

Pollen–style compatibility in cashew (*Anacardium occidentale* L.)

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Abstract The role of pollen–stigma interaction and pollen tube growth as a measure of genetic compatibility was studied in eleven clones of cashew under a tropical rainforest ecology in Southwestern Nigeria between 1999 and 2002. Diallel cross of the clones showed that 55.00% of the cross-pollinated crosses showed high pollen cross-compatibility (CC) indicating moderate genetic distance in the population. About 54.55% of the clones showed evidence of self-compatibility, therefore confirming that cashew is self-fertile. Cytological examination however suggests possible postzygotic self-incompatibility in the crop as no evidence of tube growth inhibition was recorded in both cross- and self-pollinated styles. Correlation between maternal compatibility (pollen recipients) and fruit set shows that more attention need to be focused in this direction during selection. Based on the data from this study, the use of combinations of floral quality characteristics like high pollen fertility, prolific flowering, and high flower sex ratio plus high cross-compatibility as

reliable selection criteria for cashew yield improvement is therefore suggested.

Keywords Cashew *Anacardium occidentale* · Pollen–stigma reaction · Pollen-tube growth · Self-compatibility · Cross-compatibility

Introduction

Cashew, *Anacardium occidentale* L. (Anacardiaceae), is a hardy, drought-resistant nut tree crop that is widely distributed in the tropical countries like America, Mexico, West Indies, Brazil, Peru, Africa and Asia (Johnson 1973; Ohler 1979; Nakasone and Paull 1998). It has adapted to a wide range of ecologies, ranging from tropical wet forest to tropical dry savannah zones and can tolerate annual precipitation of 7–42 dm, annual temperature of 21–28°C, and pH of 4.3–8.7 (Nambiar and Haridasan 1979). The crop was introduced into Africa by the Portuguese explorers in the 16th Century (Johnson 1973; Ohler 1979); and around the same time the crop arrived at Agege, Lagos in Nigeria (Woodroof 1979). Major nut producing countries include India, Brazil, Nigeria, Mozambique and Tanzania. In 2004, nut production in Nigeria was 213,000 mt (FAO 2004). Reports however showed that although the area of land cultivated to cashew in Nigeria increase by 6,600 ha annually since 2000, but yield per hectare

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still stands between 0.63 and 0.66 tons in 2000 and 2004, respectively (Topper et al. 2001; Aliyu 2004; FAO 2004). While demand for cashew nut is increasing in the global market, its availability is often a limiting factor for several reasons (Holanda-Neto et al. 2002). Studies on the yield in Tanzania and Nigeria have shown that about 30% of the tree population in an hectare produced 80% of the nut yield, while the remaining 20% yield comes from the 70% of the trees (Martin and Kasuga 1995; Aliyu 2004). This trend showed evidence of low yield confronting teeming cashew farmers in the continent and would require a thorough investigation into breeding system of the plant with respect to pollen–stigma compatibility and fertilization.

However, reproductive barriers are important breeding concerns in many fruit and nut cultivars, where fruit set and nut filling require efficient and predictable fertilization. The past decade has witnessed a rapid increase in the understanding of the molecular basis of incompatibility with aspect of gametophytic self-incompatibility widely reported in plant species (Wiersma 2003). Waser and Price (1991) however opined that incompatibility acting at prezygotic stages is the most efficient reproductive barriers because it saves ovules from being “usurped” by kin. The genetic recognition of pistil and pollen at prezygotic stages of reproductive systems has been reported in both gametophytic and sporophytic incompatibilities and therefore very important in the study of pollen–style compatibility in plant species (Wunnachit et al. 1992a). Style glycoproteins, a product of S-gene has been reported in the inhibition of tube growth in incompatible pollens of *Nicotiana glauca* (Jahnen et al. 1989).

Ohler (1979) and Wunnachit et al. (1992a) described cashew has an andromonoecious cross-pollinated flowering plant with spatial separation of sexes and production of hermaphrodite and male (staminate) flowers on the same flowering panicles. The crop has been reported with a wide range of features that promote out-crossing and thus reducing the loss of fitness attributed to inbreeding (Sedgley and Griffin 1989). The out-crossing nature has consequently results into a wide variability in nut yield in the crop. This out-breeding phenomenon makes a choice of compatible partners very crucial for efficient fertilization and fruiting in cashew. Understanding of this factor of mating compatibility

in terms of pollen–style interaction and pollen tube growth has important practical application to both genetic improvement and operational nut production in cashew industry. Unfortunately, very little is known about this compatibility factor in cashew thus hindering progress in its improvement.

This study therefore aim at understanding the genetic compatibility and breeding system in cashew with respect to pollen–style interaction and pollen tube growth with a view to unravel one among other limiting factors affecting crop yield in the species.

Materials and methods

This study was carried out at the Cocoa Research Institute of Nigeria (CRIN), Ibadan, South-western Nigeria between November 1999 and April, 2002. The Institute is located at Latitude of 07°10' N and Longitude 03°52' E with bimodal rainfall pattern with mean annual rainfall of 1,325 mm and a mean annual temperatures of 27°C. The rainy season is from April to October, and the dry season is from November to March. The monthly summary of the climatic conditions during the period of study is shown in Table 1. The site comprise of deep loamy soil with very high fertility. The soil is rich in organic matter and comprising sand, silt, clay and gravel of 834, 94, and 72 g/kg⁻¹, respectively (Salako et al. 1999). The soil has a pH of 6.6, available phosphorus of 30.61 ppm, 0.35% total nitrogen and 1.46% organic carbon. The exchangeable Ca, Mg, Na, K, and acidity are 0.19, 0.14, 0.58, 0.28, and 1.2 (Meq/100 g), respectively. Eleven cashew clones selected from Farmers' field in Ochaja area of Kogi State, North-central region of Nigeria were used for this investigation. Each clone is represented by three individuals vegetatively propagated by air-layering in 1988 and planted in a single row at spacing of 9.1 × 9.1 m² at the Institute's research plot. The clones are regular medium flowering and mid-season fruiting biotypes with characteristic upright-open canopy tree habit. The trees are generally high yielding varieties with mean nut per tree of 1994 and produce small to medium nut size with mean nut weight of 6.64 g (Aliyu 2004; Aliyu and Awopetu 2007). Full morphological description of the Institute accessions including these clones had been reported in Aliyu and Awopetu (2007). The materials are the progenies of early Indian

Table 1 Average weather records (temperature, rainfall and relative humidity) for the experimental location between 1999 and 2002

Month	Rainfall (mm)				Temperature (°C)				Relative humidity (%)							
	1999	2000	2001	2002	1999	2000	2001	2002	1999		2000		2001		2002	
									M	A	M	A	M	A	M	A
January	0.00	19.5	0.00	0.0	26	26	27	26	78	55	86	68	88	75.7	64	37
February	106.7	0.4	0.4	0.0	27	27	28	28	75	48	56	34	59.8	36.8	78	63
March	74.6	72.7	34.0	43.6	29	29	29	30	84	67	72	39	78	47	86	87
April	113.1	83.5	177.6	150.5	28	28	28	29	81	66	80	67	80.4	61.3	82	73
May	128.4	68.1	224.9	121.0	28	28	28	29	81	71	80	75	81.4	63.5	82	68
June	242.5	321.0	186.1	141.8	27	26	27	28	85	76	87	73	82.8	70.4	82	73
July	242.9	138.2	117.0	314.0	26	25	26	27	76	90	73	75	86	73	89	83
August	184.9	170.0	26.5	103.6	26	25	25	26	88	75	89	80	89	88	90	86
September	163.6	158.6	205.1	249.1	26	26	26	26	89	79	88	80	87	80	89	83
October	255.8	133.3	74.1	185.3	27	27	27	27	81	74	79	72	84	71	87	77
November	23.9	0.0	8.4	39.6	28	28	29	26	83	69	68	55	80	59	84	64
December	4.5	0.0	0.0	0.0	26	26	28	27	80	59	79	59	81	52	76	54
Mean	140.0	117.0	105.0	150.0	27	27	27	27	81.75	69.08	78.08	64.83	81.45	64.81	82.42	70.67

Source: Meteorological Units of the Cocoa Research Institute of Nigeria, Ibadan, Nigeria. M: Morning, A: Afternoon

Introductions in the country, which have been grown and adapted over a long planting period. A full diallel cross among the 11 clones was carried out using randomized complete block design experimental layout with tree canopy marked on the four cardinal positions (north, south, west and east) using a compass (Masawe et al. 1996; Aliyu and Awopetu 2003). There were sixty pollinations for each kind of cross (i.e. 15 pollinations/cross/side) and this was replicated thrice. The trial started in 1999/2002 and was repeated in 200/2001 and 2001/2002. Flower buds were enclosed in pollination bags one day before anthesis. Cheese cloth was found more satisfactory than brown Kraft paper. Controlled hand-pollination was carried out between 9.30 h and 11.30 h with pollens for self-pollination taken from same flower and for cross-pollination from mix derived from other clone.

To study in vivo pollen tube growth, pollinated styles were removed from the plants between 4 h and 24 h after pollination, fixed in FAA (1:1:8 v/v/v formalin: glacial acetic acid: 80% ethanol) and stored at 4°C (Erazzu et al. 1999). Styles from the crosses were rinsed with two changes of tap water, and then treated with 8 N NaOH solution for 4 h. The styles were rinsed again in two changes of tap water, stained

with 0.1% aniline blue solution in 0.1 M Potassium phosphate, mounted on a drop of glycerol on a glass slide, and gently squashed with a cover slip for 30 min (Bermawie and Pickergill 1992). The preparations were then examined under the microscope. During examination, the pistil was divided to three; stigma surface region, within style and the stylar base in order to study the pollen tube growth. Compatible clones were those with pollen tube growth found at the stylar base 24 h after pollination and photomicrographs of the pollen tubes growth within the style were taken.

However, pollinated flowers for pollen–stigma interaction were harvested between 4 h and 6 h after pollination and examined under stereomicroscope to score number of pollen tetrads adhering to the stigmatic papilla. Pollen viability was indirectly determined by staining with Alexander's staining solution and in vitro germination on 5% sucrose solution (Aliyu et al. 2001). Pollen grain size was determined under the microscope by measuring with a calibrated ocular eye-piece graticle. Records on floral characters like number of male and hermaphrodite flowers were carried out daily between 10.30 h and 12.30 h during the flowering period (September–January) following the procedure described by

Damodaran (1966) and Masawe et al. (1996) and also reported in Aliyu and Awopetu (2003). The study was carried out in three cashew production seasons (1999/2000–2001/2002). Although flowering commences as early as September, study on pollination and compatibility is often delayed till the peak of flowering between October and January in the region (Akinwale 1992; Aliyu and Awopetu 2003).

Results

The selected cashew clones showed high variation in their floral attributes and yield (Table 2). The number of staminate flowers ranged from 121.11 to 461.91 in CC09 and CC02, respectively. These two clones also had the least (12.55) and highest (128.17) number of hermaphrodite flowers, respectively. The ratio of the hermaphrodite to staminate flowers ranged between 10% and 28%. In terms of pollen fertility, CC06 produced the best fertile pollen with 98.94% and 44.89% for pollen stainability and in vitro germination, respectively. The least pollen fertility was recorded in CC09 with 53.77% and 23.30% pollen stainability and in vitro germination. Largest pollen grain size of 56.60 μm was recorded for CC01, while clone CC11 produced the smallest pollen grain (40.42 μm). Number of nuts per tree ranged between 718 for CC09 and 3,021 for CC06. Average of 6.16 was recorded for clustering of fruits per panicle with range between 3.34 and 9.75.

Compatibility status was adjudged by the cytological examinations of pollen tube growth as shown in Figs. 1, 2, 3 and 4. While Figs. 1–3 showed pollen tube germinating at different lengths (upper, middle and within ovule) of the style after 12, 16, and 24 h of pollination, Fig. 4 is an evidence of pollinated style without tube growth. The result of pollen–stigma adherence is presented in Table 3, while the in vivo pollen tube germination data were summarized in Table 4. The respective percentage values were presented in italics in both Tables. Average of 11,388 (52.29%) pollens was found adhered to the stigmatic surface and 8,890 (40.82%) pollens germinated on the styles. This indicates that not all the pollen grains that adhered to the stigma at pollination eventually germinated or could effect fertilization. Highest pollen adherence of 93% and 90% germination were recorded in the crosses between CC05 $\times$ CC06, suggesting very high pollen–style compatibility among these clones. Clones CC07 and CC05 showed some degree of self-incompatibility with their respective selfed crosses thus showing no evidence of germination. The potentials of the selected clones as either pollen or mother parents in their respective crosses were summarized in the mean values presented in Tables 3 and 4. However, to avoid repetition, the mean values of in vivo pollen germination in Table 4 will be emphasize in this text as it show similar trend with pollen–stigma adherence. Pollen germination ranging from 0% to 90% was recorded among the crosses. Crosses involving

Table 2 Floral and yield component characters of the 11 cashew clones

Clones	Male flowers	Hermaphrodite flowers	Flower sex ratio	Pollen stainability (%)	In vitro pollen germinability (%)	Pollen grain size (μm)	NNT	NNP	NWT (g)
CC01	134.46	15.03	0.11	66.07	39.00	56.50	980	3.82	8.55
CC02	461.91	128.17	0.28	94.66	42.30	52.64	2,375	8.25	6.29
CC03	274.58	61.44	0.22	82.44	43.20	53.10	2,380	9.75	5.75
CC04	168.21	20.25	0.12	67.69	41.17	52.40	1,179	3.60	7.21
CC05	350.68	84.36	0.24	97.74	41.30	53.50	2,059	8.80	6.10
CC06	454.07	109.33	0.24	98.94	44.89	52.08	3,021	9.02	7.05
CC07	247.00	46.18	0.19	78.20	40.79	52.58	1,211	6.84	7.23
CC08	152.02	15.95	0.11	56.84	29.98	51.45	1,115	4.26	6.98
CC09	121.11	12.55	0.10	53.77	23.30	53.95	718	3.34	8.14
CC10	160.90	18.50	0.11	67.20	28.54	50.45	1,071	4.68	6.60
CC11	148.98	15.50	0.10	60.75	39.40	40.42	1,020	5.40	2.88
Mean	243.08	47.93	0.17	74.94	37.62	51.73	1,557	6.16	6.35

NNT: Number of nuts/tree/year, NNP: Nuts/panicle, NWT: Average weight of individual nut

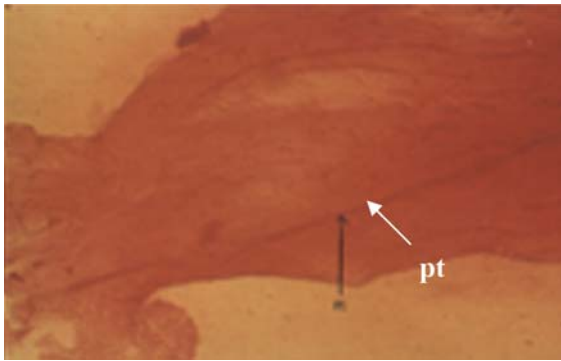


Fig. 1 Pollinated style of cross-compatible clones showing pollen tubes growth from the stigmatic position and within upper part of the style 12 h after pollination. Mag. $\times 400$. pt: pollen tube growth



Fig. 2 Pollen tube growth within the styler tissue of compatible clones observed 16 h after pollination. Mag. $\times 400$. pt: pollen tube growth

CC06 with average of 57% germination showed the highest pollen compatibility, while CC11 had the least germination with 30% as pollen parents. Based on the mean values of *in vivo* pollen germination, seven clones (63.64%) showed evidence of high cross-compatibility as pollen parents with average pollen germination $\geq 40\%$, while the remaining 36.36% gave a picture of partial compatibility as pollen parents. However, as female parents, 54.55% of the clones (6) showed high cross-compatibility with highest and least mean germinations of 61.29% and 28.76% recorded in CC06 and CC09, respectively. The coefficient of variations obtained in this study showed the extent of variability of each selection for pollen compatibility when crossed with

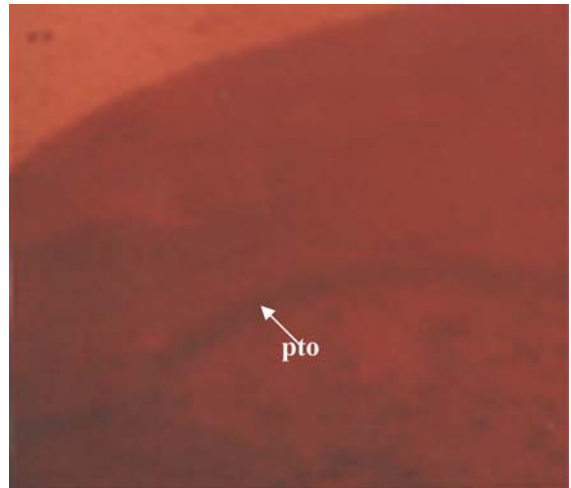


Fig. 3 Pollen tube at the base of the style of the compatible clones i.e. at the ovary site 24 h after pollination. Mag. $\times 400$. pto: pollen tube growth within the ovary

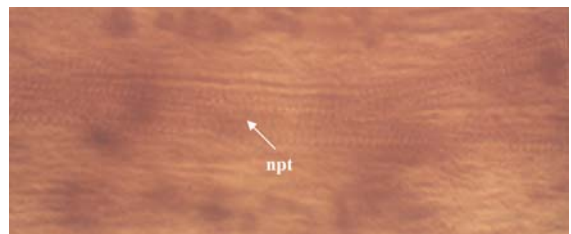


Fig. 4 Pollinated style of self-incompatible clones without pollen tube growth 12 h after self-pollination. Mag. $\times 400$. npt: no pollen tube growth

other clones. It is important to note that clones with evidence of low pollen germination as pollen parents tend to display less compatibility as female parents. This observation has implication in the selection of planting materials for the establishment of cashew plantations. Analysis of *in vivo* pollen germination among the cross-pollinated crosses showed evidence of high cross-compatibility with values ranging between 13.3% and 90.0% for (CC02 $\times$ CC11) and (CC05 $\times$ CC06) crosses, respectively. Highest cross-compatibility both as pollen and female parents was recorded in CC06 (Tables 3 and 4). Each parental clone showed preference for pollens of a particular group of clones as evident in Table 4. While some clones are very broad, other showed narrow or very selective compatibility. However, the percentage self-compatibility ranged from 0.00% to 26.70%, with eight clones showing varying level of self-compati-

Table 3 Mating matrix in diallel of 11 cashew clones showing compatibility (pollen–stigma adherence)

Female Parent	Pollen parent											Mean	CV (%)
	CC01	CC02	CC03	CC04	CC05	CC06	CC07	CC08	CC09	CC10	CC11		
CC01	51	108	144	123	141	156	114	156	99	114	72	116.18	28.78
	28.3	60	80	68.3	78.3	86.7	63.3	86.7	55	63.3	40	64.53	
CC02	123	75	165	141	114	132	144	108	81	75	66	111.27	29.84
	68.3	41.7	91.7	78.3	63.3	73.3	80	60	45	41.7	36.7	61.82	
CC03	123	150	96	123	111	138	108	90	114	54	72	107.18	26.11
	68.3	83.3	53.3	68.3	61.7	76.7	60	50	63.3	30	40	59.54	
CC04	114	174	84	48	105	129	102	69	57	78	84	94.91	37.65
	63.3	96.7	46.7	26.7	58.3	71.7	56.7	38.3	31.7	43.3	46.7	52.74	
CC05	144	120	63	117	12	150	66	108	69	84	57	90.00	46.38
	80	66.7	35	35	65	6.7	83.3	60	38.3	46.7	31.7	50.0	
CC06	162	150	159	126	168	114	96	138	105	141	114	133.91	18.34
	90	83.3	88.3	70	93.3	63.3	53.3	76.7	58.3	78.3	78.3	74.37	
CC07	78	102	90	81	45	123	0.00	111	66	57	111	78.55	45.35
	43.3	56.7	50	45	25	68.3	0.0	61.7	36.7	31.7	61.7	43.65	
CC08	162	60	75	81	120	126	69	45	72	90	54	86.72	40.85
	90	33.3	41.7	45	66.7	70	38.3	25	40	50	30	48.18	
CC09	72	84	60	45	78	102	63	108	18	84	90	73.09	35.07
	40	46.7	33.3	25	43.3	56.7	35	60	10	46.7	50	40.61	
CC10	57	69	45	60	72	93	111	108	51	24	78	69.82	38.14
	31.7	38.3	25	33.3	40	51.7	61.7	60	28.3	13.3	43.3	38.78	
CC11	57	54	108	63	75	84	78	63	75	99	54	73.64	24.40
	31.7	30	60	35	41.7	46.7	43.3	35	41.7	55	30	40.92	
Mean	103.91	104.18	99.0	91.64	94.64	122.00	86.50	100.36	73.36	81.82	77.00		
	57.72	57.88	55.00	50.90	52.57	68.00	48.00	55.76	40.76	45.46	43.00		
CV (%)	41.28	38.77	41.26	38.36	46.53	18.52	43.70	32.15	36.78	38.07	27.27		

bility (Jacob and Atanda 1972). Clones CC05 and CC07 however showed evidence of complete self-incompatibility both in terms of pollen–stigma reaction, pollen germination and tube growth as none of their pollens adhere nor germinate on their self-crosses (Tables 3 and 4). A typical self-incompatible pistil with no tube growth from CC05 and CC07 is shown in Fig. 4. Complete self-incompatibility observed in these two clones suggests that they are probably exclusively cross compatible and lack pseudo or partial self-compatibility.

Based on the modified propositions by Drewlow et al. (1973) and Jacob and Atanda (1972) for classification of compatibility in cross- and self-pollinated crosses respectively, the result of the in vivo pollen compatibility was further summarized in Table 5. It was evident that the crosses from the

selected cashew clones grouped into six breeding classes on the basis of pollen–style compatibility. The degree of cross- and self-compatibility however varied among the clones. The cross-pollinated crosses grouped into highly cross-compatible (CC), partially cross-compatible (PC) and cross-incompatible (CI), while, self-compatible (SC), partially self-compatible (PS) and completely self-incompatible (SI) individuals were recorded among the self-pollinated crosses. Among, cross-pollinated crosses, 55.00% and 46.36% showed evidence of high cross-compatibility (CC) and partial cross-compatibility (PC) respectively, while the remaining 3.4% were cross-incompatible. In the self-pollinated crosses however, 54.55% were self-compatible (SC), 18.18% partially self-compatible and 27.27% were completely self-incompatible (SI) (Jacob and Atanda 1972).

Table 4 Mating in diallel cross of 11 cashew clones showing compatibility (pollen tube germination)

Female Parent	Pollen parent											Mean
	CC01	CC02	CC03	CC04	CC05	CC06	CC07	CC08	CC09	CC10	CC11	
CC01	9	72	126	84	90	141	78	141	66	78	48	84.82
	5	40	70	46.7	50	78.3	43.3	78.3	36.7	43.3	26.7	47.12
CC02	108	30	153	129	60	108	90	72	57	63	42	82.91
	60	16.7	85	71.6	33.3	60	50	40	31.7	35	23.3	46.05
CC03	84	144	45	96	87	117	93	66	96	33	51	82.91
	46.7	80	25	53.4	48.7	65	51.7	36.7	53.3	18.3	28.3	46.08
CC04	63	159	60	24	84	114	90	60	45	60	57	74.18
	35	60	33.3	13.3	46.7	63.3	50	33.3	25	33.3	31.7	41.17
CC05	96	93	54	102	0	147	51	99	57	63	39	72.82
	53.3	60	30	56.7	0	81.7	28.3	55	31.7	35	21.7	41.22
CC06	157	135	138	111	162	48	78	120	66	126	81	111.09
	81.7	75.7	76.7	61.7	90	26.7	43.3	66.7	36.7	70	45	61.29
CC07	54	87	78	66	39	108	0	84	60	42	84	63.82
	30	48.3	43.3	36.7	21.7	60	0	46.7	33.3	23.3	46.7	35.45
CC08	150	42	60	57	111	117	60	21	57	66	42	71.18
	88.3	23.3	33.3	31.7	61.7	65	33.3	11.7	31.3	36.7	23.3	39.96
CC09	36	42	54	27	60	84	48	93	3	60	69	52.36
	16.7	23.3	30	15	33	46.7	26.7	51.7	1.67	33.3	38.3	28.76
CC10	42	60	36	54	60	78	87	99	45	15	57	57.55
	23.3	33.3	20	30	33.3	43.3	48.3	55	25	8.3	31.7	31.95
CC11	39	24	69	54	60	66	72	57	60	81	18	54.55
	21.1	13.3	46.7	30	33.3	36.7	40	31.7	33.3	45	10	31.01
Mean	76.18	80.73	79.36	73.09	73.91	103	67.90	82.91	55.64	62.46	53	73.47
	41.92	45.63	44.85	40.62	41.03	57	39.1	46.07	30.90	34.68	30.00	

Discussion

Generally, the pollens observed in these set of cashew trees are composite and their grains are mostly arranged in tetrads and with 1–2 pollen tube per tetrad. This was corroborated by the result of in vitro germination study showing less germination percentage compared to stainability test. Regular growth of pollen tube was recorded in both compatible cross- and self-pollinated styles. Pollen tube appears as bright yellow under optimum illumination while the surrounding tissues appear pale gray making pollen tubes clearly distinguishable. In fairly good preparations pollen tubes can be clearly traced from their origin (i.e. pollen grain) to their last destination in the ovule of the compatible clones (Figs. 1–3). The tubes do not show thickening (callose) or other abnormal-

ities that might be indicative of incompatible tube growth. After about 24–36 h of pollination, pollen tubes were found in the vicinity of ovules. It is noteworthy that all the pistils examined were found to have hollow styles, and cytological examination showed that in the compatible crosses either self- or cross-, the pollen tubes were found to penetrate the stylar transmitting tissue and grows down to the ovules. This however suggests that incompatibility in cashew especially in the self-pollinated crosses may probably be at postzygotic stages as no evidence of tube inhibition was recorded within the styles in this study.

The averages of 291.01, 243.08, and 47.93 for total number of flowers per panicle, number of staminate flowers and number of hermaphrodite flowers are within the range reported in previous studies in

Table 5 Summary of pollen compatibility among the clones

	Cross-compatibility						Self-compatibility		
	Female parents			Pollen parents					
Clone	++	+ -	--	++	+ -	--	++	+ -	--
CC01	8	2	0	5	4	1	0	1	0
CC02	6	4	0	6	3	1	1	0	0
CC03	7	2	1	5	5	0	1	0	0
CC04	4	6	0	5	4	1	1	0	0
CC05	5	5	0	5	5	0	0	0	1
CC06	9	1	0	9	1	0	1	0	0
CC07	5	5	0	7	3	0	0	0	1
CC08	3	7	0	7	3	0	1	0	0
CC09	2	6	2	1	9	0	0	0	1
CC010	3	7	0	3	6	1	0	1	0
CC011	3	6	1	2	8	0	1	0	0
Total	55	51	4	55	51	4	6	2	3
Percentage	55.00	46.36	3.64	55.00	46.36	3.64	54.55	18.18	27.27

Cross among the parents (Drewlow et al. 1973): -- : Cross-incompatible (CI) (<19.99% germination); + - : Partially cross-compatible (PC) (≥20.0–39.99% germination); ++ : Compatible-compatible (CC) (≥40.0% germination)

Cross within parents (selfing) (Jacob and Atanda 1972): -- : Self-incompatible (SI) (≤4.99% germination); + - : Partially self-compatible (PS) (≥5.0–9.99% germination); ++ : Self-compatible (SC) (>10.0% germination)

cashew (Heard et al. 1990; Masawe et al. 1996). The selections showed high yielding tendency with estimated nut yield of 1.2 tons/ha. The mean values for pollen fertility parameters recorded in this work are ideal for efficient pollination and fertilization in cashew. There seems to be correlations between pollen fertility index and nut yield in one hand and between nut weight (size) and pollen grain size on the other hand. Similar observation has been reported in *Cola* spp. (Adebola 2003). There was disparity between the percentage of pollen stainability and actual in vitro germination, suggesting complex interplay between germination medium and pollen metabolism. Taylor and Helper (1997) remarked that germination in vitro does not completely mimic growth *in vivo* that with highly optimized germination media, in vitro germination of 30–40% is possible. Preuss et al. (1993) opined that pollen of some species does not germinate well in vitro no matter the conditions used. These submissions however validate the result obtained in this work. Data collected on pollen compatibility across the four sides of the tree canopy do not show any significant difference and therefore were not presented. Number

of staminate and hermaphrodite flowers tend to show slight variation across the canopy with highest recorded in the southern part. Full data on this aspect of flowering pattern and distribution in Nigeria has been reported in Aliyu and Awopetu (2003). Light and temperature had been reported to influence the distribution of flowers in cashew (Charkrabort et al. 1981). The prevailing climatic conditions during the 3 years period of this investigation presented in Table 1 were however ideal for optimal cashew flowering and pollen development (Tolla 2004). Suffice that no significant influence of the season on pollen–style compatibility was recorded in this study. Kerhoas et al. (1987) and Shivanna et al. (1991) opined that extreme fluctuation in temperature and humidity during dispersal phase may result in irreversible membrane injury in the pollen and consequently affects its viability. Previous study on cashew in this location showed that environmental factors tend to be more critical during the advance stage of fruit development than at flowering and pollination (Aliyu and Hammed 2000).

Disparity between the numbers of pollen grains adhering to the pollinated stigma papillae and in vivo

germination probably suggests complex interplay between environment and genetic constitution of pollens and the stigma as opined by Ramanna and Mutsaerts (1971). Kerhoas et al. (1987) observed that when released from the anther, pollen is partially dehydrated, with water contents ranging from 6% to 60% with degree of dehydration being species-specific. Therefore, the conditions under which water loss occurs can significantly affect the subsequent adhesion and germination of pollen (Kerhoas et al. 1987; van Bilsen et al. 1994). Suffice that disparity observed in this study may be attributed to this transitory effect. There is tendency that pollinations carried out at 11.30 h (i.e. close to noon when temperature tends to rise) may probably have low chance than early one. It is therefore necessary to further scrutinize the proportion of germinated pollens at every hour. This observation is an evidence of limitation of pollen fertility index as a sole criterion for selecting high yielding varieties in cashew. Non-germination of pollens recorded in selfed crosses of CC05 and CC07 probably suggests that there is high affinity between the constituent allele in the male and female gametes thus leading to what is called self-rejection (Thompson and Kirch 1992). Successful germination of cross-pollinated crosses rule out the possible environmental influence in the poor germination of the respective self-pollinations of these two clones. The non-germination of pollens from self-pollinations of these clones suggests that the S-alleles in both the pollen and the ovule are phenotypically the same, and this probably indicates some sort of sporophytic self-incompatibility reaction which has been reported to be rare and uncommon (Wiersma 2003). According to Hughes (1996), in a sporophytic incompatibility reaction, the tube growth is inhibited on the stigma surface. However, Thompson and Taylor (1966) opined that the higher the frequencies of the recessive S-alleles in a population the higher the proportion of self-compatibility. It is therefore possible that self-incompatibility in the two clones listed above may be due to absence of recessive S-alleles, while the self-compatibility recorded in other clones probably indicates presence of recessive S-alleles. Relative low percentage of self-compatibility compare to cross- also indicates the relative low frequencies of these recessive S-alleles cohabiting with dominant S-alleles in these cashew selections. Intensive selection for morphological and physiolog-

ical characters by plant breeder has been attributed to the increased high frequencies of recessive S-alleles and self-compatibility. True-self compatibility is also known to be conferred by very rare “Sf” allele (Atwood 1942). Yamada et al. (1989) found only a single self-compatible plant in a population of 143 and demonstrated that the “Sf” allele was inherited in a simple Mendelian manner as a dominant gene. The occurrence of self-compatibility of about 55% is suggestive of the presence of recessive S-alleles although in a relative low proportion among the selections. It is not impossible that the high compatibility of clone CC06 with other clones indicates the relative high frequencies of the recessive S-alleles in the clone. Suffice that the broad high cross-compatibility exhibited by this clone with other clones, signifies the potential increase in the self-compatible S-alleles among the future progenies of the crosses involving this clone, since the allele could be inherited in a simple Mendelian manner (Yamada et al. 1989). The clone combines good floral quality characteristics with higher fruit set (yield) suggesting that combinations of good floral quality characteristics like high pollen fertility, prolific flowering, and flower sex ratio plus high cross-compatibility are good and reliable yield components index for selection in cashew yield improvement. Wunnachit and Sedgley (1992b) had opined that the number of hermaphrodite flowers can be used as a selection criterion in cashew. High self-compatibility in CCO6 suggests it as a good candidate for future molecular study on self-compatibility in cashew crop.

Observation in this study corroborates Holand-Neto et al. (2002) and Wiersma (2003) that cashew trees can set fruits from both self- and cross-pollinations. The absence of pollen tube inhibition both in the cross- and self-pollinated crosses also affirmed this assertion and further corroborated the submission of Holand-Neto et al. (2002) that cashew probably has a mechanism of selective abortion through which it discards self-pollinated fruits which may results into inbreeding depression as originally outlined by Grun and Radlow (1961). The 26.70% self-compatibility recorded in this work is comparatively higher than 15% earlier reported. Increasing human selections for high fruit set has been strongly attributed to among other factors that may result into the breaking down of self-incompatibility (SI) and increasing self-compatibility (SC) (Wiersma 2003).

This study however confirms clearly that cashew is an outright out-breeding plant with very high degree of cross-compatibility; a phenomenon that has been attributed to the evolutionary success of angiosperms (Sedgely 1994; Wiersma 2003). The genetic basis of high out-breeding couple with high cross-compatibility can be explained, that the crop tends to produce seeds with high heterosis as prerequisite for continued evolutionary existence. Reports have shown that the degree of cross-compatibility by a population can depend on the degree of genetic distance between the parental lines (Kidwell et al. 1994). The cashew clones used in this work could then be classified as a population with moderate genetic distance on the basis of cross-compatibility. About 55.00% of the cross-pollinated crosses showed high cross-compatibility (CC), while 46.30% demonstrated partial cross-compatibility (PC). This observation may not be unconnected to the fact that all the clones are selected progenies of old Indian introductions from farmers' farms in the North-central part of Nigeria. This observation is further explained by Holanda-Neto et al. (2002) that low fruit yield was recorded in orchard exclusively planted to clonal materials of narrow diverse origin, and thus recommends planting of materials from genetically diverse origin in the same orchard to enhance fruit yield.

Data from this study gave a clue to the possible factors responsible for low yield been experience in cashew as shown in the floral characteristics and pollen–style compatibility of the clones. It was evident that clones exhibited great variation in flower prolificacy, flower sex ratios and pollen fertility. A variation in flower prolificacy among the clones agrees with similar study in Tanzania Masawe et al. (1996). On the basis of floral attributes and compatibility, five clones (CC02, CC03, CC05, CC06, and CC07) can be regarded as good planting materials for cashew farmers, because they combine high pollen grain fertility, flower prolificacy with high degree of high and partial cross-compatibility both as pollen and female parents which are very essential in fruit set. Nonetheless, efficiency of pollination insects has also been implicated for good fruit set (Freitas and Paxton 1998; Wunnachit et al. 1992b. Wunnachit and Sedgley (1992) and Aliyu (2006) have reported correlation between these floral characteristics and fruit set in cashew. This study has demonstrated that about 55% of the trees used in the trial showed

evidence of high cross-compatibility and which may be less in a typical cashew orchard, thus have consequent negative effect on the overall fruit yield. Based on this observation, it would be advisable to recommend that materials to be planted into cashew orchards must combine high floral quality characteristics with high cross-compatibility and from diverse genetic origin. It is important to select varieties that combine high number of hermaphroditic flowers with higher pollen grain fertility. It was equally evident from the data that there seems to be a relationship between maternal compatibility i.e. pollen acceptability and fruit-set. This however suggests that compatibility as an index of selection for yield improvement should be more focused on the mother plant rather than the pollen fertility or pollen parent compatibility (pollen potency), since fruit set is expected from the former. It was also evident that although the crop is an out-breeder, there is likelihood for selection preference among parent trees growing in plantations for compatible pollens therefore point to the importance of compatibility as selection criterion.

The result of this work has thrown light into the spectrum of variation in fruit yield in cashew orchard. Therefore, the practical application of this work to the future research in areas of pollination biology and genetic compatibility, as well as overall crop improvement and production in cashew cannot be overemphasized.

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References

- Adebola PO (2003) Genetic characterization and biosystematic evaluation of the cultivated and wild species of *Cola* Schott and Endlicher. Ph.D. Thesis, The University of Ilorin, Ilorin, Nigeria
- Akinwale SA (1992) Implications of pollen characters to systematic delimitation of cashew (*Anacardium occidentale* L.). Plant Cell Incompatibility Newslett 24:1–6
- Aliyu OM (2004). Characterization and compatibility studies in cashew (*Anacardium occidentale* L.). Ph.D. submitted to the University of Ilorin, Nigeria, 266 pp
- Aliyu OM (2006) Phenotypic correlation and path coefficient analyses of nut yield and yield components in cashew (*Anacardium occidentale* L.). Silvae Genet 55(1):19–24

- Aliyu OM, Hammed LA (2000) A study on nut and apple development in cashew (*Anacardium occidentale* L.). Nigerian J Tree Crop Res 4(2):1–10
- Aliyu OM, Badaru K, Ibrahim AI (2001) Pollen longevity and *in-vitro* germination in cashew (*Anacardium occidentale* L.). Biosci Res Commun 13(6):595–601
- Aliyu OM, Awopetu JA (2003) Studies on flowering pattern in cashew (*Anacardium occidentale* L.). Nigerian J Genet 18:29–35
- Aliyu OM, Awopetu JA (2007) Multivariate analyses of cashew (*Anacardium occidentale* L.) germplasm in Nigeria. Silvae Genet (in press)
- Atwood SS (1942) Genetics of self-compatibility in *Trifolium repens*. J Am Soc Agron 34:353–364
- Bermawie N, Pickersgill B (1992) Pollen tube behaviour in intra- and inter specific pollination in *Caspicum* spp. Indonesia J Crop Sci 7:37–53
- Charkrabort DK, Sadhu MS, Bose TK (1981) Studies on sex expression in cashew (*Anacardium occidentale* L.). Progress Horticult 13:1–3
- Damodaran VK (1966) The morphology and biology of the cashew flower, *Anacardium occidentale* L. II. Anthesis, dehiscence, receptivity of stigma, pollination, fruit set and fruit development. Agricult J Kerala (India) 4:78–84
- Drewlow LW, Ascher PD, Widmer RE (1973) Genetic studies of self-Incompatibility in the garden Chrysanthemum, *Chrysanthemum morifolium* Ramat. Theor Appl Genet 43:1–5
- Erazzu EL, Camadro EL, Clausen AM (1999) Pollen–style compatibility relations in natural populations of the wild diploid potato species *Solanum spigazzinii* Bitt. Euphytica 105:219–227
- FAO (2004) Food and Agriculture Organization production statistics website < <http://faostat.fao.org/site/DesktopDefault.aspx?PageID=340> >
- Freitas BM, Paxton RJ (1998) A comparison of two pollinators: the introduced honey bee *Apis mellifera* and an indigenous bee *Centris tarsata* on cashew *Anacardium occidentale* in its native range of North East Brazil. J Appl Ecol 35:109–121
- Grun P, Radlow A (1961) Evolution of barriers to crossing of self-incompatible with self-compatible species of *Solanum*. Heredity 16:137–143
- Heard TA, Vithanage V, Chacko EK (1990) Pollination biology of cashew in the Northern Territory of Australia. Aust J Agricult Res 41(6):1101–1114
- Holanda-Neto JP, Freitas BM, Bueno DM, Araujo ZB (2002) Low seed/nut productivity in cashew (*Anacardium occidentale*): effects of self incompatibility and honey bee (*Apis mellifera*) foraging behaviour. J HortSci Biotechnol 77:226–231
- Hughes MA (1996) Plant molecular genetics. Henry Ling Limited, Dorchester, UK, 226 pp
- Jacob VJ, Atanda OA (1972) Compatibility and fruit setting in *Theobroma cacao* L. Proceedings of the genetic society of Nigeria, University of Ibadan, pp 98–106
- Jahnen W, Lush WM, Clarke AE (1989) Inhibition of *in vitro* pollen tube growth by isolated S-glycoproteins of *Nicotiana glauca*. Plant Cell 1:501–510
- Johnson D (1973) The botany, origin and spread of cashew (*Anacardium occidentale* L.). J Plant Crops 1:1–7
- Kerhoas C, Gay G, Dumas C (1987) A multidisciplinary approach to the study of the plasma membrane of *Zea mays* pollen during controlled dehydration. Planta 171:1–10
- Kidwell KK, Bingham ET, Woodfield DR, Osborn TC (1994) Relationships among genetic distance, forage yield and heterozygosity in isogenic diploid and tetraploid alfalfa populations. Theor Appl Genet 89:323–328
- Martin PJ, Kasuga LJ (1995) Variation in cashew tree yield in South-east Tanzania and the implication for management of cashew smallholding. Trop Agricult (Trinidad and Tobago) 72(4):261–268
- Masawe PAL, Cundall EP, Caligari PDS (1996) Distribution of cashew flower sex-types between clones and sides of tree canopies in Tanzania. Ann Bot 78:553–558
- Nakasone HY, Paull RE (1998) Tropical fruits. CAB International, UK, 432 pp
- Nambiar MC, Haridasan M (1979) Fertilizing cashew for higher yields. Indian Farm 28:16–17
- Ohler JG (1979) Cashew. Koninklijk instituut voor de Tropen. Teskin, Zutphen Co. Amsterdam, The Netherlands
- Preuss D, Lemieux B, Yen G, Davis RW (1993) A conditional sterile mutation eliminates surface components from *Arabidopsis* pollen and disrupts cell signaling during fertilization. Genes Dev 7:974–985
- Ramanna MS, Mutsaerts MCA (1971) Unusual behaviour of growing pollen tubes in the styles and ovules of *Spinacia oleracea* L. Euphytica 20:145–151
- Salako FK, Babalola O, Hauser S, Kang BT (1999) Soil macroaggregate stability under different fallow systems and cropping intensities in Southwestern Nigeria. Geoderma 91:103–123
- Sedgley M, Griffin AR (1989) Sexual reproduction of tree crop. Academic Press, London
- Sedgley M (1994) Self-incompatibility in woody horticultural species. In: Williams EG, Clarke AE, Knox RB (eds) Genetic control of self-incompatibility and reproductive development in flowering plants. Kluwer Academic, Dordrecht, pp 141–163
- Shivanna KR, Linskens HF, Cresti M (1991) Responses of tobacco pollen to high humidity and heat stress: viability and germinability *in vitro* and *in vivo*. Sexual Plant Reproduct 4:104–109
- Taylor VP, Helper PK (1997) Pollen germination and tube growth. Annu Rev Plant Physiol Plant Mol Biol 48:461–491
- Thompson KF, Taylor JB (1966) The breakdown of self-incompatibility in cultivars of *Brassica oleracea*. Heredity 21(4):637–648
- Thompson RD, Kirch HH (1992) The S-locus of flowering, when self-rejection is self-interest. Trends Genet 8:381–387
- Tolla TD (2004) Effect of moisture conditions and management on production of cashew: a case study in the Lower Limpopo basin, Mozambique. Thesis, International Institute for Geo-information Science and Earth Observation, Enschede, The Netherlands, 95 pp
- Topper CP, Caligari PDS, Camara M, Diaora S, Djaha A, Coulibay F, Asante AK, Boamah A, Ayodele EA, Adenola PO (2001) West African Regional Cashew Survey Report (Guinea, Guinea Bissau, Cote d'Ivoire, Ghana and Nigeria). Sustainable Tree Crop Programme (STCP) and Biohybrids Agrisystem Ltd. UK, vol 1, 110 pp

- van Bilsen DG, van Roekel T, Hoekstra FA (1994) Declining viability and lipid degradation during pollen storage. *Sexual Plant Reproduct* 7 303–310
- Waser NM, Price MV (1991) Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules usurped? *Am J Bot* 78:1036–1043
- Wiersma PA (2003) Reproductive barriers in tree fruit crops and nuts. *Proc xxvi international horticultural congress: genetics and breeding of tree fruit and nuts, Acta Horticulturae*, vol 622, pp 369–377
- Woodroof JG (1979) Tree nuts: production, processing, and products. AVI Publishing Company Incorporation, Westport, Connecticut
- Wunnachit W, Sedgley M (1992) Floral structure and phenology of cashew in relation to yield. *J Horticult Sci* 67:769–777
- Wunnachit W, Pattison SJ, Giles L, Millington AJ, Sedgley M (1992a) Pollen tube and genotype compatibility in cashew in relation to yield. *J Horticult Sci* 67:67–75
- Wunnachit W, Jenner C, Sedgley M (1992b) Pollen vigour and composition in relation to andromonoecy in cashew (*Anacardium occidentale* L.: Anacardiaceae). *Sexual Plant Reproduct* 5:264–269
- Yamada T, Fukuoka H, Wakamatsu T (1989) Recurrent selection programs for white clover (*Trifolium repens* L.) using self-compatible plants. I. Selection of self-compatible plants and inheritance of a self-compatibility factor. *Euphytica* 44(1–2):167–172