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Cross-compatibility Among Six Improved Cassava (*Manihot esculenta* Crantz) Varieties

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Abstract

Crosses are very vital in the development of populations for breeding purposes and the ease with which a parent can cross with other clones is very important in enhancing successful generation of progeny in a breeding programme. Sexual propagation is a promising technique in cassava breeding programmes due to its manifold advantages such as enhanced multiplication rate, longer seed viability, ease of storage and transportation. Another advantage is the high genetic heterogeneity, leading to variation among seedlings which provides the breeder opportunity to select materials with good qualities of the traits of interest. However, one of the major problems militating against breeding of cassava is the difficulty in producing recombinant seeds due to the associated limitations such as inbreeding depression, high abortion rate, low seed germinability and long breeding cycle of the crop. The objective of this study was to assess crossability among six cassava varieties in terms of seed set capability and germinability of the hybrid seeds. Nine hybrid populations were developed using three female and three male parents in a non-reciprocal cross and the resultant seeds were sown in the nursery. Seed set and germinability were recorded. The results showed that seed set and germinability were influenced by the genotypes used as parents. The percentage seed set ranged between 19.9 and 35.5 and the cross, TMS 98/0505 x NR 8083 with highest percentage seed set was about 178% better than the cross, TMS 97/2205 x TMS 30572 with the least percentage seed set. There was variation in compatibility between each female parent and the male parent. Germinability of seeds among the nine crosses ranged between 25.74 and 51.30% indicating that a broad base of variation existed in crossability among the cassava varieties. It is therefore suggested that varieties with high crossability having good qualities of breeder's trait(s) of interest should be used in cassava breeding programmes to enable breeders to generate large populations within a short period.

Key words: Crossability, germinability, percentage seed set, hybridization

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most common food crops grown in many parts of the tropics (IITA, 2000). It is the fourth most important basic food after rice, wheat and maize and is a fundamental component in the diet of millions of people (FAO/FIDA, 2000). It is a staple starchy food; the tubers can be boiled, baked, fried or dried. The tubers are also used to produce flour, bread, tapioca, sugar, laundry starch, biofuel and even an alcoholic drink (eg. Impala Beer in Mozambique brewed by SABMiller). The leaves of cassava are consumed in many countries as a preferred vegetable providing proteins, minerals and vitamins (IITA, 1992) and famine rarely occurs in areas where cassava is widely grown (Nweke et al., 2002).

Cassava can be propagated either vegetatively using stem cuttings or by the use of true sexual seeds. Sexual seeds are used mostly in breeding programmes to generate new genetic variation. However, botanical (sexual) seeds have also been used occasionally in commercial propagation schemes (Rajendran et al., 2000).

Cassava is a highly heterozygous species and has extremely high inbreeding depression (CIAT, 1974), hence, selfing is not a tool always used in cassava programmes, though it has been suggested that it would be valuable to introduce inbreeding into cassava breeding programme (Perez et al., 2005). Cassava is monoecious and allogamous, with female flowers opening 10-14 days before the male ones on the same branch (Blair et al., 2007), a characteristic called protogyny (Halsey et al., 2008). This encourages cross pollination but self pollination may occur because male and female flowers on different branches of the same plant or on different plants of the same genotype can open simultaneously (Jennings and Iglesias, 2002). Self-pollination in cassava varies from 0 to 100%, depending upon the genotype and planting distance (CIAT, 1974). A flower bud is formed every time the plant branches, hence, flowers cannot be obtained from non-branching cultivars (Kawano, 1980). The plants seldom bear receptive flowers during the first six months; therefore, most of the flower buds formed during the early growth stages are abortive in nature. Many cassava cultivars flower from 8 to 16 MAP under tropical conditions and flowering is rare during the dry season (Kawano, 1980).

Cassava seeds can be stored for about a year under ambient conditions without any serious decline in viability, and much longer at low temperature and humidity (Kawano, 1980). The seeds require fairly high temperatures (30-35°C) for optimum germination (Ellis et al., 1982) and the germination is favoured by dry heat and complete darkness (Halsey et al., 2008; Rajendran et al., 2000).

Cassava breeding has received relatively little attention, until recently, due to the associated difficulties in its breeding programme such as long breeding cycle and difficulties in the production of sexual (recombinant) seeds (Ceballos et al., 2004). The recent advances in science and technology and the tools offered by biotechnology could be very valuable in elucidating the mechanisms of incompatibility among genotypes to increase crossability among them (Ngeve, 2000). Levin (1973) measured cross-compatibility in Phlox in terms of the average seed-set per cross. The objective of this study was to assess cross-compatibility (crossability) among different cassava varieties in terms of seed set capability and germinability of the hybrid seeds.

Materials and Methods

The study was carried out in the field and screen house of the Cassava Programme, National Root Crops Research Institute (NRCRI), Umudike, Nigeria. Umudike is located at a latitude of $5^{\circ}29^{D}$ N, longitude of $7^{\circ}33^{D}$ E and altitude of 122 m above sea level. The average annual rainfall was 2169 mm in 148-155 rainy days, while average ambient temperature was 26°C with minimum and maximum temperatures of 22°C and 32°C, respectively. The relative humidity ranged between 50 and 95%. Table 1 shows the quantity of rainfall, temperature and relative humidity during the period of experimentation at the experimental station, Umudike, Nigeria during 2005 and 2006.

Nine F_1 populations (COB-1 to COB-9) were developed using three early and three late bulking cassava varieties (Table 2). The early bulking varieties (TMS 30572, TMS 97/2205 and TMS 98/0505) were used as female parents based on their ability to produce flowers profusely, while the late bulking varieties (TMS 30555, NR 8212 and NR 8083) were used as the male parents. Classification of the parent material into early and late bulking categories was reported earlier by Olasanmi (2010).

Pollination

Mature female flowers on the female plants were covered with cloth bags measuring 10 x 17 cm in the morning (between 7.30 and 10.30 am) of each day that pollination was carried out to prevent stray pollination. Mature male flowers were picked off the branches of the male plants between 10.30 am and 12 noon shortly before opening and kept in small bottles under shade till the time of pollination. The pollen were used to pollinate the designated (covered) female flowers between 1.00 and 5.00 pm using one male flower to pollinate one female flower. Any female flower found to have not opened in a raceme at the time of pollination was removed to ensure that all the fruits on the branch developed only from controlled pollination. The female flowers were left open after pollination. Each flower branch on which pollination was made was labelled with a tag measuring

Table 1. Rainfall, temperature and relative humidity at Umudike, Nigeria during 2005 and 2006

Month	Quantity of rainfall (mm)		Temperature (°C)				Relative humidity (%)			
			2005		2006		2005		2006	
	2005	2006	Max	Min	Max	Min	9.00 h	15.00 h	9.00 h	15.00 h
January	17.3	76.6	33	19	33	24	53	39	81	59
February	126.7	81.9	35	23	33	24	79	56	80	61
March	64.0	131.9	34	23	34	24	80	65	80	60
April	141.3	136.0	34	24	33	24	77	64	79	62
May	222.4	202.8	32	23	31	23	81	71	83	71
June	264.4	237.3	31	23	31	22	90	70	84	72
July	277.0	303.4	29	23	30	23	88	80	87	78
August	225.0	133.7	30	22	29	22	85	77	84	77
September	339.7	483.1	31	23	29	22	86	77	86	80
October	323.0	237.4	31	22	31	22	84	71	84	72
November	45.4	14.2	33	23	31	23	82	60	82	58
December	8.6	0.0	32	22	32	20	79	56	70	40
Total	2054.8	2038.3	-	-	-	-	-	-	-	-
Mean	171.23	169.86	32.1	22.5	31.4	22.8	80	66	82	66

Source: Meteorological Center, NRCRI, Umudike

3.5 x 4 cm indicating the cross combination (female parent x male parent); date of pollination and number of female flowers pollinated in the inflorescence. Number of female flowers pollinated in each cross was recorded daily. The data was used to calculate the total number of female flowers pollinated in each cross and the total was used to estimate the maximum number of seeds expected from the cross. The developing young fruits were covered with a cloth bag (the same type used to cover female flowers during hybridization) four weeks after pollination. The bag helped to retain mature fruits and seeds that dropped. There was no loss due to pest infestation because the fruits were protected by the bags and the field was well maintained throughout the course of the hybridization programme. The mature fruits were collected and sun-dried between December 2005 and February 2006. The schematic representation of steps employed in development of the populations is shown in Fig. 1. Seeds recovered from mature fruits were bulked for each of the nine crosses and the total number of seeds for each cross was recorded. Percentage seed set for each cross was calculated as a ratio of number of seeds recovered to the maximum number of seeds expected from the cross.

Table 2. Nine cassava hybrid populations developed from six parents

Population	Female parent	Male parent
COB-1	TMS 30572	TMS 30555
COB-2	TMS 30572	NR 8212
COB-3	TMS 30572	NR 8083
COB-4	TMS 97/2205	TMS 30555
COB-5	TMS 97/2205	NR 8212
COB-6	TMS 97/2205	NR 8083
COB-7	TMS 98/0505	TMS 30555
COB-8	TMS 98/0505	NR 8212
COB-9	TMS 98/0505	NR 8083

Germination of seeds

Seeds of the nine cassava populations were sown in nursery at the greenhouse in February 2006 to test germinability of the seeds. The seeds were sown in the nursery using perforated plastic trays filled with well prepared sandy loam soil collected from an undisturbed site at the Institute's cassava field. The seeds were drilled to a depth of about 5 cm in furrows made in the soil about 10 cm apart and the soil surface was covered with black nylon to aid germination. The trays were watered on alternate days throughout the period of the experiment. Number of emerged seedlings in each tray was recorded daily until seedling emergence ceased in all the trays. The data was used to calculate the total number of emerged seedlings in each of the nine families. Per cent germinability was calculated as a ratio of emerged seedlings to the total number of seeds sown in each family.

Fig. 1. Schematic representation of steps employed in the development of hybrid populations

Results and Discussion

Opening of mature male and female flowers

It was observed in this study that male and female flowers failed to open on cold rainy days, hence, no pollination was carried out on those days although adequate preparations (covering of mature female flowers and collection of mature male flowers) for pollination were made in the early hours of such days. However, mature flowers opened each day when there was sunshine after early morning rain. There was no variation in response to weather conditions as regards to opening of mature flowers (male and female) among the varieties used in this study. However, there was variation in time of the day when male and female flowers of the varieties opened. The observed similarity in the opening of mature flowers among the varieties used in this work in response to weather conditions suggests that the trait may not be genotype dependent in cassava. The genetic differences observed among the parents in terms of time of the day when mature flowers opened on the plants, calls for understanding of floral behaviour of varieties used as parents in cassava breeding programmes to enable good planning. Male flowers of NR 8083 plants opened before those of NR 8212 and TMS 30555 plants. The male flowers of both NR 8212 and TMS 30555 plants opened almost at the same time. It was also observed that female flowers of TMS 98/0505 plants opened before those of TMS 97/2205 plants, while female flowers of variety TMS 30572 plants opened last. Having observed this difference at the early stage of the study, covering and pollination of female flowers as well as collection of male flowers were planned in the order of earliness of opening of the flowers. Although there were few rains in the last two months of the pollination (Table 1), there was still enough moisture in the soil to support good growth of the plants and the fruits. The weather condition during this period was favourable for the pollination activity in the sense that there was not much disturbance from rain during pollination. Opening of mature flowers on days when there was sunshine after early morning rain suggests that environmental conditions had an effect on opening of flowers and not flowering alone as stated earlier (Ceballos et al., 2004). It is therefore suggested that pollination, if possible, should be carried out towards the end of rainy season when there would be little or no rain during the early hours of the day.

Percentage seed set among the cassava hybrid populations

Most of the harvested fruits in this study had three seeds at harvest, indicating that the female parents were highly fertile. However, less than one seed was obtained per pollination on an average in this study. Out of 16,179 seeds expected from all the pollinated flowers (with three possible seeds in each trilocular fruit), only 4,134 seeds (25.2%) were obtained. Many of the pollinated flowers did not develop into fruits due to abortion shortly after pollination. There was non-significant but positive correlation between number of flowers pollinated and number of seeds harvested in each population ($r = 0.54$, $p = 0.1359$, $n = 9$). Population 4 had the lowest seed set of 19.9%, while population 9 had the highest seed set of 35.5% (Fig. 2). Highest per cent seed set was obtained in the cross between TMS 98/0505 and NR 8083, which was the best female and male parent in terms of seed set, respectively. TMS 98/0505 had higher per cent seed set with each male parent than the corresponding crosses involving TMS 30572 and TMS 97/2205. Also, NR 8083 had higher per cent seed set with each female parent than corresponding crosses involving the other two male parents (TMS 30555 and NR 8212) (Table 3). There was little difference in per cent seed set among the crosses involving each female parent but much variability was observed among the crosses involving each male parent as shown by the coefficient of variation (CV) (Table 3).

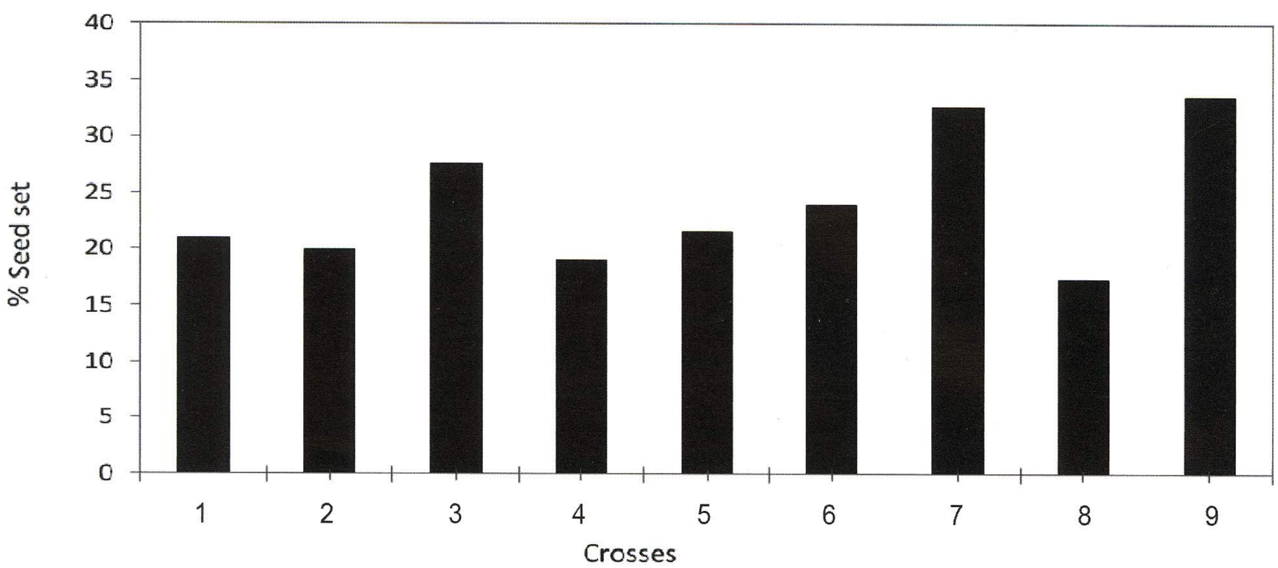
Number of seeds per female flower is genotype dependent in cassava, therefore, selection of highly fertile genotypes as female parents is a critical factor (Kawano, 1980). The average seed set observed in this study was far below the expectation of Ceballos et al. (2004) who stated that one or two seeds were obtained per pollination on an average. The non-significant correlation observed between number of flowers pollinated and number of seeds harvested in each population indicated that success of pollination programmes in cassava could not be accurately predicted from the number of flowers pollinated. Large differences in CV for seed set among the parents and significantly better performance of TMS 98/0505 and NR 8083 in terms of seed set than other female and male parents respectively suggest genetic differences in the crossability of these clones. This, therefore, indicates that both maternal effect and compatibility between male and female parents have influence on percentage seed set in cassava. Therefore, it may be necessary to investigate seed set capability of male and female parents used in the development of recombinant seeds in cassava breeding programmes. This would help the plant breeder to choose parental varieties with high seed set capability and thereby maximize resources like time, labour and funds.

This experiment was carried out at the end of rainy season, which ensured that the fruits matured during the dry season. This greatly enhanced drying of fruits on the plants and thereby minimised the need for drying, after collection of the fruits. Some of the fruits shattered on the plants to release their seeds into the bags, such seeds were recovered from the bags with little effort. This further supports the need to cover developing fruits

Table 3. Variability in seed set among populations developed from each parent

Male parent	Female parent			Mean	CV (%)
	TMS 30572	TMS 97/ 2205	TMS 98/ 0505		
TMS 30555	20.8	19.9	33.0	24.6	29.8
NR 8212	20.0	21.6	28.9	23.5	20.2
NR 8083	27.6	24.4	35.4	29.1	19.4
Mean	22.8	22.0	32.4	25.7	
CV (%)	18.3	10.3	10.1		

CV (%) = Coefficient of variation



1 = TMS 30572 x TMS 30555; 2 = TMS 30572 x NR 8212; 3 = TMS 30572 x NR 8083; 4 = TMS 97/2205 x TMS 30555; 5 = TMS 97/2205 x NR 8212; 6 = TMS 97/2205 x NR 8083; 7 = TMS 98/0505 x TMS 30555; 8= TMS 98/0505 x NR 8212; 9 = TMS 98/0505 x NR 8083

Fig. 2. Percentage seed set in crosses among six cassava varieties at NRCRI, Umudike, Nigeria

with bags on the plants to collect the fruits (Kawano, 1980) and also ensure that the seeds were not lost due to shattering. It is thus suggested that bags should also be used in the collection of open pollinated seeds from cassava plots. This would minimise loss of seeds due to shattering and thereby ensure recovery of almost all the seeds in a plot. It would also ensure that all the fruits collected from each plot are from the same female parent and thereby minimise contamination.

Percentage germinability

The first germination was observed five days after sowing (DAS) in population 2, whereas, no germination was recorded in populations 4, 6 and 9 until 11 DAS. Germination ceased in population 1, 105 DAS, but continued till 115 DAS, in crosses 2, 4 and 7. There was significant correlation between number of seeds sown and number of emerged seedlings in each population ($r = 0.84$, $p=0.0049$, $n = 9$). The highest germinability of 51.3% was noticed in population 1, while population 6 had the lowest value of 25.7% (Table 4). The CV of 2.8% for germinability among the crosses involving TMS 98/0505 suggests that germinability is more stable among the crosses (Table 5). T-test results showed significant differences in mean percentage germinability among crosses developed from TMS 30572 and those from TMS 97/2205 as well as among crosses developed from TMS 98/0505 and those from TMS 30572 (Table 5).

The higher difference in CV for germinability found among the female parents than the male parents indicated that the male parents exhibited more stability for germination rate. The differences observed in the rate

Table 4. Percentage germination in nine F_1 populations developed at Umudike

Population	Cross combination	Number of seeds sown	Number of emerged seedlings	Percentage germinability (a)
1	TMS30572 x TMS30555	460	236	51.3
2	TMS30572 x NR8212	326	161	49.4
3	TMS30572 x NR8083	331	132	39.9
4	TMS97/2205 x TMS30555	426	151	35.5
5	TMS97/2205 x NR8212	383	136	35.5
6	TMS97/2205 x NR8083	408	105	25.7
7	TMS98/0505 x TMS30555	730	279	38.2
8	TMS98/0505 x NR8212	500	200	40.0
9	TMS98/0505 x NR8083	570	217	38.1
	Total	4134	1617	39.3

$$a = \frac{\text{number of emerged seedlings X 100}}{\text{number of seeds sown}}$$

Table 5. Variability in percentage germinability of seeds developed from each parent

Male parent	Female parent			Mean	CV (%)
	TMS 30572	TMS 97/2205	TMS 98/0505		
TMS 30555	51.3	35.5	38.2	41.7	20.3
NR8212	49.4	35.5	40.0	41.6	17.0
NR8083	39.9	25.7	38.1	34.6	22.3
Mean	46.9	32.2	38.8	39.3	
CV (%)	13.1	17.5	2.8		

T-test result of comparison among crosses involving female parents for % germination

Parents	Probability
TMS 30572 vs TMS 97/2205	0.04*
TMS 97/2205 vs TMS 98/0505	0.18 NS
TMS 98/0505 vs TMS 30572	0.02*

*significantly different; NS = not significantly different; CV (%) = Coefficient of variation of germination of hybrid seeds in the nine populations might also be another consequence of compatibility between the two parents used to generate each population since the germination process was carried out at the same time under the same weather conditions. The relatively low CV for germinability recorded among crosses involving TMS 98/0505 indicated that crossability (in terms of germinability of the resultant seeds) was more stable in crosses involving this variety than those in which the other two female parents were used. High significant correlation observed between number of seeds sown and number of emerged seedlings in the F_1 populations suggested that one could predict to a large extent the number of seedlings expected in a breeding programme using the number of seeds generated.

Conclusion

In short, for a successful cassava breeding programme, information on the cross compatability of the male and female parents are of utmost importance. Here, nine hybrid populations were developed using three female and three male parents in a non-reciprocal cross and the results showed that seed set and germinability were influenced by the genotypes used as parents. The percentage seed set ranged between 19.9 and 35.5 and the cross, TMS 98/0505 x NR 8083 with highest percentage seed set was about 178% better than the cross, TMS 97/2205 x TMS 30572 with the least percentage seed set. Variation in compatibility between each female parent and the male parent was noticed. Germinability of seeds among the nine crosses ranged between 25.74 and 51.30% indicating that a broad base of variation existed in crossability among the cassava varieties. Thus it is suggested that varieties with high crossability having good qualities of breeder's trait(s) of interest should be used in cassava breeding programmes to enable breeders to generate large populations within a short period.

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