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Yield traits as selection indices in seedling populations of cassava

Henry Fred Ojulong[1], Maryke Tine Labuschagne[2*], Liezel Herselman[2] and Martin Fregene[3]

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ABSTRACT - *The cassava breeding scheme currently used is long, because initial stages concentrate mainly on improving yield, with root quality selection following later. To shorten the scheme, yield and root quality should be selected simultaneously, starting at the seedling nursery. In this study, a nursery comprising of eight cassava families and 1885 seedlings developed from parents adapted to three major agro-ecologies, were evaluated for yield related traits in Colombia. Percentage dry matter content (DMC) and harvest index produced similar ranking of the parents. Tuber yield, weight, and number showed potential of increasing yield through conventional breeding. A selection index including fresh root yield, percentage DMC, root weight and roots per plant, with heavier weights being assigned to root weight and roots per plant, should be used.*

***Key words**:* Manihot esculenta, *root characteristics, seedlings.*

INTRODUCTION

In cassava improvement, the breeder's primary concern is to increase the root yield of varieties that are resistant to major diseases and pests (Mahungu 1987, Fukuda et al. 2002, Ceballos et al. 2004). Root yield is usually assessed visually as the number, size and shape of roots. Selection for root quality is normally carried out at advanced stages of yield trials when clones being evaluated are reduced in number (Mahungu 1987). Root quality and yield related characteristics often considered are cyanogenic glycosides content, percentage dry matter content (DMC) and harvest index (HI). Percentage DMC determination is very useful because of its association with other root quality characteristics and its economic relevance (Mahungu 1987, Weçolovis et al. 2003, Lenis et al. 2006). Harvest index is used because it is highly correlated with root yield and has a high heritability (Kawano et al. 1998).

The present breeding scheme extends the breeding cycle by many years. To reduce the cycle, it might be necessary to simultaneously select for yield and root quality during the earliest stages of selection. Moderate to high heritability values have been reported for DMC in roots (52%), indicating that selection of clones can be effectively carried out at an early stage (Mahungu 1987). Kawano (1978) has shown that selecting for high HI in seedling plants as well as clones in single-row evaluation (clonal evaluation) is more effective in identifying high-yielding genotypes than using root yield itself as selection criterion. Furthermore, root number, a trait affecting yield, is known to be fixed early on during the plant's breeding cycle

[1] International Center for Tropical Agriculture, Apartado Aero 6713, Cali, Colombia.
[2] University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa. *Email: labuscm@ufs.ac.za
[3] Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, Missouri 63132, USA.

(Wholey and Cock 1974), and can be used as an early selection criterion. Quality is, however, difficult to evaluate as such, since it is a composite of many root characters.

The aims of this study were to evaluate a seedling nursery specifically developed for yield characteristics, and assess the different yield components for use as selection indices at seedling stage.

MATERIAL AND METHODS

Genotypes with high general combining ability (GCA) for percentage DMC or yield identified through studies by Jaramillo et al. (2005); were crossed following standard procedures described by Kawano (1980). Parents for crossing were planted in a crossing block in CIAT, Palmira. Entries were planted in single rows of 1 m between plants and 2 m between rows, to facilitate movement. Three weeks after pollination fruits were covered with netting bags to catch the seed when the ripe fruits dehisce explosively. Seed was collected from the field after 60 days, cleaned and labelled, ready for planting.

Seed was planted in a screen house in pots filled with sterilised soil, mixed with sand in a ratio of 1:1. Plantlets were watered regularly and fungicide was applied occasionally to prevent fungal infection arising from the humid conditions. When seedlings were 20 to 25 cm tall, they were transplanted to a well-prepared seedbed in the field, in Palmira, Colombia. Palmira is located at lat 3^0 06' N, long 76^0 32' W and 965 m asl; has an annual temperature of 24 ^{0}C with clay soil texture (Jaramillo et al. 2005, Kawano 2003). Seedlings were planted on ridges 1.5 m apart, with spacing of 0.6 m between plants. Daily irrigation was done during the first week, after which rainfall served as water supply. Hand weeding was done three times, and thereafter weeds were controlled using herbicide. Immediately after planting, nitrogen fertiliser was applied around plants to boost them after the long period in the screen house. Thereafter, fertiliser was selectively applied to weak plantlets. Pesticide was periodically applied against the prevalent pest, cassava green mite depending on the incidence.

Six months after planting (MAP) the seedlings were genotyped using microsatellites (data not shown) and families assigned following the CIAT naming system. Seedlings within a family were assigned numbers, with the first seedling assuming number one and the rest subsequent numbers. Parents were not included as they were from stem cuttings and could not be used for comparison purposes. At harvest (12 MAP), individual plants were harvested, the harvestable biomass divided into storage roots, and vegetative biomass, comprising leaves and stems. Roots were weighed to obtain fresh root yield. Roots which pass for sale in the local supermarkets were selected and counted to give number of commercial roots. Harvest index was calculated by dividing fresh root yield by total biomass. Percentage DMC of the roots was estimated using the standard CIAT procedure (Kawano et al. 1987, Jaramillo et al. 2005). Dry yield was derived as a product of fresh root yield and DMC.

Simple statistics were performed using the Excel programme. Because of the large number of progeny from each parent, progeny from a given parent was taken to represent a random population and its mean performance was used to estimate the parents' breeding value. The relative contribution of the different traits to the genotype performance was estimated by principal component analysis (PCA) and stepwise regression performed using Agrobase (2005).

RESULTS AND DISCUSSION

A total of 2935 seeds from eight crosses were produced, of which 1885 plantlets, representing 64.2% germination, were established in the field. Of the transplanted plants, 1453 (77.1%) resulted in plants vigorous enough to produce sufficient biomass for evaluation. At the seedling stage a relatively high number of roots per plant (RtPlt) was obtained (average 6.67), with clone GM 901-192 having the highest number of 19 (Table 1). The average commercial sized storage roots (ComRt) was 2.17, with clone GM 901-263 having the highest number of 15 roots. Clones with the highest number of roots, both total and commercial, were from families GM 901 and CM 9953, both of which had SM 1741-1 as a common parent. Highest root weight (RtWt) was recorded for GM 252B-159, with the top five clones all coming from the same family. Harvest index estimates ranged from 0.05 (GM 252B-215) to 0.90 (GM 853-13). Recorded DMC ranged from 16.3% in GM 252-307 and CM 9953B-030 to 69.1% in GM 901-270 (Table 1). This range was higher than what was reported by Magoon et al. (1973) of 20.0% to 47.2% in crosses between one female parent and three different high yielding male parents, but fell within the range later reported by Rajendran and Hrishi (1982) of 19.2% to 66.4% among four high, medium and low DMC parents. Highest yield was recorded in GM 536-

Table 1. Simple statistics of agronomic variables evaluated on the seedling nursery (1453 genotypes)

Variable	Minimum	Maximum	Average	Standard deviation
RtPlt[a]	1.00	19.00	6.67	3.08
ComRt[b]	0.00	15.00	2.17	1.79
RtWt[c]	0.01	1.33	0.25	0.15
HI[d]	0.05	0.90	0.48	0.14
DMC[e]	16.30	69.10	32.10	4.80
FRY[f]	0.10	14.67	2.69	0.86
DRY[g]	0.03	4.33	0.86	0.28

[a] Roots per plant; [b] Commercial roots per plant; [c] Root weight (kg); [d] Harvest index (0-1); [e] Dry matter content (%); [f] Fresh root yield ; [g] Dry root yield (t ha^{-1}).

146 (14.67 t ha^{-1}) followed by GM 536-79 (8.6 t ha^{-1}) and GM 901-304 (13.33 t ha^{-1}), while highest DRY was recorded in GM 536-79 (4.33 t ha^{-1}) and GM 901-329 (4.27 t ha^{-1}). Only genotypes with measurable biomass were evaluated, others were cloned to provide planting material for further studies.

Data was averaged per family to give an indication of a family's performance (Table 2). Family GM 256 recorded the highest average number of roots (7.6) and GM 853 the lowest (5.9). Families GM 901 and GM 847 had the highest average HI estimates, 0.53 and 0.50 respectively, while GM 256 had the lowest (0.37). Highest percentage DMC was estimated in GM 901 (33.5%), GM 256 (33.3%) and GM 847 (33.2%) and the lowest in GM 9958 (28.6%). Average fresh root yield (FRY) ranged from 1.31 to 2.01 t ha^{-1} in families GM 9958 and GM 536 respectively. Families GM 536 and GM 853 out yielded others, with DRY of 0.61 and 0.56 t ha^{-1} respectively, while the lowest, GM 9958, yielded 0.39 t/ha.

Average performance of progeny from a parent was used to estimate its breeding value (Table 3). Dry and fresh root yield, RtWt, and ComRt ranked the parents in the same direction. Percentage DMC and HI ranked parents the same but in the opposite direction for other yield characteristics. Jaramillo et al. (2005) noted that HI had the highest correlation with DMC and that in some parents there was a negative association between DMC and yield potential. Roots per plant, on the other hand, ranked parents in two groups of low and high, indicating that visual evaluation alone could be enough for selection. SM 1411-5 and MTAI-8 were ranked the poorest parents for almost all traits. Overall rating ranked MPER 183 and CM 4574-7 as best parents followed by SM 1565-15 and SM 1665-2. Jaramillo et al. (2005) reported that MPER 183 had the highest GCA for several traits. Most breeders, when selecting, do not take into consideration all traits but use a selection index which in most cases consist of FRY, HI and DMC.

The selection index (SI_j) for genotype used was:

$$SI_j = [FRY * 10] + [DMC * 8] - [PT * 3] + [HI * 5]$$

where, PT = plant type using a 1(excellent) to 5 (very poor) visual scale and the rest as described earlier.

When parents were ranked based on FRY, DMC, and HI, the best parent was MPER 183 followed by SM 1741-1, SM 1665-2 and SM 805-15. Jaramillo et al. (2005) observed that SM 1741-1 had a good performance as a parent across the different variables evaluated. Ranking by selection index followed the same ranking as for HI and DMC, while ranking by all traits followed the ranking by yield and storage root numbers. From this, it is apparent that by using the current selection index, breeders may not be maximising the potential of the genotype.

Simple correlation analysis showed DRY to be highly significantly correlated ($P \leq 0.001$) to all other traits (Table 4), signifying that all contribute to economic yield. Fresh root yield was correlated ($P \leq 0.001$) to all traits except DMC. No

Table 2. Means and standard errors of root quality characteristics of eight families evaluated at harvest

Family	Pedigree	Variable						
		RtPlt[a]	ComRt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]
GM 901	SM1741-1 x MPER183	6.7±3.0	2.2±1.7	0.25±0.13	0.53±0.13	33.5±5.0	1.61±1.2	0.54±0.4
GM 9953	SM1741-1 x SM1219-9	6.3±2.7	1.7±1.5	0.23±0.14	0.48±0.16	31.7±5.5	1.42±1.2	0.45±0.4
GM 9958	SM1411-5 x MTAI-8	6.7±2.9	1.5±1.2	0.20±0.11	0.46±0.17	28.6±6.7	1.31±0.9	0.39±0.3
GM 853	CM8027-3 x CM6754-8	5.9±2.8	2.3±1.5	0.35±0.21	0.46±0.17	29.2±3.1	1.90±1.2	0.56±0.4
GM 252	SM1665-2 x SM805-15	6.5±3.1	2.1±1.7	0.25±0.18	0.48±0.14	32.2±4.4	1.64±2.0	0.53±0.7
GM 847	SM1411-5 x CM4574-7	7.2±1.9	1.7±1.6	0.24±0.23	0.50±0.18	33.2±3.7	1.58±1.3	0.55±0.5
GM 256	SM1219-9 x SM1565-15	7.6±3.2	2.1±1.5	0.20±0.12	0.37±0.15	33.3±3.6	1.45±0.9	0.48±0.3
GM 536	CM4574-7 x SM1565-15	7.1±3.3	2.9±2.4	0.28±0.18	0.47±0.13	30.4±3.8	2.01±1.6	0.61±0.5
Total		6.7±3.1	2.2±1.8	0.25±0.16	0.48±0.15	32.0±4.8	1.64±1.6	0.53±0.5

[a] Roots per plant; [b] Commercial roots per plant; [c] Root weight (kg); [d] Harvest index (0-1); (t ha^{-1}); [e] Dry matter content (%); [f] Fresh root yield; [g] Dry root yield (t ha^{-1}).

Table 3. Means and rankings of root quality characteristics of progeny from nine parents evaluated at harvest

Parent	Variables									
	Number of seedlings	RtPlt[a]	ComRt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]	DRY[g]	Total Ranking	Ranking by index selection
MPER 183	309	6.7 (3)[h]	2.2 (3)	0.246 (4)	0.53 (1)	33.5 (1)	1.61 (3)	0.54 (3)	1	1
SM 1741-1	524	6.5 (7)	2.0 (5)	0.237 (6)	0.51 (2)	32.8 (2)	1.53(6)	0.50 (6)	6	2
SM 1665-2	536	6.5 (7)	2.1 (4)	0.248 (3)	0.48 (4)	32.2 (3)	1.57(5)	0.51 (5)	4	3
SM 805-15	520	6.6 (7)	2.0 (5)	0.240 (5)	0.49 (3)	32.0 (5)	1.58(4)	0.53 (4)	5	3
CM 4574-7	236	7.1 (2)	2.9 (1)	0.280 (1)	0.47 (5)	30.4 (7)	2.00 (1)	0.61 (1)	1	5
SM 1565-15	315	7.2 (1)	2.7 (2)	0.258 (2)	0.44 (9)	31.2 (6)	1.86 (2)	0.58 (2)	3	6
SM 1219-9	300	6.7 (3)	1.8 (7)	0.217 (7)	0.45 (8)	32.1 (4)	1.43 (7)	0.46 (7)	7	7
SM 1411-5	55	6.7 (3)	1.5 (8)	0.205 (8)	0.46 (6)	29.1 (8)	1.34 (8)	0.40 (8)	8	8
MTAI-8	49	6.7 (3)	1.5 (8)	0.200 (9)	0.46 (6)	28.6 (9)	1.31 (9)	0.39 (9)	9	9
Total	2844	6.7±3.1	2.2±1.8	0.248+0.16	0.48±0.15	32.0+4.8	1.6±1.6	0.5±0.5		

[a] Roots per plant; [b] Commercial roots per plant; [c] Root weight (kg); [d] Harvest index (0-1); (t ha^{-1}); [e] Dry matter content (%); [f] Fresh root yield ; [g] Dry root yield (t ha^{-1}); [h] Numbers in brackets are the rankings.

Table 4. Simple phenotypic correlations of yield related traits evaluated on a seedling nursery

	ComRt[a]	RtPlt[b]	RtWt[c]	HI[d]	DMC[e]	FRY[f]
RtPlt	0.610**[h]					
RtWt	0.340**	-0.060				
HI	0.180**	-0.001	0.320**			
DMC	-0.001	0.090**	0.002	0.110**		
FRY	0.680**	0.530**	0.550**	0.240**	-0.040	
DRY[g]	0.660**	0.490**	0.140**	0.250**	0.210**	0.960**

[a] Number of commercial roots; [b] Roots per plant; [c] Root weight (kg); [d] Harvest index; [e] Dry matter content (%); [f] Fresh root yield (t ha^{-1}); [g] Dry root yield (t ha^{-1}); **pd"0.001.

association was detected between FRY and DMC. Kawano et al. (1998) also observed the lack of association between FRY and DMC at earlier stages of selection and concluded that FRY and DMC can be handled largely as independent characters. This is of interest especially to national breeding programmes. In cassava breeding, large numbers of genotypes are handled at the seedling stage. Most of the programmes do not have the resources to evaluate DMC for such high numbers. Evaluation for FRY can be done at seedling stage and DMC included at a later stage, since selecting for yield alone at seedling stage would not affect DMC.

Percentage DMC was highly significantly correlated ($P \leq 0.001$) with RtPlt, HI and DRY. Harvest index was highly significantly correlated with all traits except RtPlt, an indication that it is suitable for use in indirect selection for yield. Kawano et al. (1998) observed that indirect selection for yield through HI at earlier stages of selection was more effective than direct selection using yield itself.

PCA was used to help explain the relative contribution of the various traits to the genotypes' performance. Eigenvalues of the first four PCs are presented in Table 5. The first four PCs accounted for 94.8% of the total variation. PC1 accounted for 51.0% of the total variance and had an eigenvalue of 3.56 indicating that it had at least four major contributing factors. Main factors for PC1 were FRY, ComRt, RtWt and RtPlt. All factors were positively correlated. PC2 accounted for 19.0% of the total variance, and indicated HI, RtPlt and RtWt as the next set of factors contributing to variation. PC3 accounted for 14.1% indicating DMC and RtWt as the next factors while PC4 accounted for 10.7% with HI and RtWt as the main factors.

Table 5. Principal component coefficients of the various traits with loadings of the various yield related traits evaluated on eight seedling families

Trait	PC1	PC2	PC3	PC4
RtPlt[b]	<u>0.339</u>[h]	<u>-0.555</u>	0.113	0.388
ComRt[c]	<u>0.458</u>	-0.156	0.048	0.112
RtWt[d]	<u>0.357</u>	<u>0.479</u>	<u>-0.237</u>	<u>-0.456</u>
HI[e]	0.099	<u>0.619</u>	-0.068	<u>0.775</u>
DMC[f]	0.063	0.234	<u>0.956</u>	-0.114
FRY[g]	<u>0.517</u>	-0.005	-0.097	-0.079
Eigenvalue	3.56	1.33	0.99	0.74
Percent total				
Variance	51.0	19.0	14.1	10.7
Cumulative	51.0	70.0	84.1	94.8

[a] Principal component; [b] Roots per plant; [c] Commercial roots per plant; [d] Root weight (kg); [e] Harvest index (0-1); [f] Dry matter content (%); [g] Fresh root yield (t ha^{-1}); [h] Underlined values are the loadings of traits contributing most to the principal component.

The main contributors to PC1 (FRY, ComRt, RtWt, and RtPlt) are traits used by breeders in the seedling trial stage. Major contributors in PC2. PC3 and PC4 (HI, DMC, RtPlt and RtWt) are used in seedling and advanced trial selection. Selection based on all these traits at the earliest stage would therefore save a lot of time and resources. Storage root weight was a major contributor to all PCs, while RtPlt was a major contributor in PC1 and PC2 indicating their relative importance to root yield. Varma and Mathura (1993), reported significant correlation ($P \leq 0.01$) between yield and mean storage root weight, and between yield and number of marketable storage roots in India. They suggested that effective direct selection for yield would be obtained through clump characteristics via storage root weight per clump, number of marketable storage roots per clump and indirect selection through storage root weight, length and girth. Mahungu (1987) and Ojulong et al. (2008) reported that storage root yield was highly correlated to the number of storage roots and suggested the existence of a good fit between expected and observed values for genetic progress. The negative correlation between storage root number and weight indicates that a compromise has to be made, since improving both is unlikely. Where cassava is consumed boiled, most consumers prefer medium sized tubers, indicating that by fixing tuber size, a breeder can exploit the increase in root number.

A stepwise regression, using $P \leq 0.05$ entry and exit values, included DMC, RtWt, RtPlt and FRY in the model and rejected ComRt and HI. Of the included traits, FRY had the highest coefficient (0.31), followed by RtWt (0.04), DMC (0.01) and RtPlt (0.002). Since DRY is directly derived from FRY and DMC, and as such they are expected to be highly correlated, it was excluded from the analysis. The regression analysis showed the importance of RWt and RtNo in yield determination. This agrees with the findings from PCA above.

Results from this study indicate that it is possible to select simultaneously for yield and quality characteristics at seedling stage as most of the quality characteristics have been shown to be fixed at earlier stages. Since percentage DMC is independent of yield, the two can concurrently be selected for, with no detrimental effect on either. Use of a selection index should include RtWt and RtPlt, in addition to FRY, HI and percentage DMC since these are important to root yield. Based on the relative contribution of the traits, the heaviest weight should be given to RtWt and RtPlt. Breeders should shift emphasis from HI and percentage DMC as the primary selection indices to other yield components like root numbers and weight. Lenis et al. (2006) suggested selecting for stay green as an alternative to selecting for high HI. Since total photosynthesis of the crop sets the ceiling for the dry biomass (Kawano et al. 1998) increasing the leaf life cycle by selecting stay green genotypes is likely to increase yield. Selecting for genotypes with low HI and high leaf area index will increase the amount of biomass translocated for DMC and FRY. From the performance of the families it is apparent that different families perform better for specific yield traits. This indicates that a breeder has to make specific crosses for the different yield traits.

The high variability of percentage DMC at seedling level (16.3 to 69.1%) compared to the usual 20 to 40% at advanced stages means that a lot of diversity is lost in earlier stages where up to 99% of the starting breeding population is lost before root quality selection (Kawano et al. 1998). The very high percentage DMC could be due to pronounced taproot system in some seedlings (not desirable) or it could be seedlings with high percentage DMC and low yield, which under the current system will automatically be discarded. These seedlings with high percentage DMC and low yield could actually be used as parents for increasing percentage DMC. This is also true for HI (0.05 to 0.90%) where clones with low HI (more vegetative material) can be selected for forage making.

Seedling nursery results and the diallel analysis (Jaramillo et al. 2005) both identified MPER 183 and SM 1741-1 as good parents for a number of traits. This indicates that a breeder does not necessarily have to make a diallel cross to select good parents, but can actually select from ordinary cross data, if the number of genotypes per cross is high. Ceballos (personal communication) is in agreement with this and is using it in the CIAT breeding programme for selecting parents.

CONCLUSIONS

It is possible to generate data for parental selection from progeny generated from conventional crossing provided a large number of siblings are generated from the respective crosses. Simultaneous selection of yield and quality traits can be carried out at earlier stages of selection with the help of simple statistical methods like stepwise regression and principal component analysis. Breeders should do simultaneous selection starting from the earliest stage-the seedling nursery, to avoid loss of valuable genetic material. At this stage it is also possible to identify genotypes excellent in a trait but generally too poor in general performance to be used as a parent for that specific trait. This study therefore concludes that cassava breeders should design breeding programmes where proper selection starts at the seedling nursery. The selection index should include, in addition to the traditional traits, root weight and roots per plant.

O uso de índices de colheita para seleção de populações de mudas de mandioca

RESUMO - *O método de melhoramento atualmente usado para o programa de mandioca é longo porque, na etapa inicial, dá-se maior prioridade ao rendimento e a qualidade das raízes vem depois. Para encurtar este método, ambos o rendimento e a qualidade das raízes devem ser selecionadas simultaneamente, começando no viveiro das novas mudas. Neste estudo realizado na Colômbia, uma população de viveiro constituída de oito famílias de mandioca e 1885 novas mudas desenvolvidas a partir de parentais adaptados a três grandes regiões agro-ecológicas diferentes foram avaliadas em termos de características de rendimento da cultura. A porcentagem do conteúdo de matéria seca (MS) e o índice de colheita produziram um ranking semelhante ao dos parentais. O rendimento do tubérculo, peso e número de raízes mostraram potencial de rendimento crescente através do melhoramento convencional. Recomenda-se o uso de um índice de seleção combinado incluindo o rendimento de raízes frescas, a porcentagem de MS, o peso e o número de raízes por planta; esses dois últimos com maior peso.*

Palavras-chave: *Manihot esculenta,* características das raízes, mudas.

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