Observations on Progenies in a Crossing Scheme between Cashew Clones: Establishment Characters

[1] Masawe*, P.A.L., E.P. Cundall[1,3] and P.D.S. Cali

[1]Cashew Research Project, Agricultural Research Institute, Naliendele, P.O. Box 509 Mtwara, Tanzania

[2] Department of Agricultural Botany, School of Plant Sciences, The University of Reading, Whiteknights, P.O. Box 221, Reading, RG6 6AS, UK

[3]Present Address: The Forestry Commission, Northern Research Station, Roslin, Midlothian, EH25 9SY, UK.

Abstract

A study was conducted to establish the genetical basis of the characters of importance in cashew production. Six clones held in the Tanzanian cashew germplasm collection, but of diverse origin, were selected for study. They were selected on the basis of showing phenotypic variation in a number of the main characters of agronomic interest as well as comprising one that was susceptible, and the other five putatively showing tolerance, to powdery mildew. These six clones were selfed and crossed in as many combinations as possible. This required that suitable techniques were developed to allow controlled pollination to be carried out. The crossing programme resulted in progenies of 13 hybrid combinations and 5 selfs. These were planted in 4 replications of 12-tree plots in the field at Naliendele Agricultural Research Institute, Mtwara, Tanzania along with vegetative propagules of the parent clones. Tree heights and canopy diameters were recorded three years after planting. The results showed that the growth of the seedlings was more vigorous than the clonal propagules of the parental clones. It was found that plant height was more highly heritable than canopy diameter at this stage in the establishment and hence would be more readily manipulated by selection in a breeding programme.

Key words: Cashew, crossing programme, height, canopy diameter, heritabilities

Introduction

Most of the cashew trees (*Anacardium occidentale* L.) grown in Tanzania, are planted as unselected seeds derived from parents which themselves possibly represent an inappropriate or narrow genetic base. However, the first stage in the Tanzanian cashew breeding programme was to identify superior trees from within the existing collections and farmers' fields (Masawe *et al.*, 1998). The position up to the present time in the cashew breeding programme at Naliendele has been the carrying out of sexual hybridisation between these parents chosen on the basis of their phenotypic expressions of the characteristics (Masawe and Millanzi 1996, Harries *et al.*, 1998), as is practised in other countries (Bhaskara-Rao, 1998). These chosen clones have also been used to establish "seed gardens". There is, however, still uncertainty about the characters that can be selected successfully in cashew and at what stage in the trees growth this can be carried out and how effective will particular intensities of selection be. This is because of the lack of biometrical-genetical information which would form the essential background to defining a more effective breeding strategy. Even the more simple genetical information on major gene determined traits, which might provide the means to answer basic questions, such as the natural outcrossing rate in cashew, has been absent until recently (Masawe and Caligari, 1998).

* Corresponding author

Reproduced by Sabinet Gateway under licence granted by the Publisher (dated 2012)

Similarly, there is no information about the level of selection intensity that can be applied to produce any predicted outcome. Therefore, biometrical-genetical analyses are important in allowing the future planning of suitable future breeding strategies. For example, the identification of any character with a high heritability and which is expressed early in development will clearly be useful in opening the possibility of a rapid response when subject to selection in the early establishment phase. Whereas characters having low heritability and expressed late in development will only allow slow progress to be made and efforts possibly wasted unless suitable trial designs and plot sizes are used alongside appropriate ways of measuring the character. Clearly, if such features can be established the way would then also be open to develop modern methods, for example trait tagging using molecular markers, in order to improve selection efficiency in the future (Bos & Caligari, 1995).

An ambitious programme was therefore undertaken to give evidence of the genetical basis of as many characters as possible that might be of significance in determining the agronomic performance of cashew genotypes. The present results represent the results from the early years of the material generated and planted to provide this essential information. Thus the specific aim of this part of the study was to determine which of the characters that were measurable in the first few years of growth would respond, in their own right, most readily to selection. It is also anticipated that measurements in later years would be compared and correlated measurements in the present study.

Materials and Methods

This study was conducted at the Naliendele Agricultural Research Institute is located south of Mtwara town. It occupies a piece of a flat land near the eastern edge of the Makonde plateau (Ngatunga, 1988), at a latitude of 10° 22´S, longitude of 40° 11´E and an altitude of 120m above sea level (Masawe, *et al.*, 1996. The mean annual temperature is about 26°C and mean annual rainfall is around 1160 mm which normally falls in a single six month season, November-April (Ngatunga, 1988). The soils at the Institute being derived from sandstones are deep and coarse textured in nature. Soil fertility levels are low and soil structures are weakly developed and available moisture percentages are also very low (Ngatunga, 1988). The top soils have loamy sand textures changing with depth to sand soils.

Five clones were chosen as promising parents, showing some resistance to powdery mildew (caused by the fungus *Oidium anacardii* Noack) and good commercial attribute, on the basis of previous data and breeders' notes on the germplasm collection at Naliendele. These clones which had been assembled in earlier years and had the identifiers of: AC4, AC10, AC43, AZA2 and AZA17. Where AC designates the clone as being of Sri Lankan origin and AZ from Zanzibar. In addition, ATA19 (ATA indicating origin being in Tanzania) was additionally selected as a clone which appeared to be susceptible to mildew. It was attempted to cross the six parental clones in all combinations which would have given the 6 selfs and 30 crosses, including reciprocals.

It may be noted that no earlier reports giving details of any hybridisation scheme being attempted in cashew were found and so the following protocol was developed. Preparations for crossing were made by painting a band of grease (6-12 cm wide) around the tree trunk and supporting poles, which were used to support the lower branches. To further reduce the frequency of ants and other crawling insects, weeding was carried out to create a circle of bare ground (to the edge of the tree canopy), while grass in the inter-rows was frequently slashed. At the same time the whole tree was sprayed with insecticide (*Karate*, l-cyhalothrin ULV 6) to eliminate any crawling insects. In addition, powdery mildew disease (*Oidium anacardii* Noack) was controlled using wettable sulphur at 14 day intervals.

Panicles of the selected female parents were bagged using locally made paper bags to exclude potential pollinating insects. Each bagged panicle was checked daily between 07.30h and 10.00h and all male flowers were removed using fine watchmaker's forceps. Only the hermaphrodite flowers were left, and since the stigmas

Reproduced by Sabinet Gateway under licence granted by the Publisher (dated 2012)

are always located above the anther, there was little chance of self-pollination occurring, especially having eliminated pollinating insects. To further ensure that no self-pollination occurred, given that in hermaphrodite flowers the stigma emerges before the anther, it was immediately hand pollinated on emergence.

Male parents were harvested between 10.00h and 11.00h every morning by completely detaching the male flowers and placing them in a covered petri dish ready for use. The bag containing the appropriate female flowers was opened and, using sterilised watchmaker's forceps, the anther of the male flower was touched on the stigmas of several flowers in the bag. Where necessary two or more male flowers were used per panicle. After pollination the bag was resealed.

Successful pollination was indicated by swollen ovaries which, after one to two months, gave rise to nuts which were big enough for the bags to be opened and the nuts labelled. All pollinations were carried out between June and December 1990. Not all attempted crosses or selfs were successfully achieved, as is indicated in Table 1, but the crossing programme did give rise to 13 hybrids and 5 selfs. The resulting nuts were planted in polythene pots in December 1990 and the subsequent seedlings were transplanted to the field at Naliendele Agricultural Research Institute, in March 1991 in a randomised complete block design with 4 replicates, and 12 trees per plot. Clonal propagules of the parents (scions grafted onto seedling rootstocks) were randomised around the periphery of the experiment, as 4-tree plots, to provide contemporary comparisons with the seedlings and additionally to act as guard rows.

The characters recorded were height and canopy diameter measured in cm, 3 years after transplanting. However, the distribution of both characters proved to show non-normal error distributions, and so they were transformation to $\log_{10}$. The analyses were carried using SAS (SAS Institute, North Carolina, USA), and taking the type III Sums of Squares Proc GLM because of the missing values. There were three groups of cashew material in the experiment, namely parental clones, hybrids and selfs (the later two being collectively referred to as "crosses").

Results

The results of the analyses of variance for height and canopy diameter of the "crosses" are presented in Table 2. As can be seen there was evidence of differences between "crosses" for both characters and this was evident even when judged against the significant blocks x crossed interaction. The parental clones, however, although showing significant differences for height failed to do so for canopy diameter (Table 3). From Table 4 it can be seen that the means of the seed derived material were greater than those of the parental clones for both height and canopy diameter.

The heritability of height for these lines was estimated by regression of offspring means onto midparent values (Falconer & Mackay, 1996;

Table 1: Crossing programme (Partial Diallel) schematic diagram.

Female parent (E)	Male parent (G)					
	AC4	AC10	AC43	AZA2	AZA17	ATA19
AC4	+	-	-	-	-	-
AC10	-	+	+	-	+	+
AC43	-	-	+	-	-	+
AZA2	+	+	-	-	-	+
AZA17	+	+	+	-	+	+
ATA19	+	+	-	-	-	+

note:
a.+""Crosses"" with more than 48 successfully germinated nuts.
b.-""Crosses"" with less than 48 (or no) successfully germinated nuts.

Reproduced by Sabinet Gateway under licence granted by the Publisher (dated 2012)

Table 2: Analysis of variance for the 18 "crosses"[1,2]

Source	df	Mean Squares	
		Height	Diameter
Blocks	3	0.093***	0.558***
"Crosses"#	17	0.095***	0.187***
Blockx"Crosses"	51	0.025***	0.048***
Error	737	0.011	0.024

[1] in this and table 3, ***, ** and * means significantly different at p<0,001, p<0.01 and p<0.05 respectively. #*The significance levels of "Crosses" was unchanged whether tested against the Error or Block*"Crosses" MS.*

Table 3: Analysis of variance for all 6 parental clones

Source	df	Mean Squares	
		Height	Diameter
Blocks	3	0.063***	0.032
Clones#	5	0.125*	0.109
BlockxClones	15	0.038**	0.066*
Error	155	0.016	0.036

Table 4: The means of "crosses" (hybrids and selfs), along with the clonal propagules of the parents, for height (upper number) and canopy diameter (lower number in italics) in cm. (No standard

Male / Female	AC4	AC10	AC43	AZA2	AZA17	ATA19
AC4	269.1					
	218.8					
AC10		229.1	257.0		275.4	245.5
		154.9	186.2		208.9	177.8
AC43			234.4			302.0
			166.0			234.4
AZA2	218.8	239.9	-			257.0
	169.8	204.2				204.2
AZA17	275.4	302.0	288.4	-	251.2	281.8
	213.8	245.5	239.9		199.5	239.9
ATA19	275.4	331.1	-	-	-	302.0
	213.8	275.4				223.9
Clones	138.0	154.9	186.2	134.9	158.5	190.5
	95.5	107.1	128.8	89.1	104.7	123.0

Ditlevsen; 1985; Mahlenbacher *et al.*, 1993). The slope of the regression (b, the regression coefficient) is equal to the narrow sense of heritability (h^2_n) for that character. This gave an estimated narrow heritability of 44% for height. No heritability estimate was possible for canopy diameter since no significant differences between the parental clones were detected.

Among the hybrids there were 2 families in which the reciprocal crosses had been achieved and these were therefore analysed separately. The analysis again showed highly significant differences between blocks, crosses and their inter-

Reproduced by Sabinet Gateway under licence granted by the Publisher (dated 2012)

Table 5: Analysis of variance for hybrids with reciprocals

Source	df	Mean Squares Height	Diameter
Blocks	3	0.036***	0.143***
"Crosses"	3	0.120***	0.272***
Reciprocals	1	0.299***	0.689***
Families	1	0.0015	0.000
Recip x Fam	1	0.083***	0.134***
Blockx"Crosses"	8	0.046***	0.072***
Error	166	0.012	0.023

*** = P<0.001 ** = P<0.01

action for both height and canopy diameter (Table 5). But more interestingly when they were tested for reciprocal effects both the differences between reciprocals and the interaction of reciprocals x families were highly significant (P<0.01), although there were no significant differences between the families themselves (Table 5). This indicates that the direction that the crosses were made affects the height of the subsequent progenies.

Discussion

For the first year after planting (1991) the rainy season was late and this meant that losses of trees were quite high but these were replaced by others which had been planted elsewhere at the same time and kept for replacement purposes. Therefore each hybrid or self was represented as far as possible by 48 planted trees, and the few trees that were eventually missing were mostly attributable to termite and wind damage.

The results reported here are those of vegetative measurements in the establishment phase of the trial. The results of the 1990 crossing programme have shown that hybridisation in cashew, by controlled hand pollination, is achievable. However, further investigation is required to optimise the use of a wide range of germplasm in crossing programmes. For example, clone AC4, when used as a female in cross-pollination was only moderately successful using our technique, while clone AZA2 when used as a male gave disappointing pollination results. In general, however, there appear to be no barriers to selfing in this species with only the selfing of clone AZA2 giving low numbers of fruit/nut set at maturity. Thus there is a need to refine our pollination protocols slightly but there is no evidence of any incompatibility system operating in this species.

The results for height and canopy diameter have generally shown that "crosses" differed from the parental clones. The differences may be traceable to two factors: -

(i).The "crosses" were transplanted as seedlings which were raised in polythene bags resulting in vigorous growth but the parents were tip grafted onto seedlings (Milheiro and Evaristo, 1994; Behrens; 1996; Bashiru, 1998), also in polythene bags, and transplanted later and/or
(ii)"crosses" tended to grow vegetatively while the parental clones tended to grow reproductively because the scion wood buds used was at a mature stage i.e. flowering stage (Ohler, 1978).

This is also the possibly supported by the significant differences detected between reciprocals in which the presence of maternal effects (Kearsey & Pooni, 1996) in the transmission of the character studied is suggested. For example, clone AC10 when used as a male parent on female parents ATA19 and AZA17 resulted in moderately tall progenies but when used as a female to cross with the same parents the plant height was significantly less (Table 4). The reciprocal effects (Hill *et al.*, 1998) on height and diameter strongly indicate that the direction of the cross must be taken into account in designing future cashew breeding schemes. However, the reciprocal effects observed here warrant further investigation because of the limited number of crosses so far investigated. More reciprocal crosses would help clarify the situation.

Reproduced by Sabinet Gateway under licence granted by the Publisher (dated 2012)

An important outcome of these early results is the relatively high heritability estimate found for height. At 44% this suggests that selection could be practised for this character at this stage and that a useful positive response could be obtained (Bos &Caligari, 1995). The results available from future years, particularly allied to those for yield, will be essential in further elucidating the underlying genetic control of characters in cashew and in determining breeding strategies.

Acknowledgements

Financial support from the Tanzanian Government and the British Government, through ODA (now DfID), is gratefully acknowledged. We also wish to thank the help of the technical staff of the Breeding Section, ARI Naliendele for their assistance in the experimental work.

References

Bashiru, R.A. 1998. Studies on vegetative propagation methods of cashew in Tanzania. *Proc Int* Cashew & Coconut Conf, Tanzania (1997), BioHybrids Int Ltd, Reading, UK, 302-308.

Behrens, R.1996. Cashew as an agroforestry crop. Prospects and Potentials, Tropical Agriculture- 9, (GTZ), Margraf Verlag, Weikersheim Germany, 56p.

Bhaskara Rao, E.V.V. 1998. Cashew crop improvement programmes in India. *Proc Int Cashew & Coconut Conf*, Tanzania (1997), BioHybrids Int Ltd, Reading, UK, 141- 145.

Bos, I and Caligari, P.D.S. 1995. *Selection Methods in Plant Breeding*. Chapman & Hall, London pp347.

Ditlevsen, B. 1985. Quantitative genetics. General principles and practical application in forest tree improvement. In FAO Forestry Paper 20. Forest tree improvement. *Report on FAO/DANIDA Training Course on Forest Tree Improvement, Merida, Venezuela January-February 1980. FAO Rome, 1985, 131-139.*

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics. 4th edition. Addison Wesley Longman Ltd, UK. pp 464.*

Harries, H.C., Kusolwa, P.M., Millanzi, K.J. and Masawe, P.A.L. 1998. A cashew breeding scheme for Tanzania. *Proc Int Cashew & Coconut Conf*, Tanzania (1997), BioHybrids Int Ltd, Reading, UK, 128-133.

Hill, J.,Becker, H.C. and Tigerstedt, P.M.A. 1998. *Quantitative and Ecological Aspects of Plant Breeding. Chapman & Hall, London. pp. 275.*

Kearsey, M.J. & Pooni, H.S. 1996. *The Genetical Analysis of Quantitative Traits. Chapman & Hall, London. pp. 381.*

Mahlenbacher, S.A., Smith, D.C. and Brenner, L.K. 1993. Variance components and heritability of nut and kernel defects in hazelnut. *Plant Breeding 110: 144-152.*

Masawe, P.A.L., E.P. Cundall and Caligari, P.D.S. 1996. Distribution of cashew flower sex-types between clones and sides of tree canopies in Tanzania. Annals of Botany, 78: 553-558.

Masawe, P.A.L. and Millanzi, K.J.K. 1996. Investigation into difficulties in controlled hand pollination between selected cashew clones, (*Anacardium occidentale* linn). Tanzania Agricultural Research and Training Newsletter Vol. XI. Nos. 1-4: 5-8.

Masawe, P.A.L. and Caligari, P.D.S. 1998. Investigation of cashew apple colour and out-crossing in cashew (*Anacardium occidentale L.). Proc Int Cashew & Coconut Conf, Tanzania (1997), BioHybrids Int Ltd, Reading, UK, 134-137.*

Masawe, P.A.L., E.P. Cundall and Caligari, P.D.S. 1998. Performance in south-eastern Tanzania of local cashew germplasm and selected clones. *Proc Int Cashew & Coconut Conf*, Tanzania (1997), BioHybrids Int Ltd, Reading, UK, 146-150.

Milheiro, A.V. and Evaristo F.N. 1994. Manual do Cajueiro, Cultivars-Associsção de técnicos de culturas tropicais, Portugal

Ngatunga, E.L.N. 1988. Soils of the Agricultural Research Institute TARO-Naliendele, National Soils Services, TARO Agricultural Research Institute Mlingano Tanga Tanzania. *Site Evaluation Report 98.*

Ohler, J.G. 1978. *Cashew*. Koninklijk Inst voor de Tropen, Amsterdam. pp 260.

SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc. Cary, North Carolina.

Reproduced by Sabinet Gateway under licence granted by the Publisher (dated 2012)