow flowers. The flowers are normally carried in pairs, on short peduncles which arise from the axis formed by the petioles and the stem (Doku, 1968). The flowers produced on the same peduncle do not open on the same day, although the interval does not exceed 24 hours. The flowers open in the early hours of the morning when the skies are clear. Sometimes, flower opening may be delayed due to an overcast sky or low temperature (Massawe *et al*., 2003).

Doku (1968) reports that ants play a vital role in the pollination of the flowers, but recent research suggests that bambara groundnut is mainly self-pollinated in most environments (Massawe *et al*., 2003). In bambara groundnut, fertilisation takes place on the same day as anthesis (Linnemann, 1994). Massawe *et al.* (2003) reported that the glandular apex of the bambara groundnut flower produces a sweet secretion which attracts ants. In the natural habitat the ants loosen the soil around the glandular apex while feeding on the secretion thus assisting the penetration of the peduncle into the soil.

The pods of bambara groundnut develop underground or just above the ground and may be up to 3.7 cm in diameter, depending on the landrace and number of seeds they contain. The pods are spherical or oval in shape and many contain only one seed. Pods with two seeds are also common in some landraces (Massawe *et al*., 2003). Pods with more than two seeds have also been reported (Pasquet and Fotso, 1997). Mature pods are indehiscent, ranging from yellow to reddish to dark brown or even black in colour.

Physiology of bambara groundnut

Germination of bambara groundnut generally takes 7 to 15 days (Kocabas *et al.*, 1999). Germination depend on temperature, genetic variability, seed size and age (Massawe *et al.*, 2002; Kocabas *et al.*, 1999; Sreeramulu, 1982). Kocabas *et al*. (1999) reported that there was no germination below 12 °C and above 45 ^{o}C, and is at a maximum at 32.9 ^{o}C. Massawe (2002) reported a similar response for germination to temperature, but noted that the response is landrace-dependent. Pre-sowing hydration has been reported to have a positive effect on the germination of bambara groundnut (Massawe, 1999), although, again the soaking time is landrace-dependent. Mabika (1992), however, reported that soaking the seeds from two bambara groundnut landraces for 24 hours did not improve germination. Massawe (1999) also reported that germination is faster when in continuous darkness indicating that germination of bambara groundnut is sensitive to the duration of light.

One of the most important factors influencing the physiology of bambara groundnut is photoperiod or daylength sensitivity. Photoperiod influences its reproductive development (Linnemann *et al.*, 1995; Linnemann and Craufurd, 1994; Linnemann, 1994; Linnemann, 1991; Nishitani *et al.,* 1988) both in terms of flowering and pod formation. The degree of sensitivity, however, for both of these processes varies widely between landraces, from completely insensitive to very sensitive. Harris and Azam-Ali (1993) showed in a serial sowing study that, while there was no detectable influence of photoperiod on flowering, it did have an effect on the onset of podding. Nishitani *et al.* (1988) showed a delay in flowering of 6-11 d when plants were grown under continuous light, compared to plants grown under a normal day-and-night rhythm. Four photoperiodic response types have been identified (Swanevelder, 1998; Linnemann, 1991):

1. Day-neutral for flowering, with a quantitative response to short days for podding
2. Day-neutral for flowering, with obligate response to short days for podding
3. Quantitative response to short days for flowering, with obligate response to short days for podding
4. Obligate response to short days for flowering

Leaf appearance of bambara groundnut is dependent on temperature. Massawe *et al*. (2003) reported a base temperature range for leaf appearance of 8.1 to 12.0 ^{0}C depending on the landrace. Brink (1999) and Linnemann (1994) observed that leaf production is also influenced by photoperiod. Plants grown under long photoperiods seem to produce more leaves than plants grown under short photoperiods.

Bambara groundnut is widely considered to be a drought resistant crop (Collinson *et al*., 1997 and Babiker, 1989). Begemann (1988) suggested two traits that help the crop adapt to a dry environment, namely, a short growing season, and a deep root system. Collinson *et al*. (1997) suggested that drought tolerance of bambara groundnut is as a result of osmotic adjustment, reduction of leaf area index, and low water loss through the stomata. Nyamudeza (1989) reported a high root to total dry matter ratio in bambara groundnut compared to other crops, while Shamudzarira (1996) found a high water use efficiency, both of which are characteristics linked to drought resistance. Also, paraheliotropism and higher leaf reflectivity have been observed when the crop is subjected to water stress (Collinson *et al.,* 1999). Mwale *et al.* (2003) reported preferential allocation of dry matter to the roots with increase in the intensity of drought.

Like most other legumes, bambara groundnut has the ability to fix nitrogen through a symbiotic relationship with *Rhizobium*. Gueye and Bordeleau (1988) found in a study of 24 landraces in Senegal that 12 landraces nodulated very effectively with both indigenous and introduced *Rhizobium* strains. Most effective in this study were the indigenous strain MAO 113 and the introduced strain TAL 22. A study in Nigeria showed that nodule production rate was influenced by genotype and soil (Uguru and Ezeh, 1997). Kishinevsky *et al.* (1996) studied the nodulation and nitrogen fixation of 23 indigenous Malawian landraces grown on soil free of *Vigna subterranea*-nodulating *Rhizobia*.

Agronomy and yield

Bambara groundnut is often grown as an intercrop with major crops such as maize, millet, sorghum, cassava, yam, peanut, and cowpea (Karikari *et al.*, 1997 and Ngugi, 1997) or grown in small plots as monoculture (Manthe *et al.,* 2002; Ntundu, 1997). Sesay *et al.* (1999) reported that almost all farmers in the study in Swaziland grow the crop as a sole crop. In Namibia, farmers assign a specific part of a field for growing bambara groundnut, and use

this for many years (up to 17 years) until yields start to decline (Fleissner, 2002). In Nigeria, it is widely grown in the North central states.

One of the most time-consuming aspects of growing bambara groundnut is the practice of earthing up, also known as ridging (Manthe *et al.,* 2002; Fleissner, 2002; Linnemann, 1987). This is the covering up with soil of the developing pods. Different reasons are given for this practice. For example, Sesay *et al.* (1999) cited promotion of yield, better pod development, protection of the pods against pests and protection of the pods against the sun.

Cultivating bambara groundnut is surrounded by folklore (Manthe *et al.,* 2002; Sesay *et al.*, 1999; Swanevelder, 1997). Swanevelder (1997) reported that bambara groundnut cannot be planted before a maize crop, males can not walk through a bambara groundnut plot because it could results in bad yields and bambara groundnut cannot be planted in virgin soil. However, in contradiction to this, research results in Swaziland have shown that bambara groundnut can be ideally planted in virgin soil or soils that have been fallow for over two years (Sesay *et al.*, 1999).

The current yields of bambara groundnut are extremely low and variable, because the environments in which it is normally grown are characterised by various biotic and abiotic stresses (Massawe *et al.*, 2003). However, even under optimum conditions yields are variable and unpredictable due to the variability of growth and development of individual plants within a landrace (Squire *et al.,* 1996).

According to Linnemann and Azam-Ali (1993) farm pod yields vary between 650 and 850 kg ha^{-1} for most of the semi-arid tropics. However, there are large differences between countries, with yields as low as 56 to 112 kg ha^{-1} have been reported in Zambia, while in Zimbabwe 3870 kg ha^{-1} was obtained (Linnemann, 1987). Eyzaguirre (1997) reported values of yield in West Africa, ranging from 575 to 940 kg ha^{-1}. In different studies in Southern Africa the following yields are reported: 500 to 800 kg ha^{-1} in Ghana (Doku, 1997), 50 to 660 kg ha^{-1} in Swaziland (Sesay *et al.*, 1999), and 71 to 862 kg ha^{-1} in Zimbabwe (Manyepe, 2002).

Pollen grains

Pollen grains are produced in specialized floral organs called stamens, which consist of a stalk with vascular tissue and a two-lobbed anther. They develop inside the anther until it splits and the mature grains are released into the environment (dehiscence). The pollen grains then have to be transported to a compatible stigma and they do so using one of various possible vectors. Common vectors for pollen transport are wind and various insects, depending on the species. Mature pollen grains are unusual vegetative cells that contain within themselves sperm cells, complete with cell walls and plasma membranes. This arrangement is accomplished soon after meiosis, when an asymmetric mitotic division produces a large cell that engulfs its diminutive sister, the generative cell (Twell *et al.,* 1998; Yang and Sundaresan, 2000). Subsequently, the generative cell undergoes a second mitosis to form the second sperm cell required for double fertilization; ‘‘tricellular’’ pollen completes this division before it is released from the anther, whereas ‘‘bicellular’’ pollen undergoes this division only later, within the elongating pollen tube. Pollen cells are contained within a unique pollen wall, whose construction begins when the meiocyte is newly formed, with the surface layers elaborated over time. At maturity, the pollen surface can be divided into three principal strata, with the relative amount of each varying between species: (1) an outer exine wall, itself multilayered, composed of the chemically resistant polymer sporopollenin and interrupted by openings called apertures; (2) an inner intine, also sometimes multilayered, made primarily of cellulose; and (3) a pollen coat, composed of lipids, proteins, pigments, and aromatic compounds, that fills the sculptured cavities of the pollen exine.

Pollen development inside the anther is complex and involves various processes that occur simultaneously to enable pollen development on one hand, and to prepare the anther for pollen release on the other. One special feature of pollen grains is their wall, which is completely different from the wall of any other plant cell. The pollen wall is multilayered, derived from both the developing pollen and sporophytic cells of the anther and consists of material that is highly resistant to degradation, making it an important determinant of pollen viability (Bots and Mariani, 2005). In some species, on the surface of the pollen wall a layer of material is present, known as the pollen coat, tryphine or pollen kit (Taylor and Hepler, 1997). This sticky substance may contain lipids, proteins and phenolic compounds, and is especially substantial in entomophilous species. The functions of the pollen coat are thought to include sticking to insects to facilitate pollen transfer and protection against UV-radiation, but the lipids and proteins of the pollen coat also play an important role on the stigma (Dickinson *et al.,* 2000).

Stigma

Stigmas, the receptive portions of the female tissues, bind pollen and mediate tube migration into the style. Stigmas generally are classified into two groups: wet stigmas, which are covered with surface cells that often lyse to release a viscous surface secretion containing proteins, lipids, polysaccharides, and pigments; and dry stigmas, which have intact surface cells that typically protrude as papillae and are covered by a primary cell wall, a waxy cuticle, and a proteinaceous pellicle. Stigmas have been studied extensively in plants that exhibit self-incompatibility, a process that restricts inbreeding. Self-incompatible stigmas reject self-pollen by inhibiting pollen hydration, germination, and tube invasion (Silva and Goring, 2001; Wheeler *et al.,* 2001; Hiscock and McInnis, 2003; Kao and Tsukamoto, 2004).

Angiosperm species that produce trinucleate pollen typically have dry stigmas, whereas binucleate pollens often interact with wet stigmas (Heslop-Harrison, 1977). As diverse as they may be, all stigmas perform the same functions. Stigmas capture pollen, support hydration and germination, and offer entry points and guidance to pollen tubes en route to the ovaries. Stigmas do not only discriminate between different pollen grains; they also promote outcrossing or self-fertilization by the coordinated timing of their maturation with pollen release. This phenomenon, termed dichogamy, has been investigated for >200 years. Two forms of dichogamy have been recognized: protandry and protogyny, in which the male or female structures, respectively, are the first to mature (Bertin and Newman, 1993). Standard tests for stigma maturation and receptivity are routinely conducted based on (1) the presence of simple enzymatic activity, (2) the ability to support pollen germination, and (3) the ability to support fertilization (this third assay being the most stringent) (Dafni and Maues, 1998). Stigma development is linked to the development of other floral organs. In Arabidopsis, immature stigmas appear to promote pollen tube growth, although the immature pistil then is unable to support navigation to the ovules (Nasrallah *et al.,* 1994). As the pistil matures, the papillae extend to form elongated cells receptive for pollination.

At maturity, the receptive surface of a stigma presents secretions and enzymes required for pollination. Some plants that separate male and female flowers (monoecious angiosperms) have been found to retain their stigmas in the functionally male flowers but to delay the maturation of these stigmas. Likewise, some sterile cultivars lack the stigma secretions visible in their fertile counterparts (Heslop-Harrison and Shivanna, 1977). Other structural

changes accompany late stigma development, including modifications of the papillar cells and their cuticles. Also, in plants with wet stigmas, apoptotic events release stigma fluids from internal reservoirs (Heslop-Harrison, 1977; 1981).

Pollination

Pollination, the transfer of pollen to an appropriate stigma, is an integral part of the sexual life cycle of flowering plants. It involves a series of complex cellular interactions that culminates in the fusion between male and female gametes. The process also regulates crucial invents such as pollen adhesion, hydration, pollen tube growth and guidance to the ovule. The incompatibility mechanisms that control the acceptance or rejection of pollen alighting on a recipient play a major role in pollination process. Pollination is brought about by agents of pollination (Pollinators) which include wind, insects, birds, bats. Generally, animals are attracted to a flower by an odor or by the color and shape of the corolla. The floral shapes can also either inhibit or promote visitations from particular animal pollinators.

Pollen viability and performance

The distance and success to which pollen-mediated gene flow is likely to occur is dependent not only on its dispersal in space by either wind or insect action, but also on the length of time the pollen grains retain its potency. Pollen viability is generally considered to indicate the ability of pollen grains to perform its' function of delivering the sperm cells to the embryo sac following compatible pollination (Shivanna *et al.,* 1991a). It is considered as an important parameter of pollen quality (Dafni and Firmage, 2000). Pollen viability varies with environmental conditions, particularly temperature and humidity. Fertilization does not only depend on pollen viability, but also on receptivity of pistil and pollen deposition. Poor seed set may be the result of the immature ovules, poor pollen quality, adverse environments, genotype, lack of pollination, abnormal anatomical development or any combination of these factors (Knox *et al.,* 1986). Pollen longevity, considered as the period in which pollen retains its ability to germinate on appropriate (receptive and compatible) stigma (Defni and Firmage, 2000), varies significantly from species to species, ranging from minutes after shedding to months under laboratory condition (Shivanna and Johri, 1985).

Germinability of pollen appears to be related to the nuclear state of pollen. Pollen of angiosperms may be described as either bi-or trinucleate, depending on whether anthesis occurs, before (binucleate) or after (trinucleate) generative cell mitosis (Brewbaker, 1957;

1959). Most members of the *Poaceae* are trinucleate (Brewbacker, 1967). Brewbaker and Kwack (1963) growth medium germinated a number of species, however, species with trinucleate pollen germinated at a much lower percentage or failed to germinate (Brewbaker, 1967). Trinculeate pollen is also difficult to store. Once shed, trinucleate pollen germinability decreases rapidly (Fei and Nelson 2003; Lansac *et al.,* 1994; Leduc *et al.,* 1990). The vitality of trinucleate pollen may be related to respiration rate; trinucleate pollen respires at 2 to 3 times the rate of binucleate pollen (Hoekstra and Bruinsma, 1975). The difficulty in germinating trinucleate pollen has resulted in a number of different media and culture methods that are species-specific (Fei and Nelson 2003; Leduc *et al.*, 1990).

Pollen performance, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed producing plants. Failure in pollen performance diminishes the fitness of the male parent through a reduction on its contribution to the next sporophytic generation. Differences in pollen performance could also favor reproductive isolation, by the promotion of conspecific pollen (Carney *et al.*, 1996; Campbell *et al.*, 2003) and, conversely, could be a way to avoid inbreeding depression by promoting foreign pollen (Hauser and Siegismund, 2000). Conspecific pollen appears to be quite effective in limiting gene exchange and operates through a number of different mechanisms which include: production of compounds that either facilitate or inhibit pollen germination or the growth of pollen tubes (Cruzan, 1990), physical blocking of pollen by grains that arrive earlier on the stigmatic surface (Thomson, 1989), usurption of ovules by prior penetration of pollen tubes (Waser and Price, 1991), and selective abortion of zygotes (Hauser *et al.,* 1997).

Modulation of pollen performance plays a major role in regulating the gene flow and could have significant evolutionary consequences in terms of sexual and gametophytic selection. Following this interpretation, sexual selection in plants could take place at the prepollination and postpollination levels (Stephenson and Bertin, 1983). At the prepollination level, the factors involved in sexual selection include, among others, the amount of pollen produced and the differences among genotypes in flower quantity and attractiveness for pollinating agents. At the postpollination level, both male competition and female choice can be involved in sexual selection (Snow and Spira, 1991; Marshall,1998; Mitchell and Marshall, 1998). The prezygotic stage is emerging as a scenario for the regulation of mating (Herrero, 2003) with clear evolution consequences both in plants and animals (Bernasconi *et al.*, 2004).

Paternity analyses are becoming a powerful tool to investigate competition and selection during the reproductive phase (Bernasconi, 2003). Pollen performance is clearly affected by the genotype of the pollen (Snow and Spira, 1991). It has been suggested that pollen competition should eventually lead to selection for rapid pollen tube growth and, hence, fixation of the traits controlling pollen performance (Walsh and Charlesworth, 1992). However, several lines of evidence suggest that the genotype of the female recipient can limit the likelihood of that fixation (Mulcahy *et al.*, 1996; Hormaza and Herrero, 1996, 1999) and could play a clear role in controlling the pollen tube growth (Willson and Burley, 1983; Stephenson and Bertin, 1983; Herrero and Hormaza, 1996). On the other hand, studies in a few species have shown that environmental conditions affect pollen development (Young and Stanton, 1990; Jóhannsson and Stephenson, 1998) as well as pollen tube growth (Stephenson *et al.*, 1992). If this effect is widespread in plants, it may have important evolutionary consequences. One of the most important environmental factors that could affect pollen performance is the temperature during the progamic phase. Progamic phase is a period of pollen tube growth through the pistil. It has been shown that temperature affects pollen germination (Elgersma *et al.*, 1989; Shivanna *et al.*, 1991b), and pollen tube kinetics in the style (Jefferies *et al.*, 1982; Elgersma *et al.*, 1989). By pollinating a female genotype with pollen from two different donor genotypes, it has been shown that, besides those physiological effects, temperature also affect pollen tube population dynamics and that the response to temperature during the reproductive phase is genotype-dependent (Hedhly *et al.*, 2004).

Pollen Hydration and Polarization

In many species, pollen grains undergo programmed dehydration before release from the anther. Dehydration of the pollen is accompanied by dehydration of the anther cells and sugar-starch conversions (Keijzer, 1987; Pressman *et al.,* 2002). After dehiscence, the pollen of many species is dehydrated and metabolically inactive. In some species, however, pollen grains are still partially hydrated and metabolically active, which enables fast pollen tube formation (Heslop-Harrison, 2000; Nepi *et al.,* 2001). When a dehydrated pollen grain arrives on a compatible stigma, it takes up water from it and produces a pollen tube. Depending on the species, this process may take minutes to several hours. Once formed, the tube enters the pistil and is shielded from the environment, and dependent on resources provided by the pistil to accomplish fertilization.

Most pollen grains are metabolically quiescent and highly desiccated, ranging from 15 to 35% water content, when released from the anthers (Heslop-Harrison, 1979; Buitink *et al.,* 2000). Pollen Hydration transforms pollen from a non polar cell to a highly polarized cell. Whether tubes emerge on a dry stigma surface, from a grain submerged in stigma exudates, or from pollen germinated *in vitro*, the grain organizes its cytoplasm and cytoskeleton to support the extension of a single tube. These changes occur within minutes after hydration and include the formation of filamentous cytoskeletal structures that wrap around the nuclei, cytoskeleton polarization towards the site of tube emergence (Tiwari and Polito, 1988; Heslop-Harrison and Heslop-Harrison, 1992), reorientation of the large vegetative nucleus so that it enters the extending tube before the generative cells (Lalanne and Twell, 2002), assembly of mitochondria and polysaccharides at the site of the elongating tube tip (Cresti *et al.,* 1977; Mazina *et al.,* 2002) and selection of pollen plasma membrane for secretary vesticle targeting and deposition of callose at the site of tube emergence (Johnson and Mccormick, 2001).

Regardless of the mechanism of transfer, pollen hydration often is regulated, both temporally and spatially. Inappropriate pollen hydration can have disastrous consequences, leading to premature germination within the anther (Johnson and McCormick, 2001) or germination on the wrong surface (Lolle and Cheung, 1993; Lolle *et al.,* 1998). In plants with dry stigmas, regulated pollen hydration provides an effective early barrier to incompatible pollination. This mode is active in self incompatible crosses (Sarker *et al.,* 1988) and in crosses between species (Hu¨ lskamp *et al.,* 1995). These processes are remarkably localized: the stigma can hydrate a compatible grain while restricting the hydration of foreign or incompatible pollen on the same stigma (Dickinson, 1995).

Pollen hydration and tube germination can occur very fast or may take up to one hour, depending on the degree of pollen desiccation at the time of anther dehiscence. Grass pollen, for example, is never fully dehydrated and is metabolically active when shed from the anthers. This makes it very vulnerable, but it germinates within minutes after landing on a stigma. By contrast, pollen of lily is very dehydrated when released, which enables it to survive under extreme environmental conditions, but it takes up to one hour to germinate

after hydration on the appropriate stigma (Heslop-Harrison, 2000). Once pollen is hydrated and germination occurs, tube growth is directed within the stigma. In dry stigmas, tube growth occurs through the papilla cell wall, and pollen tube penetration is accompanied by cell wall expansion or loosening in the stigma (Elleman *et al*., 1992). Tube growth seems to be facilitated by wall-degrading enzymes produced either by the pollen itself or by the stigma.

Pollen tube growth through the stigma

After crossing the exine wall, pollen tubes can only enter the style after transiting the stigma barrier. The detail of this process vary considerably from species to species. In plants with open styles, the stigma is covered with an epidermis that is continuous with the style, but in species with closed stigmas, pollen tubes grow through the outer cuticle and cell wall of the stigma papillae to enter the style. Enzymes secreted by pollen have been proposed to play an important role in pollen tube invasion of the stigma surface. Acid phosphatase, ribonuclease, esterase, amylase, and protease activity have been localized to pollen intines and tubes (Knox and Heslop-Harrison, 1970). Esterases, particularly those known as cutinases, are important for breaching the stigma cuticle; they have been identified in the pollen of Brassica, Tropaeolum, and many other taxa (Knox and Heslop-Harrison, 1970; Shaykh *et al.*, 1977; Hiscock *et al.,* 1994) as well as in the proteinaceous pellicles of dry stigma surfaces (Heslop-Harrison and Shivanna, 1977; Hiscock *et al.,* 2002). Removing or disrupting the pellicle prevents compatible pollen tubes from entering the stigma, despite normal germination (Heslop-Harrison and Heslop- Harrison, 1975; Heslop-Harrison, 1977; Heslop-Harrison and Shivanna, 1977). Enzymatic penetration of the stigma surface is precisely controlled not to expose the pistil to pathogenic or inappropriate invasion. This control likely requires constant communication between the pollen tube and the stigma. Recently, receptor kinases have been identified as candidate mediators of communication between the pollen tube and the stigma.

Incompatibilty mechanisms in plant

Plant have a variety of mechanisms to distinguish pollen and ensure that only a certain kind of pollen can germinate on their stigmas. A pollen grain might be rejected, as it is genetically different from pistil. On the order hand, a pollen grain might be rejected as well as it is too closely related to the pistil. The latter kind of recognition is called self incompatibility (SI). Self incompatibility flowering plants, while possessing normal male and female garmetes,

fail to form zygote after pollination and cross pollination with certain other plants of same species. SI is an important genetic mechanisms preventing inbreeding and gene pool simplification. The growing pollen tube is shielded from the environment and dependent on the resources provided by the pistil to accomplish fertilization (Bots and Mariani, 2005). However, both on the stigma surface and in the pistil, molecular mechanisms may function to prevent fertilization by genetically distance or closely related pollen (Mccubbin and Kao, 2000).

Post-Pollination Selection

Post-pollination mate choice is essential for sexual selection in plants, because opportunities to choose mates prior to pollination are limited. Selection at this stage operates directly on the gametes. Bernasconi *et al.,* (2004) emphasized that this is in contrast to animals, where post-copulatory mechanisms discriminate among available ejaculates rather than sperms. Intra-ejaculate competition among individual sperms can not be strong because of limited gene expression at this stage. Pollen, unlike sperm, does not move in a fluid. After a pollen grain lands on a stigma, its vegetative cell elongates producing a pollen tube, which grows into a structure of the style and delivers a generative cell to the ovule. The complexity of this process, direct interaction with the female tissues and also high levels of gene expression in pollen (Becker *et al*., 2003) suggest that there is ample opportunity for sexual selection to act at this stage. Gametophytic self-incompatibility is a clear example of such selection favouring outbreeding (Richards, 1997). However, sexual selection may discriminate also among compatible pollen that is genetically heterogenous. The term pollen competition has been widely used in pollination studies to describe differential fertilization success of such pollen.

Pollen competition is believed to be responsible for cryptic self-incompatibility (CSI): a slow growing self-pollen may fertilize all ovules in single donor pollinations, but when it is applied in a mixture with a faster growing outcross pollen its fertilization success is greatly reduced. Bateman (1956) reported CSI for the first time in *Cherianthus cheiri* – a species producing full seed set when pure self-pollen is applied. He performed two mix pollination treatments on one yellow flowered individual: in the first treatment he used a mixture of self-pollen and pollen from a red flowered donor, in the second treatment – a mixture of outcross pollen from two donors: yellow and red flowered. The proportion of red flowered offspring was 92.2% and 22.7% in the first and second treatment respectively. This result suggests a strong disadvantage of self-pollen in *C. cheiri.*

Cryptic self-incompatibility has been studied by means of pollen tube growth measurements or/and paternity analysis following mixed pollinations. So far, most of the evidence for CSI comes from studies on heterostylous species. In these species, self-pollination is a kind of illegitimate pollination and self-pollen may have more disadvantages than only a slower growth. Other CSI studies showed a number of other methodological flaws like low number of genotypes or using morphological characters for paternity analysis.

Embryo abortion in plant

After fertilization, embryos can be selectively aborted depending on their genotype. This is however rather difficult to prove in plants. First, one has to prove that ovules that did not develop into seeds were indeed fertilized, embryos were potentially viable and moreover, that the observed abortion was selective. A common observation in plants is that they produce more ovules than seeds (Bawa and Webb, 1984; Stephenson, 1981). Seed-ovule ratios equal on average 85% for annuals and 50% for perennials (Wiens, 1984). However, low fruit or seed production can not be attributed directly to embryo abortion, because not necessarily all of the ovules are fertilised in the field. Burd (1994) reviewed pollen limitation in field studies by comparing fruit or seed production after natural and after hand pollination with outcrossed pollen. He assumed that after hand pollination excess of pollen were applied and thus seed-set and fruit-set were not pollen limited. Burd (1994) reported that 62% out of 258 analysed species was significantly pollen limited in the field. The frequency distribution of percent fruit set for the species indicated that for pollen limitation was not found (fruit set is defined as fraction of flowers setting fruits). This distribution indicates a great potential for embryo abortion. However, fruit abortion may be related to seed number rather than seed quality or genetic constitution, even when pollen is not limiting seed production.

Selection of embryo abortion

There are three hypotheses explaining this apparent "overproduction" of ovules in plants. First, in hermaphroditic plants the production of "excess" flowers can result from selection on optimal division of resources into male and female function. Flowers that do not contribute to seed production may disperse pollen and contribute to siring seeds (Sutherland and Delph, 1984). Second, it has been suggested that in variable environments it is impossible for the

plant to predict how much of the resources will be available during seed maturation. The overproduction of zygotes gives an opportunity of adjusting the seed set to the number that is optimal in a certain reproduction period. Such a strategy is called bet-hedging (Kozlowski and Stearns, 1989; Lloyd, 1980). Third, embryo abortion may serve to increase female fitness by providing the possibility to select for superior offspring and avoid investment of resources in seeds that produce offspring with a low fitness later in life (Willson and Burley, 1983). The selective embryo abortion (SEA) hypothesis received a lot of attention in 1980s (Casper, 1988; Stephenson, 1981; Willson and Burley, 1983). However, experiments designed to test it on the level of the phenotype were not able to discriminate between artifacts resulting from a treatment and effect of SEA (Casper, 1988).

Breeding mechanisms in plants

Plants can not choose their mates directly. However, they may have many adaptations that lead to an increase of pollen export or variation of pollen that land on the surface of a stigma. Such adaptations are: prolonged receptivity of the stigma and characters increasing the number of pollinators' visits like high nectar production rate and attractive floral display (Stephenson and Bertin, 1983).

Almost seventy five percent of the flowering plant species are hermaphroditic (Charnov, 1982). Therefore, mate choice in self-compatible plants often involves the avoidance of self-pollination. Various morphological and physiological features like: position of the stigma (herkogamy), timing of its receptivity (protandry and protogyny) can minimize self-pollination within the same flower (autogamy). However, there may be still a lot of self-pollination, which can not be prevented, as pollinators tend to visit neighboring flowers on the same genet (geitonogamy). As a result self-compatible plants may experience a considerable degree of inbreeding depression. Apart from autogamy and geitonogamy, crosses between related individuals (biparental inbreeding) may increase inbreeding. Such crosses can be common in populations with similar genetic structure because pollinators tend to visit neighboring plants. The level of biparental inbreeding depends on the genetic structure of the population.

MATERIALS AND METHODS

Two field 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. 06^0 52'N; Long. 07 24' E; Alt. 447.2 m a.s.l.). The monthly rainfall distribution, temperature and the relative humidity are presented in Table 1. The 13 bambara groundnut genotypes that were used in the present study were sourced from bambara

groundnut producing areas of Nigeria. The genotypes were classified based on the seed coat colour (Massawe *et al.,* 2000) and were given accession numbers/names and the place of collection (Table 2). Plate 1 shows the variations in the seed coat colour among the genotypes. The genotypes were grown in a randomized complete block design (RCBD) with three replications. The plot size was 180 m^2 and the seeds were planted at a spacing of 30 x 75 cm. Four kilograms of well cured pig dung (equivalent to 222 kg/ha) was applied to each plot before planting. The plots were weeded manually to keep weed pressure low.

Pollen germination and pollen tube growth: Flowers were randomly collected from the thirteen bambara groundnut genotypes at anthesis between 0900hrs and 1000hrs. Freshly matured bambara groundnut flowers were collected from twelve plants per genotype, and immediately placed in the plastic containers and carried to the laboratory. Pollen germination and pollen tube growth were tested using a medium containing 10g succrose, 100 mg/L boric acid and 300 mg/L calcium nitrate made up to 100 ml with deionized water. This medium is a standard for *in vitro* 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 minutes before germination *in vitro*. Using dissecting forceps, scalpel and a needle, anthers of various genotypes were opened to allow extraction and subsequent transfer of pollen dust into a drop of *in vitro* germination medium that was placed on a glass slide. Mature anthers were crushed and pollen grains mixed thoroughly with the medium. This procedure was replicated three times. The glass slides were then put in petri dishes with moist whatmann filter paper and were incubated in the dark under laboratory conditions.

Pollen germination was scored after 80 minutes of incubation by direct microscopic observation (Nikon Scientific, Kanagawa, Japan) using Grid squre ocular 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 in percent. The measurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece of the compound microscope. Pollen tube length was recorded for 10 randomly selected pollen

tubes in each replicate at 20, 40, 60 and 80 minutes of pollen incubation and the mean values were used for analyses.

Floral and Agronomic traits

Data on the floral traits include the stigma diameter, anther diameter, anther length, pistil length and stamen length. They were collected using an ocular micrometer. The stigma-anther separation (herkogamy) was computed by subtracting the stamen length from the pistil length (Mal and Lovett-Doust, 1997; Faivre and McDade 2001). Positive values of stigmatic exertion (stigma longer than longest anther) would indicate exerted stigma position, while negative values of stigmatic exertion (stigma shorter than the longest anther) represent inserted stigma position (PereraO and Poulos,1993). Positive and negative herkogamy values point to the existence of approach herkogamy and reverse herkogamy, respectively (Asier *et al.,* 2009). Data on the agronomic and yield traits were collected on days to emergence, days to 50% flowering, canopy spread (m^2), number of flowers per plant, number of pods per plant, number of leaves per plant, seed weight per plant (g), plant height. The leaf count and plant height were collected at the onset of flowering.

Table 1: Mean rainfall (mm), temperature (^{0}C), and the relative humidity during the experimental period.

Month	Temperature (^{0}C)		Rainfall (mm)	Relative humidity (%)	
	Min.	Max.		At 10 am	At 4 pm
April	22.67	32.67	121.66	74.53	64.53

May	21.9	31.73	193.55	76.32	70.81
June	21.83	30.97	327.66	77.53	72.93
July	21.2	30.50	62.99	78.74	73.61
Average	**21.9**	**31.47**	**176.47**	**76.78**	**70.47**
August	21.87	27.65	323.6	79.06	74.29
September	21.37	28.27	169.67	78.07	73.54
October	20.71	29.71	267.2	76.61	71.77
November	20.71	30.4	55.12	76.33	68.11
Average	**21.17**	**29.01**	**203.9**	**77.52**	**71.93**

Accession Number	**Name of the Accession**	**Place of collection**	**Ecological Zone**

Table 2: Genotypes number, names of the genotype and place of collection of the bambara groundnut genotypes

V1	Bg-01	Quaanpan, LGA	Southern Guinea savanna
V2	Bg-02	Mikang, LGA	Southern Guinea savanna
V3	Bg-03	Langtan South, LGA	Southern Guinea savanna
V4	Bg-04	Langtan North, LGA	Southern Guinea savanna
V5	Bg-05	Pankshin, LGA	Southern Guinea savanna
V6	Bg-06	Shendam, LGA	Southern Guinea savanna
V7	Bg-07	Langtan South, LGA	Southern Guinea savanna
V8	Bg-08	Mikang, LGA	Southern Guinea savanna
V9	Bg-09	Shendam, LGA	Southern Guinea savanna
V10	Bg-10	Quaanpan, LGA	Southern Guinea savanna
V11	Bg-11	Pankshin, LGA	Southern Guinea savanna
V12	Bg-12	Langtan North, LGA	Southern Guinea savanna
V13	Bg-13	Langtan South, LGA	Southern Guinea savanna

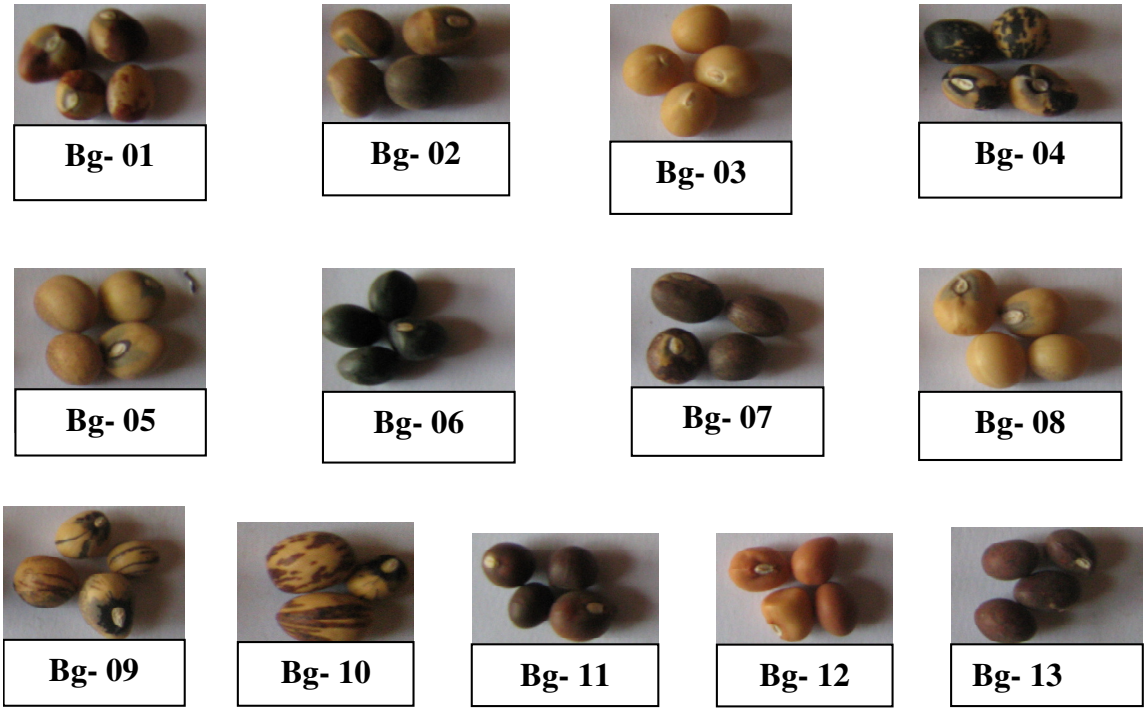

Plate 1. Variations in the seed coat colour of the 13 bambara groundnut genotypes evaluated.

Statistical analyses

Pollen germination and tube growth: The Genstat 7.22 release data analysis software (Lewis Agricultural Trust, Rothamsted Experimental Station) was used for all statistical analyses on the pollen data collected. Square root transformation was made on the percentage data (pollen germination percentage) before the analysis (Snedecor and Cochran 1967; Sokal and Rohlf, 1995). Data collected were analyzed using the two-way ANOVA procedure. Separation of treatment means for significant effect was by Fishers' least significant difference at 5% probability level.

Curve fitting and analysis: The mean pollen tube length recorded among the genotypes after 20, 40, 60, and 80 minutes of incubation, at each pollen exposure, were analysed using linear and nonlinear regression techniques to quantify developmental responses to different pollen exposure durations (Kakani *et al.,* 2005). 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, Gompertz were applied to the data and examined to determine the best-fit model. These models were explained in the equations 1 to 3. The prediction and goodness of fit of these models were examined by computing the coefficient of determination (R^2) among the genotypes. The best model is one with the highest R^2 value (Kakani *et al* ., 2002). The pollen tube growth model that performed best was used to fit the pollen data collected.

- **Exponential growth model:**

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

- **Logistic growth model:**

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

- **Gompertz growth model:**

$$y_i = \alpha + y \exp(-\exp(-\beta(x_i - \mu))) + E_i \quad . \; . \; . \; . \; . \; \textbf{(3)}$$

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) and Kakani *et al.* (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 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. Based on the values of the PC1 and PC2 scores, a two dimensional scatter plot using component score 1 as horizontal axis and component score 2 as vertical axis was constructed.

Floral and Agronomic traits: Analysis of variance was conducted using Genstat software version 7.22 to determine the effect of planting dates, genotypes and planting date x genotype interactions on all the floral and agronomic traits collected. The Fisher's Least Significant Difference (F-LSD) was used to detect significant differences between the means. Factor analyses, based on principal component (PCA) and cluster analyses, were performed (Johnson, 1998; Manly, 1994) on the floral and agronomic traits. Correlation coefficients were also calculated to determine the relationships among the floral, agronomic and yield traits.

RESULTS

Floral structre of bambara groundnut

The bambara groundnut flower has a bract or cap-like operculum (Plate 2a) that covers the staminate and pistillate parts of the flowers. This whithers away after fertilization. The bract or cap-like operculum structure functions not only to protect the male and female parts of the flowers but also to discourage cross pollination. Plate 2(b) shows the arrangement of anthers and the stigma of bambara groundnut flower. The stigma is positioned a little below the longest anther (Plate 2(b)) (reverse herkogamy or inserted stigma). The observed floral features were same for all the 13 bambara groundnut genotypes evaluated.

***In vitro* pollen germination**

Table 3 shows the effect of genotypes on the pollen germination at both early and late planting dates. Genotypes had significant effects ($p<0.05$) on pollen germination only at the late planting date. Although, genotypes did not differ signifficantly in pollen germination at early planting, the genotype, Bg-13 recorded the highest pollen germination percentage (30.1%) followed by Bg-07 (26.9%), Bg-04 (23.4%), Bg-03 (21.6%), Bg-05 (20.4%) and the lowest pollen germination percentage was observed in Bg-12 (11.0%). However, during the late planting, the genotype Bg-12 produced the highest pollen germination percentage (20.3%) which did not differ significantly from the pollen germination in Bg-03 (15.5%), Bg-04 (20.1%) and Bg-08 (19.8%). The Pollen grains did not germinate in the genotypes, Bg-02, Bg-05, Bg-09, Bg-10, Bg-11 and Bg-13 in the late planting. Pollen germination percentage of bambara groundnut was low at both planting dates.

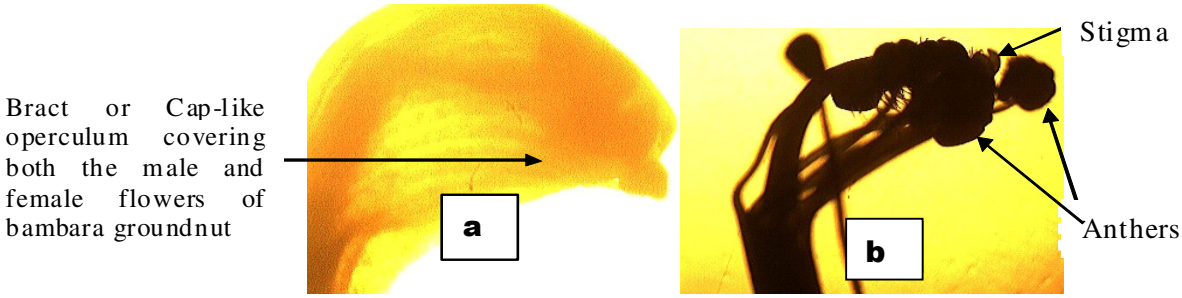

Plate 2: The floral structure of bambara groundnut showing (a) cap-like operculum and (b) anther and stigma positions

Table 3. Effect of different bambara groundnut genotypes on the pollen germination immediately after harvest. Values within the parentheses are transformed data.

Genotypes	Pollen germination (%)	
	Early planting	Late planting
Bg-01	20.9 (4.62)	5.0 (2.09)
Bg-02	16.1 (4.07)	0.0 (0.71)
Bg-03	21.6 (4.70)	15.5 (3.99)
Bg-04	23.4 (4.88)	20.1 (4.17)
Bg-05	20.4 (4.56)	0.0 (0.71)
Bg-06	18.8 (4.39)	0.3 (0.88)
Bg-07	26.9 (5.21)	6.0 (2.55)
Bg-08	13.2 (3.68)	19.8 (4.44)
Bg-09	12.7 (3.61)	0.0 (0.71)
Bg-10	13.5 (3.72)	0.0 (0.71)
Bg-11	17.6 (4.19)	0.0 (0.71)
Bg-12	11.0 (2.98)	20.3 (4.52)
Bg-13	30.1 (4.63)	0.0 (0.71)
F-LSD$_{0.05}$	**NS**	**(1.25)**

Figures 1a and b show the effect of duration of pollen exposure on the pollen germination of thirteen bambara groundnut genotypes after 80 minutes of incubation. Pollen germination of the thirteen bambara groundnut genotypes were tested after 0, 5, 10 and 15 minutes 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, the pollen grains that were incubated immediately after harvest had the highest germination percentage across all the genotypes. However, pollen germination dropped greatly when exposed for five minutes before incubation. The pollens from all the genotypes lost viability and did not germinate at 10 and 15 minutes 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 minutes of pollen exposure. Unlike what happened in the early planting when no pollen germination was recorded at 5 minutes exposure, about 3% of the pollen grains germinated in the late planting. There was no pollen germination recorded at 10 and 15 minutes of pollen exposure (Figure 1b).

Pollen tube growth

Table 4 shows the variation in pollen tube length at 20, 40, 60 and 80 minutes of incubation among the genotypes during the early and late planting. In the early planting, genotype differences were observed in the pollen tube length at 20, 40, 60 and 80 minutes of incubation. At 20 minutes 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 minutes of incubation was 57.10 µm in Bg-10. This value did not differ significantly from the 51.00 µm in Bg-05, 50.00 µm in Bg-04, 49.70 µm in Bg-01, 49.70 µm in Bg-07 and 42.50 µm in Bg-12 while Bg-02 produced the shortest pollen tube length. At 60 minutes of incubation, Bg-10 produced

the longest pollen tube (81.80 µm). This value did not differ statistically from the pollen tube length of Bg-05 (75.00 µm), Bg-07 (63.00 µm), Bg-12 (60.70 µm), Bg-04 (56.40 µm) and Bg-01 (54.50 µm). The genotype, Bg-02 also produced the shortest tube length (13.50 µm). A similar trend was maintained at 80 minutes of incubation.

Genotype differences in pollen tube growth were also observed at the late planting at 20, 40, 60 and 80 minutes of incubation (Table 4). At the first 20 minutes of pollen incubation, the genotypes differed significantly ($P<0.05$) in pollen tube length. Pollen tube growth was more rapid in the genotype, 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 minutes of incubation, Bg-04 also gave a highly significant pollen tube growth increase of 239.91 µm, 239.91 µm and 268.65 µm, respectively. Five genotypes namely; Bg-06, Bg-09, Bg-10, Bg-11 and Bg-13 did not produce viable pollens in the late planting and therefore no pollen tube growth was recorded for them.

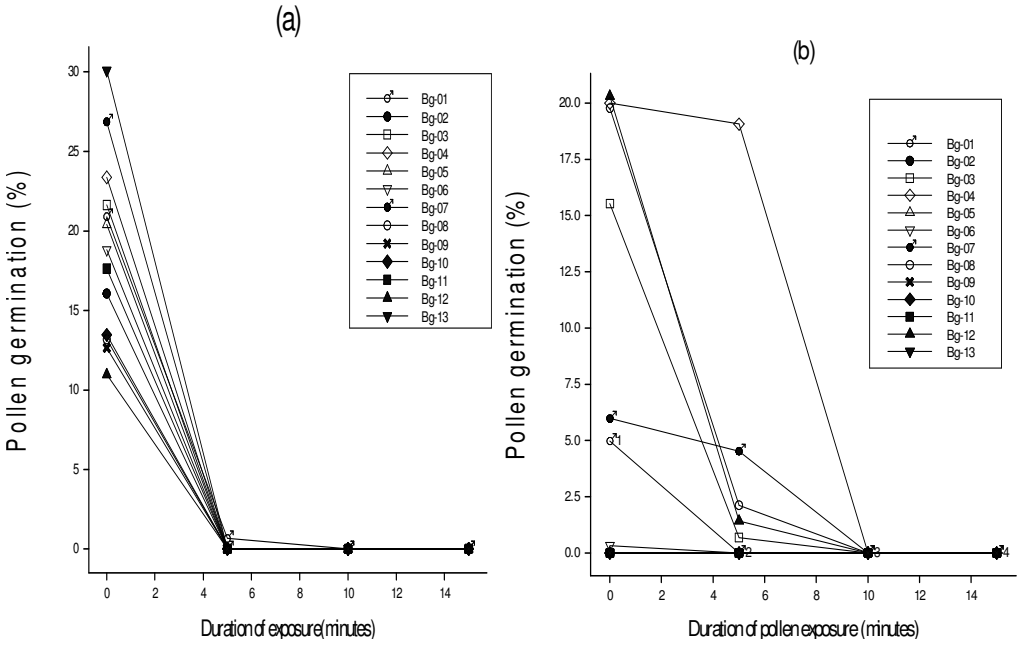

Figure 1. Effect of duration of pollen exposure on the pollen germination of thirteen bambara groundnut genotypes after 80 minutes of incubation in the (a) early and (b) late planting after 80 minutes of incubation.

Table 4. Effect of genotype on the pollen tube growth of 13 bambara groundnut genotypes during the early and late planting.

Genotypes	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	49.70	54.50	54.50	20.64	58.33	87.30	87.30
Bg-02	10.08	12.70	13.50	13.50	9.52	15.47	23.81	23.81
Bg-03	27.95	37.30	42.10	42.10	18.54	30.16	40.39	40.39
Bg-04	40.52	50.00	56.40	56.40	66.22	239.91	239.91	268.65
Bg-05	29.36	51.00	75.00	75.00	20.64	20.64	20.64	20.64
Bg-06	23.81	29.00	34.50	34.50	0.00	0.00	0.00	0.00
Bg-07	30.55	49.70	63.00	63.00	21.43	49.72	56.26	65.75
Bg-08	15.08	28.20	28.10	28.10	28.17	28.17	36.14	36.14
Bg-09	27.78	34.40	35.00	35.00	0.00	0.00	0.00	0.00
Bg-10	39.47	57.10	81.80	81.80	0.00	0.00	0.00	0.00
Bg-11	23.50	34.50	43.10	43.10	0.00	0.00	0.00	0.00
Bg-12	20.24	42.50	60.70	60.70	41.57	78.97	78.97	78.97
Bg-13	22.22	33.30	39.40	39.40	0.00	0.00	0.00	0.00
F $LSD_{0.05}$	**7.08**	**15.20**	**27.94**	**27.94**	**2.86**	**8.15**	**8.95**	**9.133**

Growth models

Table 5 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 genotypes 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 5). 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 6 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%) while the least prediction value of 91.5% was observed in exponential model (Table 6).

Growth rates: Figure 2 shows the increamental pollen tube growth of bambara groundnut during the early and late planting dates. The logistic growth curves were fitted to the pollen data and the observed pollen tube growth followed a sigmoidal pattern with time. At both planting dates, pollen tube growth rate increased rapidly (exponential and linear growth phase) from 0 to 40 minutes after incubation, increased minimally (adaptive period or lag growth phase) from 40 to 60 minutes, and showed non significant increase (stationary growth

phase) thereafter (Figure 2). The logistic growth model equation is as presented in equation 2 above. The fitted equation constants estimated for the pollen tube growth of bambara groundnut are: B = 0.0502; M = -10.5; C = 136 and A = -85 for the early planting and, B = 0.1051; M = 22.88; C = 51.33 and A = -4.28 for the late planting. The equation model acounted for 99% of the variance and Standard error of observations of 1.9.

Principal Component Analysis (PCA)

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 (*i.e.* 0, 5, 10 and 15 minutes). The pollen germination and tube growth paramters at 10 and 15 minutes duration of pollen exposure were not included in the PCA analysis because no germination occured at both exposure durations. At early planting, the first three principal component vectors (PC1, PC2 and PC3) accounted for 100% of the total variation (Table 7). 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 pollen germination percentage at 5 minutes exposure (PG % E_{5t}) and pollen tube length at 5 minutes exposure (PTL E_{5t}). The genotypes with higher and lower pollen germination at 5 minutes pollen exposure were placed on the right and left of the plot respectively (Figure 3). The PC2 had high positive loadings for pollen germination percentage at 0 minutes exposure (PG % E_{0t}) and pollen tube length at 0 minutes exposure (PTL E_{0t}). Based on the scores of the first two principal components, the genotypes were grouped into three (Table 8; Figure 3): Group 1 genotypes as moderate pollen survival with positive PC1 and negative 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 9). PC1 accounted for 83.32% of the variation among the genotypes while PC2 and PC3 accounted for only 14.75% and 1.14% respectively. The PC1 eigenvector contrasted genotypes 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}. Genotypes with higher pollen germination and pollen tube growth at 0 and 5 minutes duration of pollen exposure were placed on the left side of the plot while genotypes with low values were placed on the right flank of the plot (Figure 4). The genotypes were divided into four groups based on the scores of the first two principal components (Table 10; Figure 4): Group 1 genotypes as 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.

Table 5. Equation constants of exponential, logistic and gomperzt growth models 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	γ	μ	R^2	α	β	γ	μ	R^2
Bg-01	56.72	-56.88	0.952	**99.7**	-44.37	0.078	99.47	2.781	**99.9**	69	0.075	69	6.3	**99.9**
Bg-02	13.64	-13.64	0.935	**100**	-89.32	0.073	102.9	-26	**100**	-68	0.071	81	-24	**100**
Bg-03	43.24	-43.23	0.95	**99.8**	-220.3	0.057	263.3	-28.6	**99.6**	-1649	0.052	1693	-70.17	**99.7**
Bg-04	56.87	-56.77	0.942	**99.6**	-817.9	0.062	874.7	-42.7	**99.1**	-92539	0.06	92596	-123.2	**99.2**
Bg-05	101.3	-102.2	0.981	**96.7**	-27.8	0.055	108.9	19.2	**96**	-10.5	0.045	93.3	17.3	**95.6**
Bg-06	34.93	-34.8	0.948	**98.8**	-363.9	0.055	398.8	-42.5	**97.4**	-70862	0.053	70897	-143.1	**97.6**
Bg-07	72.05	-72.49	0.971	**98.8**	-42.7	0.057	108.6	7.6	**98.7**	-15.7	0.052	82	9.6	**98.7**
Bg-08	30.43	-30.88	0.958	**94.2**	-0.012	0.395	28.15	19.63	**100**	0	0.476	28.13	19.01	**100**
Bg-09	35.38	-35.4	0.925	**99.9**	-15.56	0.13	50.59	6.24	**100**	-2.34	0.128	37.36	7.98	**100**
Bg-10	98	-97.9	0.976	**97.2**	-214.7	0.034	307.4	-25	**94.6**	-126	0.032	219	-19	**94.5**
Bg-11	47.1	-47.16	0.966	**99.2**	-104.7	0.045	150.3	-18.4	**98.5**	-51	0.044	97	-10.3	**98.5**
Bg-12	86.6	-88	0.983	**95.7**	-9.5	0.072	73.3	25.92	**98**	-1.84	0.057	66.8	22.27	**97.5**
Bg-13	42.31	-42.44	0.962	**99.5**	-46.7	0.058	87.3	-2.4	**99.4**	-19.2	0.056	60	2.3	**99.4**
Mean	-	-	-	**98.4**	-	-	-	-	**98.6**	-	-	-	-	**98.5**

Table 6. Equation constants of exponential, logistic and gomperzt growth models on pollen tube growth of bambara groundnut at late planting

	Exponential growth model				Logistic growth model					Gomperzt growth model				
Cultivars	**α**	**β**	**ρ**	**R^2**	**α**	**β**	**y**	**μ**	**R^2**	**α**	**β**	**y**	**μ**	**R^2**
Bg-01	153	-157	0.988	**92.1**	-4.22	0.092	94.8	31.92	**98.7**	-0.01	0.065	92.9	26.76	**97.7**
Bg-02	32.93	-33.12	0.982	**96.1**	-11.5	0.048	37.8	16.9	**93.9**	-5.3	0.039	32.4	15	**93.6**
Bg-03	48.08	-48.36	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	356	-370	0.979	**84.7**	-3.84	0.159	273.2	26.69	**100**	0.01	0.124	270	22.74	**100**
***Bg-05**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bg-06	30.1	-31.1	0.994	**77.2**	0	0.56	9.52	38.76	**100**	0	0.386	9.52	37.66	**100**
Bg-07	85.8	-87	0.981	**96.6**	-12.9	0.072	77.7	22.6	**96.8**	-2.09	0.062	67.5	20.51	**97.3**
Bg-08	35.03	-34.81	0.934	**93.3**	-123.6	0.077	158.6	-16.52	**85.7**	-42025	0.069	42060	-103.3	**85.7**
***Bg-09**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***Bg-10**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***Bg-11**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bg-12	85.8	-87.2	0.959	**94**	-0.033	0.394	79.01	19.73	**100**	0	0.408	78.97	18.91	**100**
***Bg-13**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean				**91.5**					**96.6**					**96.6**

* - = No pollen germination was recorded

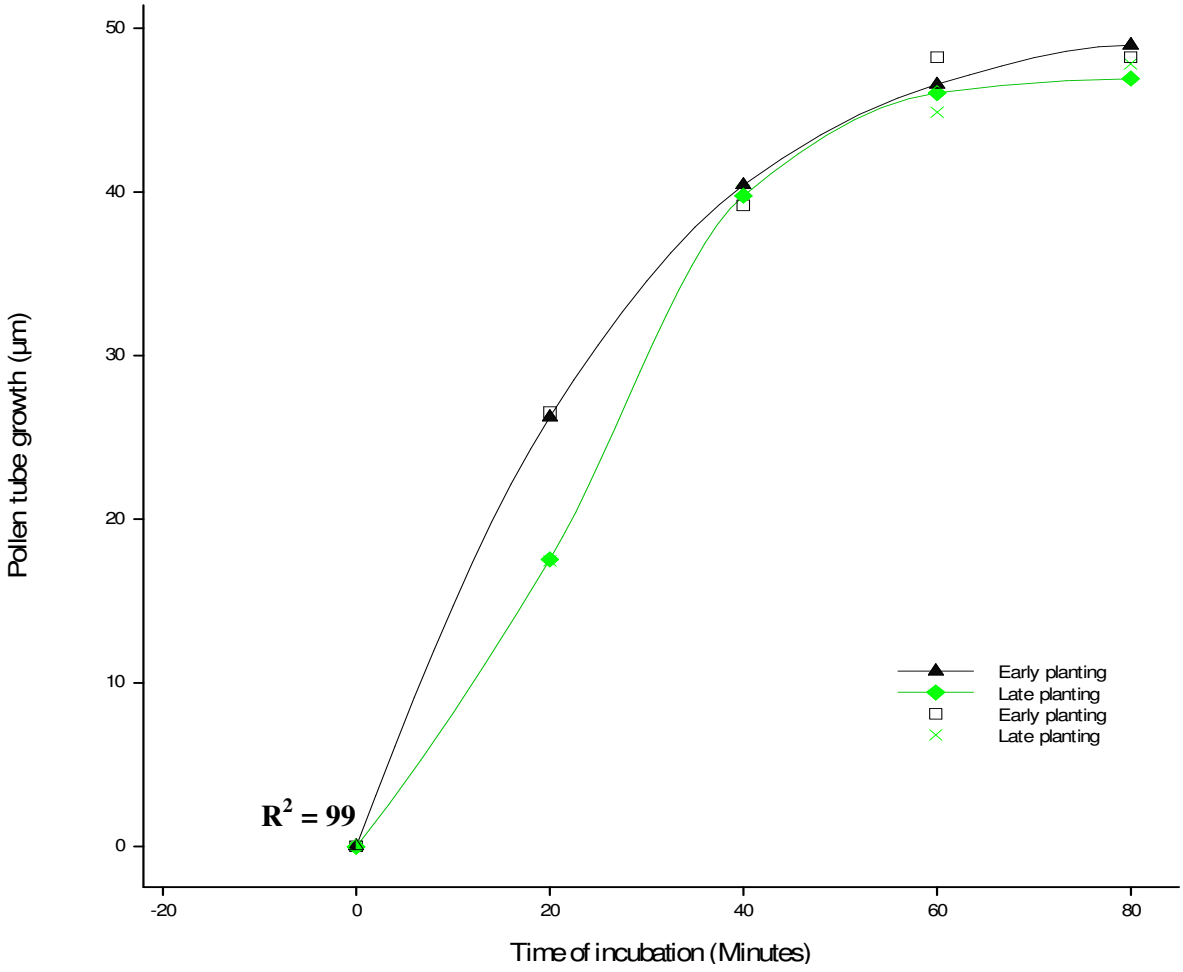

Figure 2. Increamental rate of bambara groundnut pollen tube from 20 to 80 minutes after incubation for the early and late planting dates. Logistic curves have been fitted to the data.

Table 7. Principal Component Analysis eigenvectors PC1, PC2 and PC3 of 13 bambara groundnut genotypes 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 E_{0t}	-0.08662	**0.73132**	**-0.67651**
PTL $_{E5t}$	**0.69799**	-0.02034	-0.11136
% Variation	**50.72**	**26.77**	**22.51**

PG % $_{E0t}$ and PG % $_{E5}$ = Pollen germination at 0 and 5 minutes duration of pollen exposure, respectively. PTL $_{E0t}$ and PTL $_{E05}$ = Pollen tube growth at 0 and 5 minutes duration of pollen exposure, respectively.

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

High pollen survival (+PC1, +PC2)	Moderate 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)	

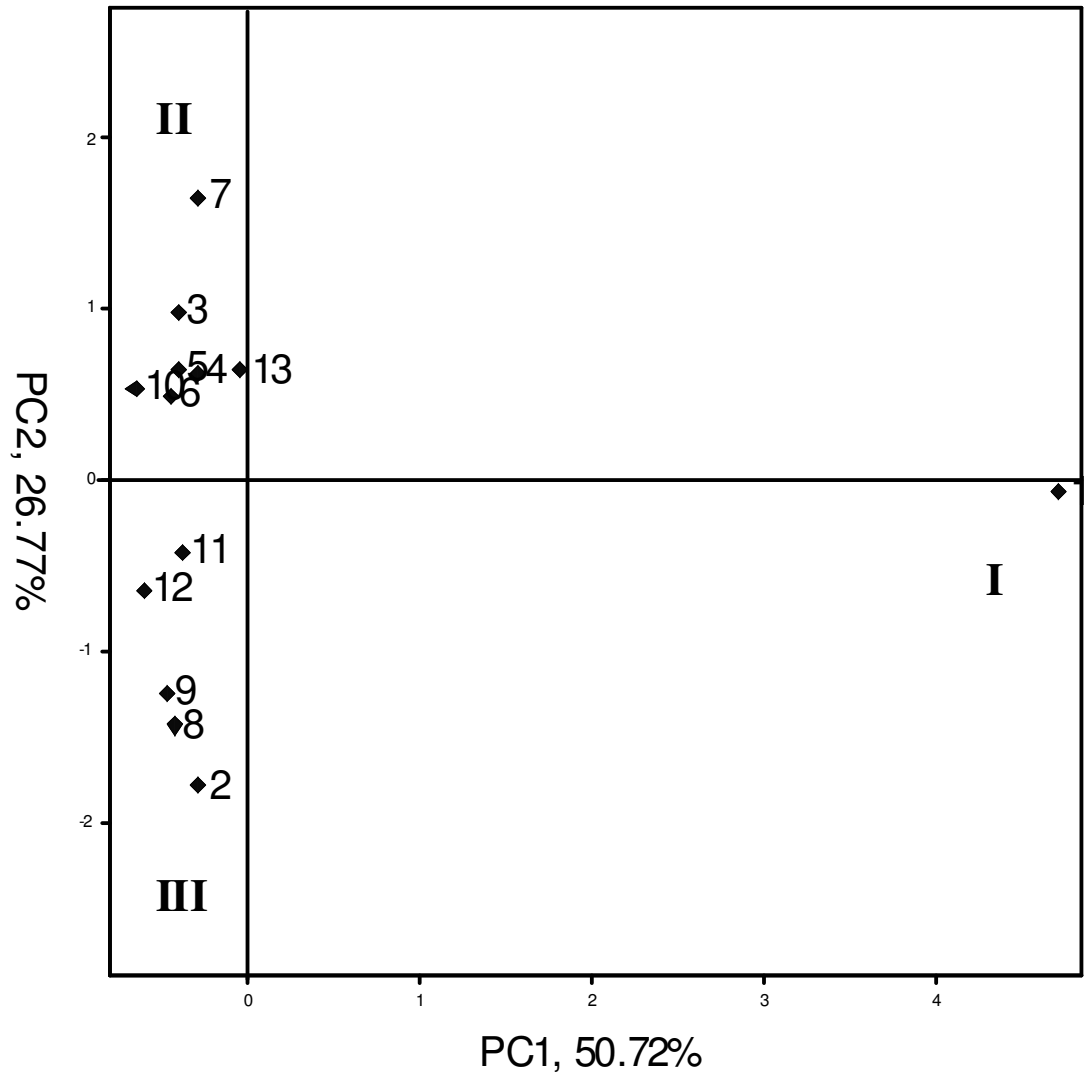

Figure 3. First and second principal component scores (PC1 and PC2) for the identification of bambara groundnut genotypes response to length of pollen exposure during the early planting. Where the values in the plot represent the bambara groundnut accession numbers.

Table 9. Principal component analysis eigenvectors PC1, PC2 and PC3 of 13 bambara groundnut genotypes for PG % E_{0t}, PG % $E_{5t,}$ PTL E_{0t}, and PTL E_{5t} and the variation accounted for by each eigenvector for late planting.

Parameters	PC1	PC2	PC3
PG % $_{E0t}$	-0.41055	0.85793	0.25295
PG % $_{E5t}$	**-0.52243**	-0.33338	0.61997
PTL $_{E0t}$	**-0.53920**	0.04269	**-0.74071**
PTL $_{E5t}$	**-0.51747**	**-0.38858**	-0.05478
% Variation	**83.32**	**14.75**	**1.14**

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

Table 10. Classification of 13 bambara groundnut genotypes based on the scores of first two principal components (PC1 and PC2) during the late planting date.

High pollen survival (-PC1, -PC2)	Moderate 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)	

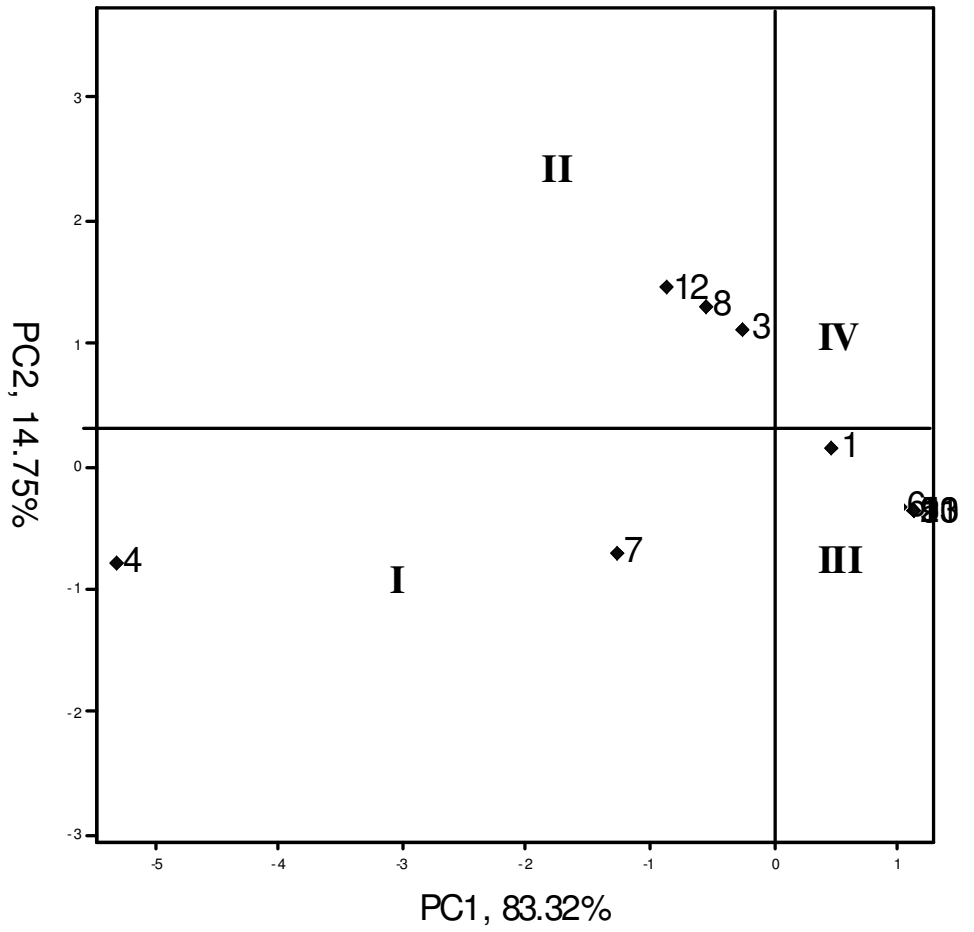

Figure 4. First and second principal component scores (PC1 and PC2) for the identification of bambara groundnut genotype response to duration of exposure during the late planting. Where the values in the plot represent the bambara groundnut accession number.

Floral traits

The analyses of variance on the floral traits are presented in Table 11. The two planting dates had significant effects ($P<0.05$) on all the floral traits measured with exception of stigma diameter. There were also significant differences among the genotypes on pistil length, stamen length and stigma-anther separation. The interactions of cultivar and planting dates showed significant influence on pistil length, stamen length and stigma-anther separation (Table 11).

The mean values of all the floral traits are shown in Table 12. The results show that planting date had significant effect on anther length, stamen length, pistil length, anther diameter, stigma-anther separation, number of pods per plant and seed yield per plant. All the traits evaluated recorded higher mean values during the early planting than in the late planting, except for anther length, anther diameter and stigma-anther separation. Variations among genotypes were significant ($p<0.05$) in pistil length, stamen length and stigma-anther separation. The pistil length was significantly higher in Bg-13 (5.77 mm) but did not difer statistically from Bg-11 (5.75 mm). These genotypes were followed by Bg-02 (5.62 mm), Bg-05 (5.60 mm), Bg-06 (5.48 mm) and Bg-04 (5.43 mm). The genotype, Bg-11 produced the longest stamen (5.85 mm) which did not differ significantly from the stamen lengths of Bg-13 (5.85 mm), Bg-02 (5.75 mm) and Bg-05 (5.72 mm). The genotypes varied in thier stigma-anther separation ranging from 0.02 mm in Bg-12 to 0.13 mm in Bg-02, Bg-07 and Bg-09.

Planting date and genotype interaction showed significant effect on pistil length, stamen length and stigma-anther separation (Table 12). Mean length of the pistil varied from 4.75 mm in Bg-03 to 6.00 mm in Bg-11. The genotypes produced longer pistil during the early planting when compared with the values obtained during the late planting. Stamen length varied from 4.79 mm in Bg-03 to 6.49 mm in Bg-05, the shortest was recorded during the late planting. The stigma-anther separation was very marginal and varied from 0.01 mm in Bg-12 to 0.19 mm in Bg-09.

Table 11. Mean square analysis of variance for floral and yield traits of bambara groundnut genotypes averaged over two planting dates.

SV		Mean squares					
	df	SD	AD	AL	PL	SL	SAS
Planting date (PD)	1	109360^{ns}	159007**	19188*	1.672**	1.339**	0.0185**
Genotype (G)	12	42988^{ns}	1703^{ns}	3270^{ns}	0.354**	0.380**	0.0061**
PD X G	12	53186^{ns}	4753^{ns}	2564^{ns}	0.050*	0.055*	0.0061**
Error	50	53634	3160	2712	0.017	0.017	0.00014

SD = stigma diameter, AD = anther diameter, AL = anther length, PL = pistil lenghth, SL = stigma length, SAS= stigma-anther separation.

Table 12. Effect of cultivar, planting date and cultivar x planting date interactions on the floral and yield traits of 13 bambara groundnut genotypes.

Genotype	SD (mm)			AD (mm)			AL (mm)			PL (mm)			SL (mm)			SAS (mm)		
	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
Bg-01	0.94	0.24	**0.59**	0.37	0.50	**0.44**	0.54	0.56	**0.55**	5.20	5.14	**5.17**	5.33	5.20	**5.27**	0.13	0.06	**0.09**
Bg-02	0.28	0.27	**0.27**	0.44	0.49	**0.47**	0.50	0.54	**0.52**	5.73	5.51	**5.62**	5.85	5.85	**5.75**	0.12	0.13	**0.13**
Bg-03	0.30	0.27	**0.29**	0.38	0.53	**0.46**	0.55	0.53	**0.54**	5.16	4.75	**4.96**	5.27	4.79	**5.03**	0.11	0.05	**0.08**
Bg-04	0.28	0.24	**0.26**	0.39	0.51	**0.45**	0.50	0.54	**0.52**	5.57	5.30	**5.43**	5.63	5.43	**5.53**	0.06	0.13	**0.10**
Bg-05	0.31	0.28	**0.30**	0.43	0.51	**0.47**	0.48	0.57	**0.53**	5.84	5.36	**5.60**	6.49	5.49	**5.72**	0.10	0.13	**0.12**
Bg-06	0.29	0.29	**0.29**	0.42	0.47	**0.44**	0.45	0.52	**0.48**	5.71	5.26	**5.48**	5.94	5.42	**5.58**	0.03	0.16	**0.10**
Bg-07	0.31	0.24	**0.27**	0.33	0.54	**0.44**	0.50	0.60	**0.55**	5.38	5.23	**5.30**	5.52	5.34	**5.43**	0.14	0.11	**0.13**
Bg-08	0.31	0.27	**0.29**	0.43	0.55	**0.49**	0.59	0.54	**0.57**	5.55	4.95	**5.25**	5.60	5.03	**5.31**	0.05	0.08	**0.06**
Bg-09	0.29	0.27	**0.28**	0.47	0.46	**0.47**	0.52	0.54	**0.53**	5.28	5.14	**5.21**	5.33	5.33	**5.33**	0.06	0.19	**0.13**
Bg-10	0.31	0.29	**0.30**	0.41	0.50	**0.45**	0.53	0.53	**0.53**	5.24	5.22	**5.23**	5.32	5.38	**5.35**	0.08	0.17	**0.12**
Bg-11	0.29	0.28	**0.28**	0.41	0.47	**0.44**	0.48	0.55	**0.52**	6.00	5.50	**5.75**	6.10	5.62	**5.86**	0.11	0.12	**0.11**
Bg-12	0.29	0.29	**0.29**	0.45	0.51	**0.48**	0.57	0.57	**0.57**	5.42	5.20	**5.31**	5.43	5.21	**5.32**	0.01	0.02	**0.02**
Bg-13	0.31	0.29	**0.30**	0.45	0.49	**0.47**	0.52	0.56	**0.54**	5.91	5.63	**5.77**	5.97	5.73	**5.85**	0.06	0.10	**0.08**
Mean	**0.35**	**0.27**		**0.41**	**0.5**		**0.52**	**0.55**		**5.54**	**5.24**		**5.62**	**5.36**		**0.08**	**0.11**	
F-LSD$_{0.05}$ for:																		
Genotype (G)			**NS**			**NS**			**NS**			**0.05**			**0.15**			
Planting date (PD)			**NS**			**0.26**			**0.24**			**0.02**			**0.06**			
C X PD			**NS**			**NS**			**NS**			**0.07**			**0.21**			

SD = stigma diameter, AD = anther diameter, AL = anther length, PL = pistil lenghth, SL = stigma length, SAS = stigma-anther separation.

Agronomic traits

The results of analysis of variance for the planting dates, genotypes, and planting date x genotype interactions are presented in Table 13. The results indicated that planting date had significant effects ($P< 0.01$) on all the agronomic traits measured. The genotype and genotype x planting date interaction effects were significant for days to 50% flowering.

Table 14 shows that the crop performed better in the early than in the late planting in all but one agronomic trait measured. The genotypes emerged 8 and 9 days after sowing for the early and late planting, respectively. Early planting date gave significantly higher vegetative growth as compared to the late planting. The number of leaves per plant was about 60% (165 leaves) higher in the early planting when compared with the 42 leaves produced during the late planting. In the early planting, the crop took significantly shorter time (34 days) to attain 50% flowering. However, in late planting, the crop attained 50% flowering at 36 days after planting. The number of flowers per plant was 63 during the early planting and 17 at the late planting. The average number of pods per plant recorded in the early and late planting were 44 and 4, respectively. Early planting gave a substantial yield increase over that of the late planting. The seed yield from the early planting was 19.0 g/plant (844.44 Kg/ha) as against the 1.0 g/plant (44.44 Kg/ha) that was produced under the late planting.

Genotype had significant effect on days to 50% flowering (Tabe 14). The genotype, Bg-02 was the earliest to attain 50% flowering at 33 days after planting but did not differ statistically from Bg-05, Bg-06, Bg-09 and Bg-13 genotypes. Flowering was delayed by five days in Bg-08. There was no significant genotype effect on the days to emergence, number of leaves per plant, plant height, number of pods per plant and seed weight per plant. The highest number of leaves was produced in Bg-04 (140 leaves), followed by Bg-05 (133 leaves), Bg-03 (132 leaves), Bg-01 (117 leaves) and the least was Bg-13 (61 leaves). The genotype, Bg-12 produced the highest number of flowers (49 flowers) and was followed by Bg-06 and Bg-03 (48 flowers), Bg-04 (45 flowers), Bg-01 and Bg-10 (41 flowers). The lowest number of flowers (28 flowers) was recorded in Bg-02. The genotype Bg-06, produced the highest number of pods per plant (36 pods), and the least number of pods (12 pods) was recorded in Bg-07. Seed weight per plant was highest (23.88 g) in Bg-06 and lowest (4.92 g) in Bg-07. Among the genotypes, Bg-08 was the tallest (21.25 cm) followed by Bg-01 and Bg-04 (20.50 cm), Bg-03 (20.34 cm), Bg-12 (20.00 cm).

The interaction of cultivar and planting date also had significant effects on days to 50% flowering. Days to 50% flowering varied from 33 days to 40 days. All the genotypes except Bg-13 flowered earlier during the late than in the early planting (Table 14).

Principal Component Analysis (PCA)

The results of the principal components analysis of the 13 floral and agronomic traits measured at both early and late plantings are presented in Table 15. The principal components in the early planting differed from those of the late planting. In the early planting, the first three components contributed 70.54% of the variability among the 13 genotypes evaluated. The PC1, PC2 and PC3 accounted for 30.41%, 25.42% and 14.71% of the total variation, respectively. The traits affecting the PC1 were anther diameter, number of pods per plant, stigma–anther separation and seed weight per plant while anther length and days to 50% flowering affected the PC2. The PC3 had high loading for number of leaves per plant. The two-dimensional scatter plot (Figure 5) apparently grouped the genotypes into three clusters with Bg-06, Bg-11 and Bg-12 as an outlier that did not belong to any of the clusters. The cluster means (Table 16) shows that cluster I comprise of genotypes with large anther diameter, very marginal stigma–anther separation and high potentials for the production flowers, pods and seed yield. The cluster II are essentially early flowering genotypes characterized with smaller anther diameter, wide stigma–anther separation, good vegetative growth (*i.e.,* number of leaves per plant and plant height) and low seed weight per plant. The cluster III are early flowering genotypes with long pistil and stamen. Although, Bg-06 did not belong to any of the clusters, it has large anther diameter and small stigma–anther separation and also performed well in the yield traits (*i.e.,* Number of pods per plant, seed weight per plant and number of flowers per plant). The Bg-11 is a late flowering genotype linked with high vegetative growth (number of leaves per plant and plant height). The genotype Bg-12 is associated with long anthers, marginal stigma–anther separation and is high seed yielding.

In the late planting, the principal component analysis revealed that the first three components contributed 72.96% of the total variation. The PC1, PC2 and PC3 contributed 47.74%, 12.77% and 12.45% of the total variation, respectively. The traits affecting the first principal component axis were number of flowers per plant, number of leaves per plant, number of pods per plant, plant height, stamen length, stigma–anther separation, and seed weight per plant. The traits representing the genotypes along the second principal component axis were

anther diameter, anther length and pistil length, while stigma diameter affected the third principal component axis. The scatter plot of the first two principal components revealed that the genotypes were grouped into two clusters (Figure 6). The cluster mean values are presented in Table 16. It shows that cluster I comprised genotypes with large anthers, very marginal stigma – anther separation, high vegetative growth and high yield attributes. The cluster II genotypes are associated with long pistil and stamen but performed poorly in pod prodoction and seed yield. The genotype, Bg-03 produced long stamen, small stigma – anther separation with high yielding ability while genotype Bg-07 is linked with long anther and high leaf production. However, the genotype Bg-13 did not produce pods but produced large anthers and long stamen.

Table 13. Analysis of variance for seven agronomic and yield traits of bambara groundnut cultivars showing the degrees of freedom (df), and the mean squares only.

SV	df	Mean squares						
		DTE	NLP	PH (cm)	NFP	D50%F	NPP	SWP (g)
Planting date (PD)	**1**	24.82**	292928**	2987.0**	40256.2**	123.13**	31400.3**	6341.1**
Genotype (G)	**12**	0.404^{ns}	3756^{ns}	17.0^{ns}	255.7^{ns}	11.54**	252.4^{ns}	223.8^{ns}
PD X G	**12**	0.293^{ns}	2876^{ns}	8.2^{ns}	271.6^{ns}	6.16**	248.1^{ns}	204.8^{ns}
Errror	**12**	0.235	2542	8.82	258.9	1.18	225.7	147.6

DTE = Days to emergence, NLP = Number of leaves per plant, PH = Plant height, NFP = Number of flowers per plant, D50%F = Days to 50% flowering, NPP = Number of pods per plant and SWP = Seed weight per plant

Table 14. Effect of genotype, planting date and genotype x planting date interactions on the agronomic and yield traits of 13 bambara groundnut genotypes

Genotype	DTE			NLP			PH (cm)			NFP			D50%F			NPP			SWP (g)		
	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
Bg-01	8.0	9.0	**8.5**	178.0	56.0	**117.4**	27.5	13.5	**20.5**	64.0	17.0	**40.5**	38.0	35.0	**35.5**	36.0	5.0	**20.5**	10.50	1.92	**6.21**
Bg-02	7.0	9.0	**8.0**	168.0	25.0	**96.3**	20.8	11.0	**16.0**	40.0	17.0	**28.5**	33.0	33.0	**33.0**	52.0	4.0	**28.0**	12.10	0.09	**6.10**
Bg-03	8.0	9.0	**8.5**	202.0	59.0	**130.5**	26.7	14.0	**20.3**	69.0	27.0	**48.0**	37.0	33.0	**35.0**	53.0	7.0	**30.0**	14.80	1.92	**8.36**
Bg-04	8.0	9.0	**8.5**	226.0	54.0	**140.0**	27.0	14.0	**20.5**	63.0	26.0	**44.5**	38.0	35.0	**35.0**	45.0	5.0	**25.0**	16.30	1.56	**8.93**
Bg-05	8.0	9.0	**8.5**	232.0	34.0	**133.0**	23.5	11.0	**17.3**	57.0	17.0	**37.0**	34.0	33.0	**33.5**	25.0	2.0	**13.5**	9.60	0.49	**5.05**
Bg-06	7.0	9.0	**8.5**	146.0	26.0	**86.0**	24.5	10.0	**17.3**	85.0	11.0	**48.0**	34.0	33.0	**33.5**	72.0	0.0	**36.0**	47.40	0.36	**23.88**
Bg-07	8.0	9.0	**8.5**	120.0	56.0	**87.7**	25.8	12.5	**19.2**	55.0	20.0	**37.5**	38.0	33.0	**35.5**	22.0	2.0	**12.0**	9.30	0.54	**4.92**
Bg-08	8.0	9.0	**8.5**	162.0	59.0	**110.5**	29.0	13.5	**21.3**	65.0	16.0	**40.5**	40.0	35.0	**37.5**	42.0	4.0	**23.0**	20.90	2.06	**11.48**
Bg-09	7.0	9.0	**8.0**	159.0	28.0	**93.3**	23.6	13.0	**18.3**	55.0	10.0	**32.5**	34.0	33.0	**33.5**	44.0	0.0	**22.0**	20.40	0.00	**10.20**
Bg-10	8.0	8.0	**8.0**	179.0	38.0	**108.3**	25.8	13.0	**19.4**	73.0	8.0	**40.5**	38.0	33.0	**35.5**	45.0	2.0	**23.5**	18.40	0.22	**9.31**
Bg-11	8.0	9.0	**8.5**	188.0	44.0	**116.0**	26.5	11.0	**18.8**	67.0	8.0	**37.5**	38.0	33.0	**35.5**	45.0	0.0	**22.5**	10.30	0.00	**5.15**
Bg-12	8.0	9.0	**8.5**	82.0	44.0	**63.3**	24.0	16.0	**20.0**	68.0	30.0	**49.0**	35.0	34.0	**34.5**	48.0	5.0	**26.5**	40.70	2.15	**21.43**
Bg-13	7.0	8.0	**7.5**	99.0	23.0	**61.0**	21.3	12.0	**16.7**	51.0	13.0	**32.0**	33.0	34.0	**33.5**	40.0	0.0	**20.0**	16.70	0.00	**8.35**
Mean	**7.7**	**8.8**		**165**	**43**		**25.00**	**12.70**		**62.7**	**17.3**		**36**	**34**		**44.0**	**4.0**		**19.00**	**1.00**	
F-LSD$_{0.05}$ for:																					
Genotype (G)		**NS**		**NS**				**NS**			**NS**			**1.26**			**NS**			**NS**	
Planting date (PD)		**0.2**		**22.93**				**1.35**			**7.32**			**0.5**			**6.8**			**5.53**	
C X PD		**NS**		**NS**				**NS**			**NS**			**1.79**			**NS**			**NS**	

DTE = Days to emergence, NLP = Number of leaves per plant, PH = Plant height, NFP = Number of flowers per plant, D50%F = Days to 50% flowering, NPP = Number of pods per plant and SWP = Seed weight per plant

Table 15. Eigenvector values for principal components using agronomic traits in early and late planting date.

	Early planting			Late planting		
Parameters	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Anther diameter	**0.463**	0.057	0.133	0.048	**0.462**	0.429
Anther length	0.005	**0.382**	0.364	0.061	**0.481**	-0.412
Days to 50% flowering	-0.193	**0.421**	-0.238	0.234	0.287	0.013
Days to emergence	-0.213	0.030	0.289	0.171	-0.146	-0.295
Number of flowers /plant	0.226	0.270	-0.383	**0.320**	0.153	-0.098
Number of leaves/plant	-0.243	-0.006	**-0.497**	**0.341**	-0.090	-0.152
Number of pods /plant	**0.403**	0.010	-0.278	**0.348**	-0.097	-0.049
Plant height	-0.130	0.439	-0.325	**0.320**	0.153	0.193
Pistil length	0.029	-0.437	-0.173	-0.291	**0.432**	-0.209
Stamen length	-0.053	-0.452	-0.210	**-0.318**	0.300	-0.241
Stigma–anther separation	**-0.419**	-0.051	-0.061	**-0.308**	-0.320	-0.089
Stigma diameter	-0.148	0.079	0.234	-0.195	0.085	**0.604**
Seed weight/plant	**0.462**	0.061	-0.021	**0.386**	0.031	0.111
Percentage variation	**30.410**	**25.420**	**14.710**	**47.740**	**12.770**	**12.450**

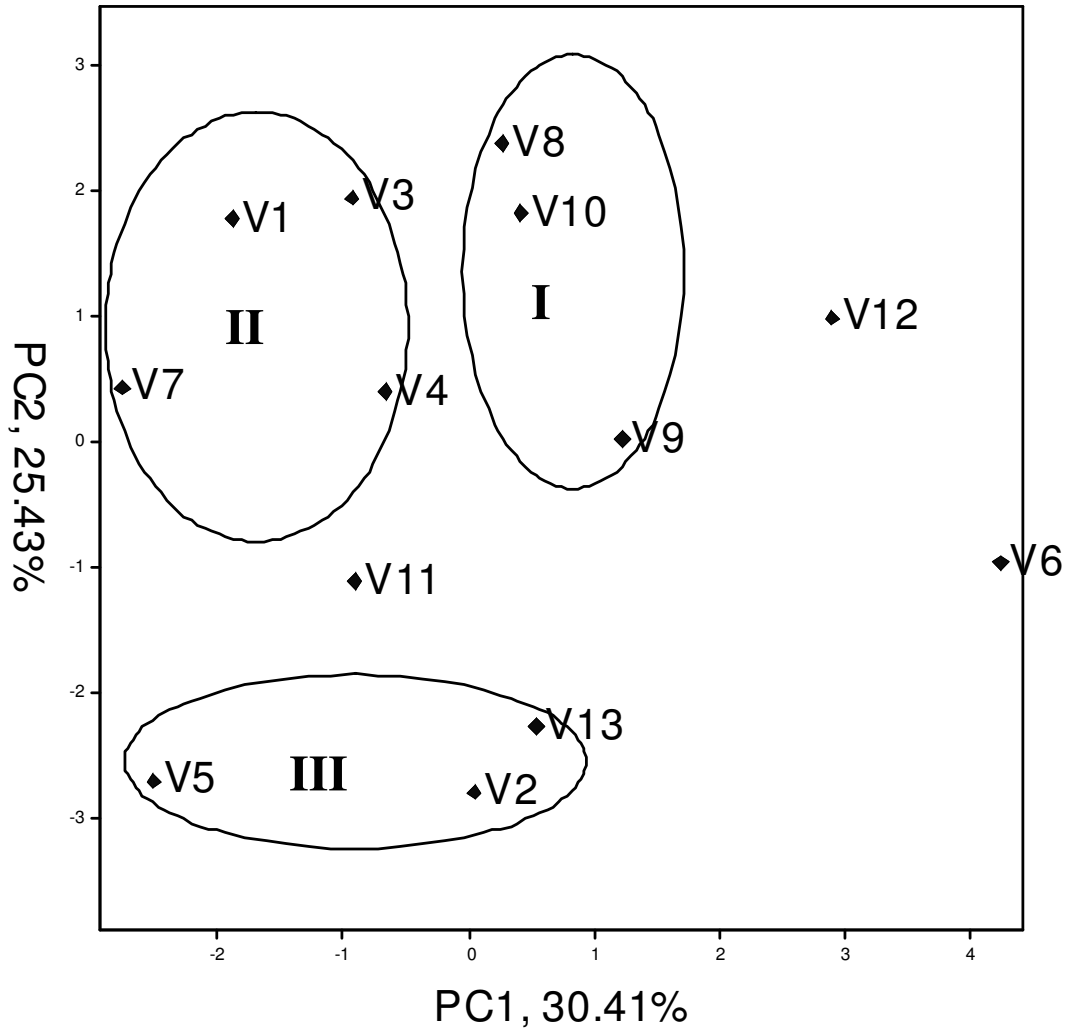

Figure 5. Scatter plot showing the clustering of the bambara groundnut genotypes during the early planting.

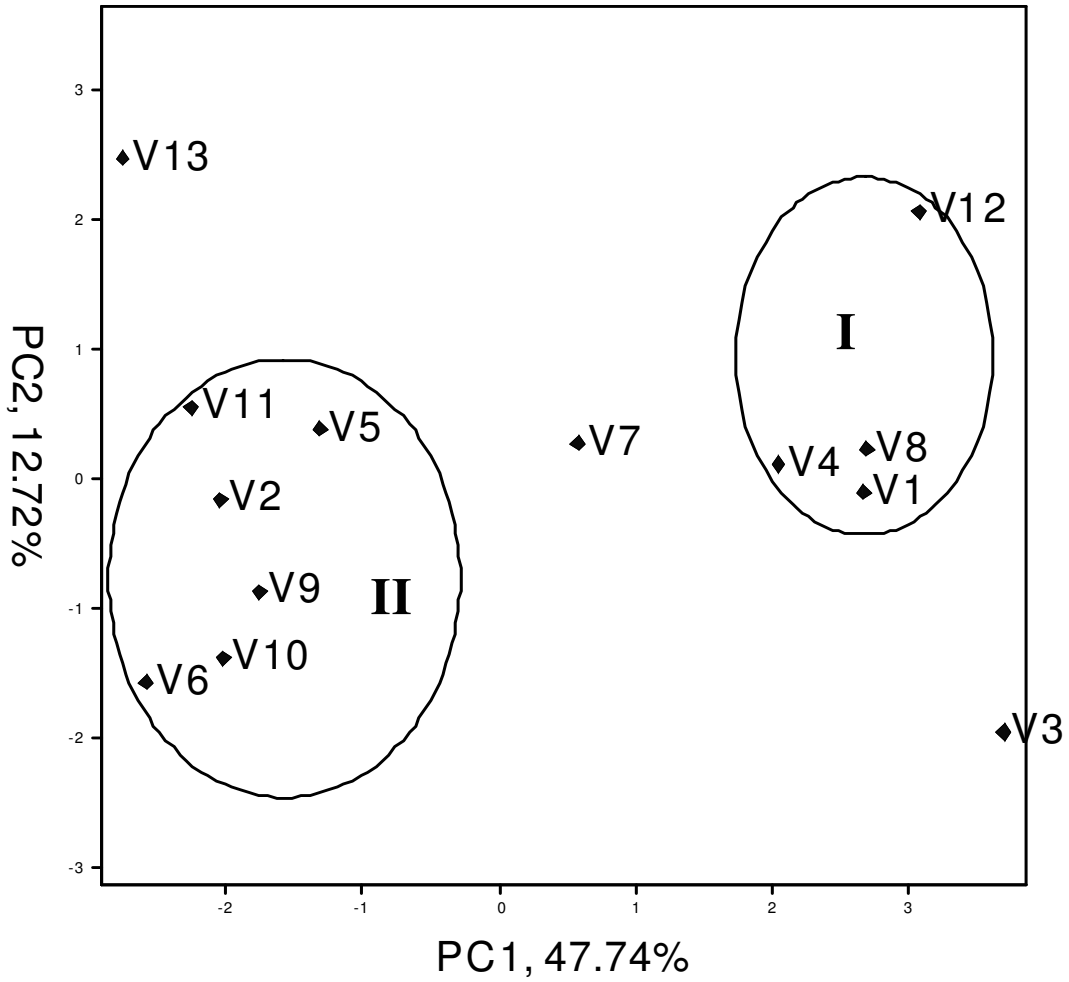

Figure 6. Scatter plot showing the clustering of the bambara groundnut genotypes during the late planting.

Table 16: Clusters means for eight traits in 13 bambara groundnt genotypes during the early and late planting.

	Clusters				
	Early planting			**Late planting**	
Traits	**I**	**II**	**III**	**I**	**II**
Stigma diameter	0.30	0.30	0.30	0.26	0.28
Anther diameter	**0.50**	**0.37**	0.42	**0.44**	0.39
Anther length	0.55	0.52	0.50	0.55	0.54
Pistil length	5.36	5.33	**5.83**	5.15	**5.33**
Stamen length	5.42	5.44	**6.10**	5.22	**5.52**
Stigma–anther separation	**0.06**	**0.11**	0.09	**0.07**	0.15
Days to emergence	7.00	8.00	7.67	9.00	8.83
Number of leaves/plant	166.67	**181.50**	166.33	**53.25**	32.50
Plant height	26.13	**26.75**	21.87	**14.25**	11.50
Number of flowers/plant	**64.33**	62.75	49.33	**22.25**	11.83
Days to 50% flowering	37.33	37.75	**33.33**	34.75	**33.00**
Number of pods/plant	**43.67**	39.00	39.00	**4.75**	1.33
Seed weight/plant	**19.90**	**12.73**	12.80	**1.92**	0.19

Correlation

The correlation coefficients (r) among the various floral, agronomic and yield traits are presented in Table 17. Seed weight per plant expressed highly significant positive correlation with anther diameter (r = 0.51**) and number of pods per plant (r = 0.87**). Stamen diameter, stamen length and pistil length had non-significant but positive correlations (r = 0.08, r = 0.26 and r = 0.35, respectively) with seed weight per plant. Anther length had negative and non-significant correlation with seed weight per plant (r = -0.348). However, seed weight per plant had significant negative correlation with stigma-anther separation (r = -0.61**). The number of pods per plant also had a significant positive correlation with anther diameter (r = 0.41*) and pistil length (r = 0.45*) and a significant negative correlation with stigma-anther separation (r = -0.48*) and anther length (r = -0.45). The number of leaves per plant has positive correlation with pistil length (r = 0.40) and stamen length (r = 0.40).

There was also highly significant but negative correlation of stigma-anther separation with anther diameter (r = -0.55**). Stigma-anther separation correlated positively with stigma diameter (r = 0.12). Anther length and pistil length had weak negative correlation values (r = -0.05 and r = -0.07, respectively) with stigma-anther separation. The results showed a strong but negative relationship between the pistil length and anther length (r = -046*) and a highly significant positive relationship between pistil length and stamen length (r = 0.94**).

Highly significant and positive correlations were found between seed weight per plant and number of leaves per plant (r = 0.53**), plant height (r = 0.70**), number of flowers per plant (r = 0.83**), and number of pods per plant (r = 0.87**). The relationships between seed weight per plant and days to 50% flowering (r = 0.32) was positive but non significant. The number of pods per plant correlated strongly with number of leaves per plant (r = 0.81**), plant height (r = 0.88**), number of flowers per plant (r = 0.93**) and days to 50% flowering (r = 0.49*). However, the results show that days to emergence had significant negative correlation with all the agronomic and floral traits measured except anther length (r = 0.459*) and stigma–anther separation (r = 0.27). The relationships between days to 50% flowering and number of leaves per plant (r = 0.64**), plant height (r = 0.77**) and number of flowers per plant (r = 0.65**) were positive and significant.

Table 17. Correlation coefficient between different floral/agronomic and yield traits.

	ST.D	AN.D	AN.L.	PT.L	SM.L.	SAS	NPP	SWP	DTE	NLP	PH	NFP	D50F
Stigma diameter (ST.D)	1												
Anther diameter (AN.D)	-0.088	1											
Anther length (AN.L.)	-0.017	0.054	1										
Pistil length (PT.L)	-0.067	0.087	-.456*	1									
Stamen length(SM.L)	-0.048	-0.068	-.524**	.943**	1								
Stigma-anther separation (SAS)	0.123	-.547**	-0.046	-0.072	0.062	1							
Number of pods/plant (NPP)	0.191	.407*	-.477*	.452*	0.346	-.454*	1						
Seed weight/plant (SWP)	0.075	.509**	-0.348	0.348	0.256	-.605**	.868**	1					
Days to emergence (DTE)	-0.143	-0.31	.459*	-.530**	-.425*	0.265	-.842**	-.677**	1				
Number of leaves/plant (NLP)	0.289	0.013	-.496**	.402*	.399*	-0.247	.811**	.528**	-.732**	1			
Plant height (PH)	0.354	0.179	-0.369	0.364	0.285	-0.375	.877**	.702**	-.789**	.897**	1		
Number of flowers/plant (NFP)	0.283	0.258	-.423*	0.385	0.316	-.487*	.928**	.827**	-.769**	.847**	.951**	1	
Days to 50% flowering (D50F)	0.343	0.017	-0.041	0.096	0.001	-0.186	.486*	0.317	-.491*	.635**	.768**	.654**	1

DISCUSSION

The observed cap-like opercula or bract which covered the anther and stigma is the natural mechanism the crop has to ensure adequate protection of the male and female flowers from harsh weather conditions. The position of the stigma in relation to the anthers as observed suggests the existence of inserted stigma (stigma shorter than the longest anther) in bambara groundnut. This indicates the occurrence of homostyly, which provides an elegant explanation for the self-compatibility system in bambara groundnut (Doku, 1968; Free, 1993; Roubik, 1995). This type of floral arrangement makes autogamy possible (*i.e.* increasing the possibility for natural self- pollination), through the contact between the stigma and the anthers. This could explain why bambara groundnut is an inbreeder.

Artificial germination of pollen grains is a reliable test of pollen fertility. The shelf life of pollen grains is 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. Both pollen germination and pollen tube growth are influenced by the environment. Temperature and other factors like humidity and 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 the genotype 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) reported that pollen viability is species dependent and in many partially hydrated pollen, dehydration leads to rapid loss of viability. Stanley and Linskens (1974) observed that pollen grains 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. The pollen germination dropped rapidly with delay in incubation. 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. The failure of artificial hybridization in bambara groundnut could therefore be attributable to this very short life span of pollen grains. The proximity and the position of the stigma below the anther would enforce self pollination and fertilization. The nearness of both structures suggests minimal time interval between the shedding of pollen and its arrival on the stigmatic surface. 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 minutes have been reported to have caused between 30 to 70% decrease in the pollen germination (Pallais *et al.,* 1988). Ayalor (2003 and 2004) reported a continuous loss in pollen germination of *Zea 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) reported that the trinucleate pollen grains are very difficult to germinate *in vitro* and when germinated, germination is at a much lower percentage. The above characteristics were observed in the bambara groundnut pollen grains. In genotypes 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 *O. sativa* (Song *et al.,* 2001); *Arum italicum* and *A. 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 consequence of its exceptionally low water content at anthesis. Loss of water leads to irreversible changes in the pollen membranes (Shivanna and Heslop-Harrison, 1981 and van Bilsen *et al.,* 1994) and partially hydrated pollens remain viable for a very short period (Bots and Muriani, 2005). Pollen longevity according to Shivanna and Johri (1985) varies significantly from species to species, ranging from minutes after shedding to months under laboratory condition.

The observed differences in pollen tube growth in the present study is a reflection of genotype variability. Genotype differences have been implicated in the pollen tube growth of many crops such as; Strawberry (*Fragaria annanassa*) (Bots and Muriani, 2005); Cherry (*Prunus avium* L.) (Hedhly *et al.*, 2004) and Cotton *(Gossypium hirsutum)* (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 trait, as the tube would reach and transmit the male nucleus into the embrayo sac at a faster rate thereby facilitating fertilization. This will make for 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 are viable and 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 pollination and rapid fertilization. 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 before it dies.

Pollen tube growth of bambara groundnut is nonlinear. The pollen tube growth models revealed that the logistic equation provided the highest R^2 value for the pollen tube growth prediction. Athough logistic growth model performed slightly better than gompertz and exponential growth 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 prediction of the bambara groundnut pollen tube growth. This is because the three models have an acceptable coefficients of determination (R^2). The result shows that the pollen tube growth rate presented sigmoid growth curves with the exponential, linear, lag and stationary phases at both planting dates. The rapid growth phases (exponential and linear phases) occurred during the first 40 minutes 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 40 and 60 minutes of pollen tube incubation. The stationary phase

of pollen tube occurred between the 60 and 80 minutes. 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 genotype pollen variability in response to the pollen exposure durations in early and late plantings, respectively. The PC1 vectors indicated that genotypes that loaded highly for 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 basic tool to identify high pollen survival in bambara groundnut genotypes. The cluster analysis divided the cultivars into three and four distinct groups in the early and the late planting, respectively. In the early planting, Bg-01 was classified as moderate pollen survival genotype; Bg-02, Bg-03, Bg-04, Bg-05, Bg-06, Bg-07, Bg-10 and Bg-13 as low pollen survival genotypes and, Bg-08, Bg-09, Bg-11 and Bg-12 as poor pollen survival genotypes. At late planting, Bg-04 and Bg-07 was classified as high pollen survival genotypes; Bg-03, Bg-08 and Bg-12 as moderate pollen survival genotypes; Bg-02, Bg-05, Bg-06, Bg-09, Bg-10, Bg-11 and Bg-13 as low pollen survival genotypes and Bg-01as low pollen survival genotype.

It is evident that bambara groundnut exhibits a considerable variation with respect to the anther diameter, style and stamen lengths. Although part of this variation can be attributed to ontogenic changes in the flower traits, some of the variations could be associated with the environmental changes at both planting dates. These floral traits are quantitative traits which are under the continuous influence of the environment. The signifficant environmental effect on floral traits have also been reported on crops such as *Brassica rapa* (Williams and Corner, 2001; Yoshioka *et al.*, 2004). Similar result has also been reported in the invasive weed, *Lythrum salicaria* (Mal and Lovett-doust, 2005). Some floral traits were significantly affected by the interactions of genotypes and planting dates indicating the a significant effect of the environment on the gentypes. Similar report has been reported in pepper (PereraO and Poulos , 1993). The marginal stigma-anther separation observed among the cultivars was not

only to ensure autodeposition efficiency and inbreeding success but also to reduce the chances of outcrossing

The reduced stigma–anther separation in the genotypes may provide an opportunity for reproductive assurance when ‘‘legitimate’’ mating partners (or pollinators) are not available. The distance between anther and stigma often affects the selfing efficiency (Jacquemart and Thompson, 1996; Karron *et al*., 1997; Affre and Thompson, 1998; Pailler *et al.,* 1998). The inherent inbreeding behaviour of bambara groundnut (Massawe *et al*., 2003) is therefore largely attributed to the highly reduced stigma-anther separation, which facilitates the natural transfer of pollen to the stigma within the flower. Moreover, the ease of transfer of the pollen grains to the stigmatic surface favours fruit set. Thus, reduced stigma-anther separation as obtained in the present study is advantageous for increased pod and seed yield.

The differences observed in days to 50% flowering could be attributed to the varietal characteristics and germination date. Or *et al.* (1999) reported that flowering in bambara groundnut is species dependent and depends on germination date, the seasonal temperature profile as well as the photothermal response of the plant.

The vegetative growth of the bambara groundnut genotypes was higher in the early planting than in the late planting, apparently due to the slightly warmer temperatures. All genotypes have shorter vegetative growth in late planting showing that the crops' growth requirements that support growth were inadequate. The slightly lower temperatures and lower rainfall of September through October to November initiated less biomass and vegetative growth in the late planting, which resulted in reduced plant height. This is in aggrement with the previous research reports on the crop (Massawe *et al*., 2003) and Peanut lines (Banterng *et al.,* 2003). Frimpong (2004) reported that plant height was significantly affected by planting date and environmental factors, which is similar to the present findings. The higher number of leaves obtained in the early planting could be attributed to the higher temperatures recorded during the period. This finding is in line with the earlier report obtained by Azam-Ali and Squire (2002) and Brink (1999). Increase in number of leaves per plant with increasing temperature between 20 ^{0}C to 32 ^{0}C has been reported in wheat (Slafer and Rawson, 1997). The genotypes were exposed to shorter photoperiods in late planting and this could explain why flowering was earlier in most of the genotypes. The report of Nishitani *et al.,* (1988) showed a delay in flowering of 6-11 days when plants were grown under continuous light, compared

to plants grown under a normal day and night rhythm. Mkandawire (2007) also reported that bambara groudnuts are typically short day plants and that flowering is delayed under long days.

The early planting date resulted in a significant yield advantage over the late planting dates. Much of this advantage could be linked to the higher vegetative growth, which resulted in the improvement of several agronomical characters that contributes to yield. The late planting date did not allow high pod production in bambara groundnut, because less number of flowers per plant was produced during the period. The number of pods per plant of all the genotypes was less in harvest at late planting which was in line with the earlier report on groundnut, *Arachis hypogaea* L., by Laurence (1983); Gardner and Auma (2003) and Çalışkan *et al.,* (2008).

This research showed that early planting produced greater seed yields when compared to the late planting. In groundnut, plant growth and pod-filling are mostly affected by precipitation and temperature during the late planting (Mozingo *et al.,* 1991; Lazarini *et al.,* 1998; Kasaı *et al.,* 1999; Jordan *et al.,* 2003; Naab *et al.,* 2004). The seed yield produced was about 92.0 % more in the early planting than in the late planting. Collinson *et al*. (1999) reported that 30 days delay in sowing caused more than 60% reduction in pod yield at Dodoma, Tanzania, and a further 30 days delay resulted in no pods at all. Similarly, Canavar and Mustafa-Ali (2008) also reported that early sown groundnut *(Arachis hypogaea L.)* under rainfed conditions gave substantially greater yield during the early planting when compared with the late planting. Soybean seed yield decreased by 33% when sowing was delayed (Beaver and Johnson, 1981) from May to July and 50% when delayed from April to July (Beatty *et al.*, 1982). The reduction in yield was associated with reduction in the some of the agronomic and vegetative traits. The substantial crop yield reduction observed in the late planting is also in line with earlier report on groundnut (Muldoon, 2002). These differences were largely related to the number of developing pegs and time of pod filling which was less in the late planting. Similar findings have been reported in groundnut *(Arachis hypogaea* L.) by Çalışkan *et al.* (2008). Arıoğlu *et al.* (2001) reported that the seed weight of groundnut (*Arachis hypogaea* L.) was affected by environmental conditions, which is consistent with the present findings. Zhang (2006) also reported that later plantings (after June) of soybeans resulted in significantly fewer flowers, pods and seeds per plant and signifficantily lower yield. Date of planting is therefore, found to be the most important factor affecting yield and some other traits.

Information obtained through principal component analysis (PCA) may assist the plant breeders to indentify a limited number of highly differentiated population for use in hybridization and selection programs. The principal components obtained during the early planting differ from those of the late planting indicating some degree of interaction of the genotypes with the environmental factors. The PC1 and PC2 obtained for the agronomic and floral traits indicated that traits such as anther diameter, anther length, stigma–anther separation, stamen length, pistil length, number of leaves per plant, plant height, days to 50% flowering, number of flowers per plant, number of pods per plant and seed weight per plant were the characters for distinguishing the cultivars. Anther diameter, anther length, stigma–anther separation, stamen length and pistil length have been the principal source of taxonomic characteritization in some vegetable crops (Chweya, 1997) and in *Narcissus cyclamineus* (*Amaryllidaceae*) (Asier *et al.,* 2009). The variability of some bambara groundnut cultivars have been described based on the some of the above agro-morphological (Begemann, 1988; Collinson *et al.,* 1997; Mkandwire, 2007; INCO-DC, 2007). The principal component analysis for the early and late planting revealed that the principal components I and II for anther diameter, anther length, pistil length, number of leaves per plant, plant height, days to 50% flowering, number of flowers per plant, number of pods per plant, and seed weight per plant were positive. Such results indicated that the 10 traits had significant contribution towards diversity. The greater diversity in the present materials is due to the 10 characters which will offer a good scope for improvement of the seed yield through rational selection of the parent genotypes. It could be concluded that the members of the cluster I genotypes at both planting dates and, genotypes Bg-03, Bg-06, Bg-12 are promising in number of pods and seed weight per plant and therefore recommended for selection for further hybridization program.

Correlation coefficient helps the breeder to select an efficient traits. The stigma-anther separation had significant but negative correlation with number of pods per plant (r = -0.45*), seed weight per plant (r = -0.61**) and anther diameter (r = -0.55**). The negative correlation obtained indicated inverse relationship between the stigma-anther separation and the above three traits. Spencer *et al.* (1987) reported a large negative correlation between

stigma-anther separation and seed set (r = - 0.87) in *Turnera ulmifolia* complex. Plants with reduced separation between stigma and anther appear to produce higher number of pods and seed weight than those with wider separations. One possible explanation could be that lesser degree of herkogamy increases rates of pollination with the plant's own pollen and produces substantially higher number of pods and seed yield. Herkogamy has been deemed a mechanism for reducing self-fecundity (Barrett, 2002). A strong negative link has been reported between the degree of herkogamy and out-crossing rates (Holtsford and Ellstrand 1992; Belaoussoff and Shore 1995; Karron *et al.,* 1997).

A positive correlation shows that the changes of two variables are in the same direction, i.e., high values of one variable are associated with high values of the other. The positive and significant relationship observed for anther diameter with number of pods per plant and seed weight per plant means that increase in anther diameter increases the number of pods and seed yield per plant. This indicates that selection of crops with large anthers is a reliable strategy for pod and seed yield improvement in bambara groundnut. It is therefore very probable that bambara groundnut lines with larger anthers produce higher number of pollen grains for enhanced pollination and seed set. The positive, though non-significant relationship observed between stigma diameter and number of pods per plant indicates that the larger the stigma diameter the higher the number of pods produced. Large stigma diameter provides a larger receptive surface area for pollen deposition. Webb and Lloyd (1986) reported that large receptive area of the stigma is an advantage as it is able to capture higher number of pollen grains and therefore, enhance the pollination, fertilization and seed set in crops. Similarly, Vasudeva *et al.* (2004) had also reported strong positive correlation between stigma diameter and average number of pollen captured which had resulted in higher seed yield in teak (*Tectona grandis* Linn. F). The pistil length had highly significant positive correlation (r = 0.94**) with stamen length indicating that both traits could be increased simultaneously. The significant negative correlation between the pistil length and anther length obtained in this research agrees with the earlier report in teak, *Tectona grandis* Linn. F (Vaudeva *et al.,* 2004).

Seed weight per plant was positively correlated with number of leaves per plant, number of flowers per plant, number of pods per plant and plant height which illustrated that higher mean values for these traits can increase the seed weight per plant. Positive correlation of seed weight per plant with days to 50% flowering showed that delayed flowering in the crop

would increase the seed yield. Similar result have been reported in soybean (*Glycine max* L.) by Sandhu and Singh (1972), Jagtap and Choudhary (1993), Malik *et al*., (2006) and Farshadfar and Farshader (2008). The correlation between the seed weight per plant and number number of pods per plant was strongly significant and positive which agrees with the results obtained in crop such as Lentil, *Lens culinaris* Medik (Manora and Manara, 1988; Salehi *et al.,* 2008), Mungbean (Shah *et al*., 1993) and Chickpea, *Cicer arientinum* L. (Farshadfar and Farshader, 2008). The positive and significant association of seed weight per plant with number of pods per plant revealed that increase in number of pods per plant would contribute to higher seed weight per plant. Hence, for effective yield improvement in bambara groundnut, selection should be made for higher values of number of pods per plant. The significantly positive correlations of days to 50% flowering with number of leaves per plant, number of flowers per plant, number of pods per plant and plant height indicated that the above traits were largely controlled by genes that govern days to 50% flowering. Bizeti *et al.* (2004) earlier report on soybean showed that days to 50% flowering were positively associated with number of pods per plant. The negative but significant correlation of days to emergence observed with number of leaves per plant, number of flowers per plant, number of pods per plant, and plant height and seed weight per plant indicated that early emergence in the crop would lead to a significant increase in the above agronomic traits. The negative and significant association of days to emergence with number of pods per plant and seed weight per plant gave an understanding that genotypes with shorter days to emergence (early emerging genoypes) would give a significant increase in the number of pods and seed weight as compared to the late emerging genotypes. The present results are confirmed by the earlier research findings on garden peas, *Pisum sativum* L. (Ramesh *et al.,* 2002, Chaudhary and Sharma, 2003, Kumar *et al.,* 2003, Kumar and Sharma, 2006 and Nawab *et al.*, 2008).

CONCLUSION

Prolonged exposure of pollen grains to ambient weather conditions markedly reduced the pollen germination and pollen tube growth. 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. The floral, agronomic and yield traits of bambara groundnut genotypes were affected by planting date. Pod number and seed yield are the most important yield components associated with the final yields. The results of the present study show that stigma–anther separation and anther diameter are important in determining the level of pollination success and subsequent pod yield in bambara groundnut. The present study also suggests a strong correlation between seed weight per plant and number of leaves per plant, number of flowers per plant, number of pods per plant and plant height. These traits are major yield contributing traits that could be emphasized in any selection programme aimed at seed yield improvement. Early planting had more number of pods per plant and higher seed weight per plant when compared with the late planting. For optimal pod yield therefore, farmers should plant at the on set of the rains in the derived savanna ecological zone of Nigeria.

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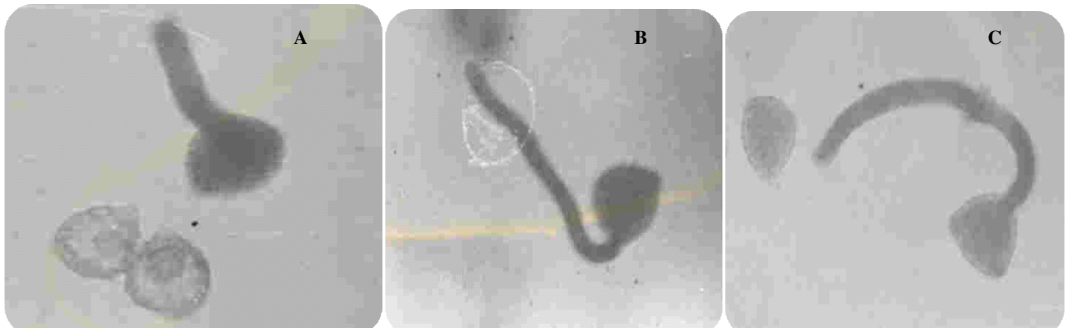
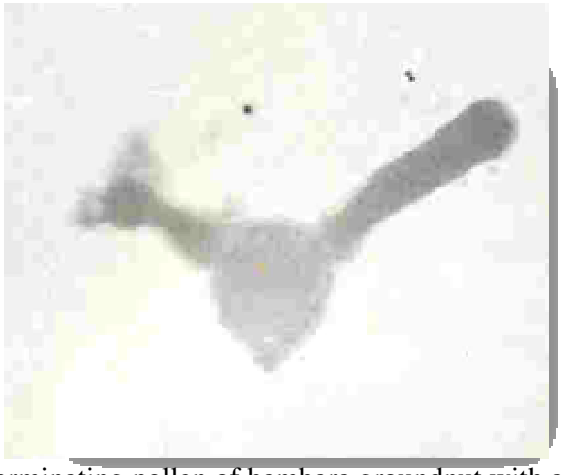
APPENDIX I



Pollen of bambara groundnut germinated in a medium containing 10g succrose, 100 mg/L boric acid and 300 mg/L calcium nitrate made up to 100 ml with deionized water. Pictures were taken from a Motican 1000 digital microscope camera after **(A)** 20 minutes, **(B)** 40 minutes and **(C)** 60 minutes of pollen incubation.



Germinating pollen of bambara groundnut with a bifurcated pollen tubes

APPENDIX II

Calibration of the ocular micrometer:
Ocular micrometers are calibrated by comparing the ocular micrometer scale with a calibrated stage micrometer. The stage micrometer is a microscopic slide that has a carefully calibrated scale which is divided into 0.1 mm and 0.01 mm units.

Procedure:

1. Install the X 10 ocular containing the ocular micrometer disc in the microscope.

2. Place the calibrated stage micrometer slide on the stage and focus on the scale.

3. Adjust the field so that the zero line of the ocular disc scale is exactly superimposed upon the zero line of the stage micrometer scale.

4. Without moving the stage micrometer, locate the point as far to the extreme right as possible where any two lines are exactly superimposed upon each other.

5. Count the number of divisions (mm) on the stage micrometer between the zero line and the superimposed line to the far right.

6. Count the number of ocular divisions between the zero line and the superimposed line to the far right.

7. Divide the distance determined in step 5 by the number of ocular divisions in step 6 and multiply by 1000 to give the ocular micrometer unit in µm.

$$\frac{\text{Stage micrometer divisions (mm)}}{\text{Ocular micrometer}} \times \frac{1000\ \mu m}{mm} = \mu m \text{ per ocular unit}$$

8. Repeat step 1 through 7 for each objective on the microscope to obtain the µm per ocular unit.