Study of the Reproductive Characteristics of Nine Cassava Accessions

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Abstract

Reproductive behaviour of two cultivars (AF and AN) and seven breeding lines (BA, AS, LA, BS-1, HO-008, ME and SE) of cassava (*Manihot esculenta* Crantz) was studied to obtain information pertaining to flowering habits and other reproductive characteristics of these potential parents required for future hybridization programmes. The accessions were grown on the Research Farm of the Biotechnology and Nuclear Agriculture Research Institute in the coastal savanna agro-ecological zone of Ghana between April 2008 and December 2009. For each accession, 40 stem cuttings, each bearing five to eight nodes, were prepared from the mid-section of healthy cassava stems and planted at a spacing of 1.5 m × 1.0 m while accessions were separated by a distance of 2 m. Ten plants were tagged per accession for the collection of data on key reproductive characteristics. All accessions flowered, suggesting that flower production may not be a limiting factor under the prevailing climatic conditions. Light microscopy revealed that one accession (BA) produced dysfunctional male flowers which were devoid of pollen. Mean days to flowering and fruiting varied significantly ($P \leq 0.05$) among the accessions, indicating the need to use different planting dates for different accessions to ensure synchronization of flowering. The accessions also differed significantly ($P \leq 0.05$) with respect to plant height at various levels of branching, as well as number of inflorescences, staminate and pistillate flowers, and fruit produced per branching level. There was also variation in percent seed set, embryo formation and fruit drop. The extensive variability observed among the accessions provides breeders with immense opportunities for carrying out cross combinations to generate new genotypes to meet specific objectives.

Introduction

Cassava (*Manihot esculenta* Crantz) is an important crop grown mainly for its starchy, tuberous roots throughout much of the tropical regions of the world. It constitutes a staple for about 800 million people worldwide (Hahn & Keyser, 1985). Apart from its use as a major staple food, it is very useful as a livestock and poultry feed and in the production of many industrial products such as syrups, ethanol and biodegradable plastics (Pranamuda *et al.*, 1996; Garcia & Dale, 1999). With such a wide use to which cassava can be put, the main objective of breeding cassava is to develop varieties superior to those currently cultivated, especially for the economically and, or biologically important traits such as high starch content, low cyanogenic potential, earliness to maturity, and pest and disease tolerance that are accepted by producers, processors and consumers (Fukuda *et al.*, 2002). Artificial sexual hybridization presents the most common conventional solution to generating segregating population for selection of desirable genotypes (Acquaah, 2007).

Currently in Ghana, none of the cultivated cassava varieties possesses all the desired agronomic traits; these include landraces and improved varieties. There is, therefore, the need to develop hybrid varieties that would combine desirable traits present in selected parents into one genetic matrix.

In artificial sexual hybridization, it is crucial for the breeder to be familiar with the flowering habits and other reproductive characteristics of the parents selected to supply desirable characteristics or traits that are to be combined in the progeny. Such vital breeding information is not available on the cultivated cassava varieties. The aim of this study was to document useful breeding information in relation to the reproductive behaviour among nine cassava accessions that could be used as parents in a breeding programme.

Materials and methods

The experiment was carried out at the Research Farm of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) at Kwabenya between April 2008 and December 2009.The experimental site, 76 m above sea level, lies within the coastal savanna zone and receives less than 1000 mm of rainfall annually. The predominant soil type found in the area is a well-drained Savanna Ochrosol derived from quartzite schist.

Two old cassava cultivars, *Afisiafi (*AF) and *Ankra* (AN), popular with farmers throughout the major cassava-growing regions of Ghana, and seven breeding lines (*Bamboo Akwetey* (BA), *Asare* (AS), *Larbi* (LA), *BNARI Selection 1* (BS-1), HO-008, *Megyewontem* (ME) and *Security* (SE),with variously desirable agronomic characteristics, were selected from the field gene bank at the research farm of BNARI and used for the plots establishment. For the sake of simplicity the cultivars and the breeding lines together are referred to as accessions in this study. Nodal cuttings bearing five to eight nodes were prepared from the mid-section of healthy cassava stems. Forty cuttings were planted per accession in a block at an inter-row and intra-row spacing of 1.5 m and 1.0 m, respectively. Ten plants were tagged and the following data collected on them: mean number of days to 50% flowering, mean number of inflorescences produced per branch, mean number of male and female flowers produced per branch, mean number of fruits per plant, percent fruit drop, mean days to fruit maturity, mean plant height at branching level, percent seed set and percent embryo formation. Percent seed set and percent embryo formation were determined on 20 fruits obtained at random from the 10 selected plants per accessionas follows;

$$\% \text{ Seed set} = \frac{\text{TNS observed}}{\text{TNS expected}} \times 100$$

$$\% \text{ Embryo formation} = \frac{\text{TNE observed}}{\text{TNE expected}} \times 100$$

where TNS and TNE observed are total number of seeds and embryos observed in 20 fruits, respectively. TNS and TNE expected are the total number of seeds and embryos expected in 20 fruits, respectively.

Illuminated Stereo Microscope (Leica Zoom 2000™ USA) was used at a magnification of ×40 to observe anthers for presence of pollen. Statistical analyses on parameters studied were carried out using analysis of variance (ANOVA) in Statistical Package for Social Scientists (SPSS). Minitab Statistical Software (version 15.0) was used where Chi-square analysis was performed.

Results

Mean number of days to 50% flowering

The mean number of days taken by the cassava accessions to attain 50% flowering is presented in Table 1. Generally, days to 50% flowering varied significantly ($P \leq 0.05$) among the cassava accessions used in the study. Cassava accession AN recorded the longest mean days to flowering (131.2 days). In comparison, the other accessions took significantly shorter periods to attain 50% flowering, with SE taking the shortest period of 69.9 days.

Number of inflorescences produced per branch

Mean number of inflorescences produced varied significantly ($P \leq 0.05$) among the accessions (Table 1). The highest number of male inflorescences produced was observed in SE which recorded two inflorescences. In contrast AS produced a mean of 1.1 inflorescences per branching, being the least among the accessions. Variation in the production of hermaphroditic inflorescences among the accessions was also statistically significant ($P \leq 0.05$). AN was the most prolific in the production of hermaphroditic inflorescences with a mean of 2.8 inflorescences. On the other hand, HO-008 produced the least number of 1.4 hermaphroditic inflorescences.

Flower production among the accessions

The number of male (staminate) and female (pistillate) flowers produced per branching indicated that the cassava accessions generally produced more male flowers than female ones (Table 1). There was highly significant variation ($P \leq 0.05$) in the number of male flowers produced among the accessions, ranging from 35.4 in BA to 58.6 in ME. In the case of pistillate flower production, AF recorded the highest with a mean of 5.5 flowers while AS produced the least of 2.6. Differences in the number of pistillate flowers produced among the accessions were found to be statistically significant ($P \leq 0.05$).

TABLE 1
Flowering characteristics among nine cassava accessions

Accessions	*Days to 50% flowering*	*Number of inflorescences*		*Number of flowers*	
		Male	*Hermaphroditic*	*Staminate*	*Pistillate*
ME	83.9^{bc}	1.3^{b}	2.2^{abcd}	58.6^{a}	4.4^{abc}
BA	90.6^{b}	1.3^{b}	2^{cde}	35.4^{c}	3.1^{cd}
AN	131.2^{a}	1.6^{ab}	2.8^{a}	40^{bc}	5.1^{ab}
BS-1	72.4^{c}	1.5^{ab}	2.1^{bcd}	45^{abc}	4.2^{abc}
AF	73.6^{c}	1.2^{b}	2.7^{ab}	52^{ab}	5.5^{a}
SE	69.9^{c}	2^{a}	2.1^{bcd}	43.3^{bc}	4.1^{bc}
LA	123.3^{a}	1.6^{ab}	2.4^{abc}	36.7^{c}	4.6^{ab}
AS	83.6^{bc}	1.1^{b}	1.6^{de}	48.9^{abc}	2.6^{d}
HO-008	95.7^{b}	1.6^{ab}	1.4^{e}	42^{bc}	3.1^{cd}

Means in a column with the same superscript are not significantly different according to Tukey's HSD test *($P \leq$* 0.05)

Number of fruits produced per branching level

Table 2 shows the mean number of fruits produced per plant at 8 months after planting (MAP). Generally, the number of fruits formed increased with branching level among the accessions. None of the accessions produced fruits at the first branching level. Nonetheless, significant differences($P \leq 0.05$) were observed in the number of fruits produced at branching levels 2–6. At the second branching level, ME, AS and HO-008 still did not record any fruit formation. SE, however, produced the highest number of 7.2 fruits while LA recorded the least number of 0.4 fruits at this level. Fruit formation at this level in SE was significantly higher compared to the other accessions. Indeed, the cassava accession SE consistently produced the highest mean number of fruits at all branching levels followed by BS-1. The accession AF which produced a mean of only 0.5 fruits at the second branching level emerged as the third highest by the 4th branching level and retained the same position at the 5th branching level. HO-008 produced fruits only at the 5th and 6th branching levels and recorded a mean of 1.25 and 0.5 fruits, respectively.

Fruit drop

Variation in fruit drop in the cassava accessions showed significant differences ($P \leq 0.05$) among them (Table 2). The highest fruit drop of 83.3% was recorded in ME. This was, however, not significantly different from values recorded for AS (82.1%), AN (81.8%) and HO-008 (81.8%). BS-1 retained the highest proportion of its fruits by recording the lowest fruit drop of 11.7%.

Mean number of days to fruit maturity

Results obtained indicate significant differences ($P \leq 0.05$) in the mean number of days to fruit maturity among the nine cassava accessions following open pollination (Table 2). Fruit maturity period ranged from

TABLE 2
Fruiting characteristics among nine cassava accessions

Accession	*Number of fruits per branching level*						*Fruit drop (%)*	*Fruit maturity (days)*
	1	2	3	4	5	6		
ME	0	0^{c}	2.6^{d}	4.3^{cd}	6.5^{bc}	–	83.3^{a}	62.5^{ab}
BA	0	2.3^{cb}	10.4^{bcd}	11.7^{cd}	13.25^{bc}	–	52.5^{ab}	55.7^{d}
AN	0	1^{cb}	5.11^{cd}	18^{bcd}	–	–	81.8^{a}	59.7^{abcd}
BS-1	0	3.7^{b}	20.4^{ab}	41.6^{ab}	49.7^{ab}	46.4^{ab}	11.7^{c}	59.8^{abcd}
AF	0	0.5^{c}	15.5^{bc}	28^{abc}	32.5^{abc}	–	28.4^{bc}	62.2^{abc}
SE	0	7.2^{a}	28.5^{a}	50.4^{a}	60.5^{a}	62.88^{a}	25.5^{bc}	58.3^{bcd}
LA	0	0.4^{c}	6.2^{cd}	14.5^{cd}	–	–	27.6^{bc}	58.5^{bcd}
AS	0	0^{c}	4^{cd}	4.44^{cd}	12^{bc}	–	82.1^{a}	62.9^{a}
HO-008	0	0^{c}	0^{d}	0^{d}	1.25^{c}	0.5^{b}	81.8^{a}	58.2^{cd}

Means in a column with the same superscript are not significantly different according to Tukey's HSD test ($P \leq 0.05$)

55.7 days in BA to 62.9 days in AS. Fruits of BA, thus, matured earlier than fruits of all other cassava accessions studied.

Plant height at branching level

The nine cassava accessions exhibited significant variation ($P \leq 0.05$) in height at which branching occurred. Four levels of branching occurred in AN and LA at 206.4 cm, 252.9 cm, 273.7 cm, 283.0 cm and 136.5 cm, 173.0 cm, 206.2 cm, 212.0 cm, respectively. Similarly, ME, BA, AF and AS produced five levels of branching at various heights above ground level (Fig.1). Only BS-1, HO-008 and SE entered the sixth branching level, attaining mean heights of 204.9 cm, 257.5 cm and 199.5 cm, respectively.

the cassava accessions. Seed set was generally high in all the accessions investigated, ranging from 73.33% in AN to 100% in AF.

Embryo formation

The proportion of seeds developed by the accessions that formed embryos following open pollination are presented in Fig. 2. Significant differences ($P \leq 0.05$) in embryo formation were observed among the cassava accessions. In BA, embryos were formed in every seed and, therefore, this accession recorded 100% embryo formation. Seeds formed in SE developed endosperms but most of them were devoid of embryos. SE, therefore, recorded the lowest proportion of

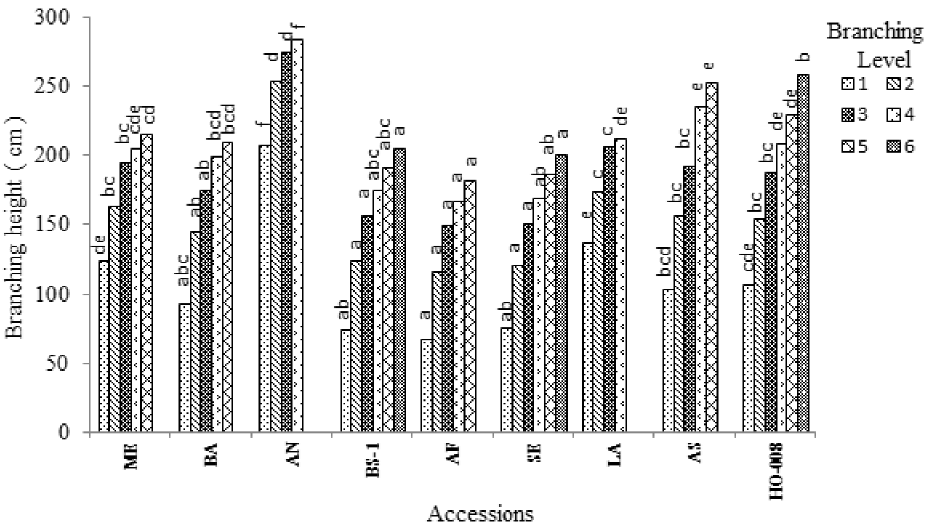

Fig. 1. Variation in plant height at various levels of branching in nine accessions of cassava. Different letters on top of bars indicate significant differences at $P \leq 0.05$ according to Tukey' HSD test.

Percent seed set

Fig. 2 shows percent seed set following open pollination among the cassava accessions. Significant differences ($P \leq 0.05$) in percent seed set were observed among

embryos (60.42%). Similar occurrences were observed in BS-1, AN, AS, ME, LA, HO-008 and AF, all of which recorded varying levels of embryo formation.

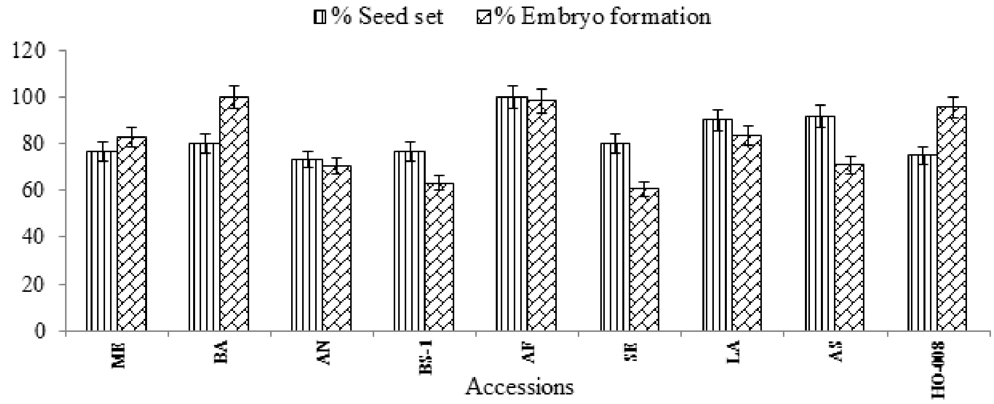

Fig. 2. Variation in seed set and embryo formation among nine cassava accessions

Discussion

All the nine cassava accessions exhibited ability to flower under the prevailing climatic conditions although there were individual differences. Onset of flowering generally occurred between 2–4 months after planting, indicating that the accessions consisted of a mixture of early and medium flowering types.

Flowering and its control is one of the most important challenges in cassava breeding. Non-synchronized flowering can result in an inability to use cassava genotypes with desirable characteristics in breeding programmes (Davies *et al.*, 2008). Lebot (2009) observed that in some cassava clones, flowering can begin as early as 6 weeks after planting while in others it will occur only after at least 10 months after planting. To synchronize flowering between early- and medium-flowering accessions used in this study and, hence, facilitate artificial hybridization, the medium-flowering types have to be planted approximately 2 months ahead of the early- flowering types.

All the accessions studied produced functional male and female flowers except BA which produced dysfunctional male flowers with anthers devoid of pollen as revealed by light microscopy. It cannot, therefore, be used as a source of pollen for crosses. This phenomenon could probably be attributed to the occurrence of male sterility in this cassava accession. Male sterility has been frequently observed among cassava cultivars, and flowers produced by such clones produce empty anthers (Lebot, 2009).

The large numbers of male flowers produced in all the accessions confirm the observation made by earlier workers (Kawano 1980; Ogburia & Okele, 2001), who reported that male flowers of cassava usually outnumber the female flowers. Fukuda *et al.* (2002), however, pointed out that in a manual pollination programme, on the average only one male flower is needed to pollinate three to five female flowers. This suggests that for all the cassava accessions studied (except BA) male flower production may not be a limiting factor to mass hybrid seed production under manual and open pollination systems.

Unfavourable soil and climatic conditions, pests and disease incidence, pollination or fertilization failures and embryo abortion are the major causes of fruit drop in plants (Boateng, 1994). In this study, the very high rates of percentage fruit drop obtained in ME, AS, AN and HO-008 are similar to those reported by Boateng (1994) in TMS 4(2) 1425 (2×) and TMS 84/00136 (4×) under open pollination conditions at IITA. Nassar (1989) also indicated that hybrids of *Manihot pohlii* and other species of cassava can be obtained, but at very low frequencies due to inter-specific crossing barriers. The low rate of fruit drop observed in BS-1 could possibly be attributed to a very weak barrier to fertilization.

Since all the accessions were subjected to relatively similar edapho-climatic conditions embryo abortion might have been the principal cause of fruit drop observed in the cassava accessions. During fruit development embryos derive their supply of nutrients from the endosperm. Embryos, however, abort when they are deprived of nutrient supply as a result of abnormal endosperm development (Hogenboom, 1972; Thomas &Pratt, 1981; Bhojwani & Razdan, 1983).

None of the cassava accessions formed fruits at the first branching level. This confirms Doku's (1969) report that most flowers of cassava produced within the axil of a primary branch by varieties which branch at 1/3 or 1/2 way up the stem usually drop off long before the flowers are fertilized, compared to varieties whose primary branching occurs at the apex or at 2/3 of the plant's height which have their flowers persisting and fertilized within the primary, secondary or tertiary branch axils.

Among all the cassava accessions SE recorded the highest number of fruits at each level of branching. This could be attributed to the high number of branches developed. In contrast, AN and LA produced few fruits because they branched late and could neither produce large amounts of secondary and tertiary branches nor attain the level of branching observed in the other cassava accessions. In HO-008 the low rate of fruit production may be attributed to fertilization impairment coupled with fewer number of branches produced.

Among the cassava accessions studied, fruits matured between 55.7 and 62.9 days. On the other hand, Ghosh *et al*. (1988) reported that fruit maturity in cassava generally occurs 75–90 days after pollination. This disparity could be due to the fact that the accessions used in the current study were early-maturing than those they worked on.

Percentage seed set among the accessions was very high. The minimum seed set of 73.33% obtained in this study (for AN) far exceeds 33.3% obtained by Ogburia & Okele (2001) in their study of 10 cassava cultivars under natural pollination conditions. This could probably be attributed to the inherent quality of the female parents as suggested by Hershey (1981). The effectiveness of the pollination process as activities of pollinating agents especially bees were very intense during flower anthesis in all the accessions or absence of barriers to fertilization among the accessions (Jennings & Iglesias, 2002) as seed set was principally dependent on number of filled ovaries. However, seeds produced by the accessions recorded variable proportions of embryo formation.

Conclusion

Out of the nine cassava accessions studied eight (i.e. *Megyewontem* (ME), *Ankra* (AN), *BNARI Selection 1* (BS-1), *Afisiafi* (AF), *Security* (SE), *Larbi* (LA), *Asare* (AS) and *HO-008*) produced functional anthers and could be used as pollen parents in hybridization programmes. The accession *Bamboo Akwetey* (BA) was male sterile. It did not produce functional anthers and this poses limitation for its use as a pollen parent in future hybridization programmes. The number of male flowers produced per branching generally ranged from 35.4 to 58.6 among the cassava accessions, indicating that obtaining pollen for hybridization may not be a limiting factor provided planting of different cassava accessions is scheduled to synchronize flowering.

All the cassava accessions produced functional pistillate flowers and this resulted in fruit production among all the accessions. High number of pistillate flower production did not necessarily translate into high number of fruit production. *Afisiafi* produced the highest number of pistillate flowers per branching level but *Security* persistently produced the highest number of fruits at branching levels 2–6. In all the cassava accessions, no fruit production occurred at the first branching level even though there was flowering. Fruit production started at either the secondary or tertiary branching level. Fruit production per branching level among the cassava accessions was similar at the primary branching level but varied significantly at higher levels, with no obvious trend.

Though no clear relationship existed between seed set and embryo formation among the accessions, these generally ranged from 73.3% to 100% and 60.4% to 100%, respectively. Similarly, fruit drop rate ranged from 11.7% to 83.3%, and it was highest in *Megyewontem* (ME). Fruits of *Bamboo Akwetey* matured earlier than fruits of all other cassava accessions used for the study, taking 55.7 days. Fruits of *Asare* were, however, late-maturing and took 62.9 days.

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