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Introgress genes from wild progenitors of cassava for increased root protein yield

Elon Francois Botha

Department of Plant Sciences, University of the Free State, P. O. Box 339, Bloemfontein, South Africa.

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Manihot esculenta spp *flabellifolia* is a potential progenitor for cassava root protein content and yield improvement. Storage roots of cassava landraces are low in protein content due to the fact that past breeding objectives concentrated mainly on yield and resistance to diseases. The improvement of cassava through its wild progenitor is of importance for the full utilization of the potential of the wild progenitor. An interspecific F₁ was crossed to a cultivated variety (MTAI - 8) to generate a backcross population. Root protein, yield and other quality traits were evaluated. High root protein content of 9.61%; fresh root yield of 60.00 ton ha⁻¹; dry root yield of 34.75 ton ha⁻¹; and dry matter content of 59.45% was found in this population. High broad-sense heritability was obtained for all the traits evaluated which is a good indicator that genetic improvement can be achieved in this population. This first backcross population had protein values higher than the earlier documented values in the landraces.

Key word: Cassava, *Manihot esculenta* ssp *flabellifolia*, interspecific, protein and yield.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) has enormous potential to reduce hunger and malnutrition for millions of people that thrive on it as a food security crop. Wild relatives of cassava have become a source of improving the crop by introgressing useful genes from it (Fregene et al., 2007). One of the drawbacks of cassava root is its low protein content (Fregene et al., 2006).

In general, breeding programmes seek to improve crop productivity and quality, widen the genetic base, and maintain its adaptation to specific agro - ecologies. The

potential for genetic improvement of cassava has been demonstrated and progress made in increasing yield potential and stability (Ngoan et al., 1995; Kawano, 1998; Ojulong et al., 2008; Okechukwu and Dixon, 2009). However, world mean yield (12.2 ton ha⁻¹) for cassava is still far below the yield potential (90 ton ha⁻¹) from the experimental field evaluations of the released varieties across growing regions (Fermont et al., 2009; Lebot, 2009; Ziska et al., 2009).

Despite the progress already made by breeders,

*Corresponding author: E-mail: elon.botha22@gmail.com

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additional gains in productivity are demanded at a faster pace because of demographic pressures, changes in agricultural practices, consumer preferences, biotic and abiotic stresses. Other root quality traits relevant to different cassava breeding programmes world-wide are the cyanogenic potential in the root (Dixon et al., 1994a; Balyejusa-Kizito et al., 2007), early bulking capacity (Okogbenin and Fregene, 2002; Olasanmi, 2010), and high protein content in the roots (Fregene et al., 2006). Unfortunately, the genetic variability for the latter two traits is relatively small in *M. esculenta*, therefore, inter-specific crosses with other *Manihot* species are necessary to introgress useful alleles from them (Ceballos et al., 2004).

In an earlier study reported by Asiedu et al. (1992), the introgression of root protein from its wild progenitor was not successful. Wild relatives of cassava are known sources of resistant genes to virtually all cassava pests and diseases as well as high root protein content (CIAT 2002; Fregene et al., 2006; Ojulong et al., 2008; Carabali et al., 2010).

Cassava cultivars are sometimes deficient in some economically important characters such as resistance to pests and diseases, drought tolerance and have low protein content in the root (Nassar and Dorea, 1982; Nassar and Grattapaglia, 1986; Okogbenin et al., 1998) due to the selection that occurred during domestication. Lost genes can be restored to the gene pool of the cultigen by inter-specific hybridization with wild relatives which possess these genes (Nassar et al., 1986). Wild species of cultivated crops have been frequently used as an important source of genetic diversity and have been employed effectively in a variety of breeding programmes (Tanksley and McCouch, 1997; Hajjar and Hodgkin, 2007; Okogbenin et al., 2007). The objective of this study was to introgress genes from wild progenitors of cassava for increased root protein yield and quality traits into commercial cassava.

MATERIALS AND METHODS

Population development

An inter-specific F₁ hybrid CW 198 - 11 was earlier developed at the International Centre for Tropical Agriculture (CIAT), Cali, Colombia (CIAT, 2002). Genetic crosses of open pollinated seeds from *M. esculenta* ssp *flabellifolia* OW230 - 1 (Morantes et al., 2002) and CW30 - 65, (an inter-specific hybrid between an improved cassava variety SG427 - 87 and an accession of *M. esculenta* ssp *flabellifolia* (MESCFLAX - 80)). The inter-specific cross was 'backcrossed' to MTAI 8 to generate a B₁P₂ family with 225 individuals. The male parent (MTAI 8) is a successful elite Thailand cultivar with high dry matter content, good tuber formation, and cream coloured roots from the breeding programme at the Thailand Agricultural Research Centre.

Geographical location of the experiment and evaluations

Embryo axes of sexual seeds from the B₁P₂ family were cultured *in*

vitro and micro-propagated to produce six to eight plantlets per genotype (Akinbo et al., 2010). The plantlets were transferred to the screen house in 2005 for hardening. After 60 days of hardening in the screen house, the seedlings were transplanted to the field at CORPOICA field experiment station, Palmira, Colombia. At 10 months after planting (MAP), one to two roots were 'milked' from each genotype and used to evaluate the genotype for protein content. At 10 MAP, matured stem cuttings from the plants harvested were used to establish a preliminary yield trial, of 225 genotypes in a randomized complete block design, three replicates and eight plants per row, with border plants on the edges. Planting was on ridges at a spacing of 0.7 m (within rows) x 1.4 m (between rows). The plants were not fertilised or sprayed with insecticide, but weeded when necessary. The field trial was conducted at CIAT - Palmira in 2006/2007 season, at Palmira in Valle del Cauca Department (elevation 965 m, 3°49'N, 76°36'W), located in the mid altitude tropics of Colombia, and repeated in CIAT and Quilichao in the 2007/2008 season for second year evaluation. The sites have bimodal rainfall, although there are yearly variations, with peaks usually between March - June and October - December (Table 1). Yield and quality traits were evaluated using the six middle plants to minimize border effects and means were calculated. MTAI 8 was used as the national check over a period of two years.

Data collection

The 6 internal plants in each row were harvested and their storage roots were weighed to determine root yield. Samples of roots from 6 plants of each genotype were collected for dry matter content (DMC) determination. DMC assessment was done by peeling of the fresh roots, chopping them into small pieces, mixing uniformly in a petri dish and oven dried at 60°C for 48 h after which the weight difference between the fresh weight and dry weight was measured and the percentage dry matter was calculated. Percentage dry matter content was determined using the formula:

$$\%DMC = \frac{\text{Weight of the oven dried sample}}{\text{Weight of the fresh sample}} \times 100$$

The dry root yield was calculated as follows: %DMC x fresh root yield.

Harvested plants were assessed for number of storage roots per plant. The aerial part (stems and leaves) of the plants were weighed to determine fresh shoot weight. Harvest index was computed as the ratio of root yield to the total harvested biomass per genotype on fresh basis.

For protein analysis, all samples were analysed at the plant tissue analytical laboratory at CIAT. Nitrogen determination was based on a modification of the Kjeldahl method (Skalar, 1995). The digestion of the samples began with hydrogen peroxide and with this step, the larger part of the organic matter was oxidised. After decomposition of the excess of H₂O₂, the digestion was completed by concentrated sulphuric acid at elevated temperature (330°C) with selenium as catalyst (Novozamsky et al., 1983; Walinga et al., 1989). The root samples were digested with a mixture of sulphuric acid, selenium and salicylic acid. The salicylic acid formed a compound with the nitrates present to prevent losses of nitrate-nitrogen. The supernatant was then 'coloured' using the salicylate (molarity), nitroprusside (catalyst) and active chlorine were added to form a green colour complex with the ammonium ion. Nitrogen was quantified based on colorimetric measurement of the supernatant on a segmented flow analyzer. The absorption was measured at 660 nm (Krom 1980; Searle 1984).

Hock-Hin and Van-Den (1996) reported that, in the case of cassava roots, the conversion factor for protein contents based on N concentrations should probably range between 4.75 and 5.87. An

Table 1. Meteorological data at Palmira and Quilichao in 2006/2007 and 2007/2008 seasons.

Climatic factors	Palmira		Quilichao	
	2006-2007	2007-2008	2006-2007	2007-2008
Precipitation (mm)	104.5	82.85	-	171.42
Evaporation (mm)	135.73	135.08	-	117.35
Radiation (MJ m ⁻²)	17.68	16.86	-	14.87
Maximum temperature (°C)	30.14	30.23	-	28.90
Minimum temperature (°C)	19.32	18.94	-	18.51
Mean relative humidity (%)	76.79	76.72	-	79.69
Mean wind velocity (m/sec.)	56.58	58.96	-	30.62

Table 2. Range of values for agronomic traits of 225 progenies of a cassava backcross population grown in CIAT 2006/2007, and CIAT and Quilichao 2007/2008 season.

Variables	CIAT 2006-2007				CIAT 2007-2008				Quilichao 2007-2008			
	^a Min	^b Max	^c Aver	^d SD	Min	Max	Aver	SD	Min	Max	Aver	SD
^e Rt/plt	1.87	16.50	6.67	2.33	0.50	30.00	4.07	2.03	0.33	25.00	4.33	2.28
^f ComRt	0.00	9.00	1.63	1.63	0.00	12.00	0.69	0.96	0.00	8.62	1.21	1.48
^g FRY	18.75	58.75	45.88	9.28	16.66	60.00	37.82	10.38	15.71	58.75	37.83	11.42
^h DRY	5.18	19.39	11.88	2.78	3.66	21.05	9.53	2.97	4.23	34.75	10.17	3.45
ⁱ HI	0.30	0.88	0.42	0.08	0.29	0.91	0.41	0.09	0.27	0.80	0.50	0.12
^j DMC	27.01	47.04	39.03	4.01	25.01	59.45	40.24	5.43	23.57	50.01	37.82	4.50
^k PC	0.84	9.61	3.02	1.21	1.10	8.13	2.91	1.26	0.69	7.75	2.13	0.86

^aMinimum; ^bMaximum; ^cAverage; ^dStandard Deviation; ^eRoots per plant; ^fCommercial Roots; ^gFresh root yield (ton ha⁻¹); ^hDry root yield (ton ha⁻¹); ⁱHarvest Index (0-1); ^jDry matter content (%); ^kProtein content (%).

average of 5.31 was the standard being established and used for the cassava roots procedure at CIAT.

Data analysis

SAS (2002) statistical programmes were used for analysis of variance, correlation and frequency distributions of phenotypic classes. Only genotypes which had complete data from the three replications were used. Since roots per plant, root weight and fresh and dry root yield data were not normally distributed, data sets were transformed by the square root method using the formula: $y = \sqrt{(x+0.5)}$. The percentage of dry matter content and protein content were transformed by the square root method using the formula: $y = \sqrt{x}$, where y is the resulting transformation and x is the data point. The SAS correlation (proc corr.) procedures were used to estimate correlation and regression coefficients between different parameters. Analyses of variance (ANOVA) of yield, yield components and quality traits across environment were performed using the general linear model procedure in the SAS software. Genotypes and environments were considered fixed and random effects, respectively (Griffing, 1956). The following model was used for the combined data:

$$Y_{ij} = \mu + G_i + E_j + GE_{ij} + e_{ij}$$

Where μ is the general mean, G_i , E_j and GE_{ij} represent the effect of the genotype, environment and G x E interaction respectively; e_{ij} is the average of random errors associated with r^{th} plot that receives the i^{th} genotype in the j^{th} environment (Crossa, 1990).

Estimates of broad sense heritability were determined using Agrobases (2005). Principal component analysis (Iezzoni and Pritts, 1991) was used to investigate the relevant traits contributing to the phenotypic variation among genotypes.

RESULTS

A relatively high number of roots per plant was obtained (average = 5.02), with genotype B₁P₂ - 6 having the highest number of 30. The average number of commercial sized storage roots (5 to 10 cm in diameter at the top and 15 cm to 30 cm long) was 1.17 with genotype B₁P₂ - 6 having the highest number of 12 commercial sized roots. The highest fresh root yield was recorded in B₁P₂ - 252 (60.00 ton ha⁻¹) while highest dry root yield was recorded in B₁P₂ - 62 (34.75 ton ha⁻¹). Recorded dry matter content ranged from 23.57% in B₁P₂ - 77 to 59.45% in B₁P₂ - 247 (Table 2).

Dry root yield was highly significantly correlated with number of commercial sized storage roots, roots per plant, harvest index, root weight, and fresh root yield (Table 3). Fresh root yield was highly significantly correlated with roots per plant, commercial roots and harvest index. Harvest index was highly significantly correlated with number of commercial sized storage roots

Table 3. Simple correlation coefficient matrix of yield components and quality traits for a cassava backcross population evaluated in CIAT 2006-2007, CIAT and Quilichao 2007-2008 season.

Variables	Root per Plant	Commercial Root	Harvest Index	Fresh Root Yield	Dry Root Yield	Dry Matter Content
Commercial Root	0.40**					
Harvest Index (0-1)	0.17**	0.38**				
Fresh Root Yield (ton ha ⁻¹)	0.99**	0.40**	0.17**			
Dry Root Yield (ton ha ⁻¹)	0.91**	0.41**	0.21**	0.91**		
Dry Matter Content (%)	0.09	-0.07	-0.12	0.09	-0.30**	
Protein Content (%)	-0.04	-0.13	0.26**	-0.04	-0.12	0.19**

**P<0.0001.

Table 4. Principal component coefficients of the various traits with principles of the various yield and quality related traits evaluated in a cassava backcross population at CIAT 2006/2007, and CIAT and Quilichao 2007/2008 seasons.

Traits	PC1 ^a	PC2	PC3
Roots per plant	0.52	0.23	-0.02
Commercial Root	0.33	-0.22	0.37
Harvest Index (0-1)	0.20	-0.49	0.49
Fresh Root Yield (ton ha ⁻¹)	0.52	0.23	-0.02
Dry Root Yield (ton ha ⁻¹)	0.52	0.01	-0.30
Dry Matter Content (%)	-0.05	0.52	0.71
Protein Content (%)	-0.10	0.55	-0.05
Eigenvalue	3.23	1.43	0.98
Percent total variance	46.21	20.50	14.03
Cumulative	46.21	66.71	80.74

^aPrincipal component.

and roots per plant. Protein content was highly significantly correlated with harvest index and dry matter content and negatively correlated with number of commercial sized roots, roots per plant, and dry matter content.

The relative contribution of the various traits to the genotype performance was explained by principle component analysis (Table 4). The first three principal components explained most of the variation and accounted for 80.74% of the total variation. The first principal component accounted for 46.21% of the total variation. Most of the variables were positively correlated which is an indication that they all contributed to total variation, except protein content. Based on the PC1 coefficients, four variables made a major contribution to variation (roots per plant, commercial roots, fresh root yield, and dry root yield). PC2 explained 20.50% of the total variation, with major contribution from harvest index, dry matter content, and protein content. PC3 explained 14.03% of the total variation with major contribution from commercial roots, harvest index and dry matter content.

Combined analysis of variance in CIAT and Quilichao trial sites over two years indicated that genotype was

highly significant for roots per plant, root weight, harvest index, fresh root yield, dry root yield, protein content and for dry matter content of the traits evaluated (Table 5). Year was highly significant for all traits evaluated. Genotype by year interaction was highly significant for fresh root yield, dry root yield; root weight and root per plant.

The combined analysis of variance in two locations of CIAT trial sites is presented in Table 6. There were highly significant differences in the genotype main effects for root per plant, harvest index; fresh root yield, dry root yield and dry matter content. Interaction between genotype and location was significant for only dry matter content. After the yield and protein data were transformed, all the traits showed a good coefficient of determination (R^2) of 0.99 across the three environments. There was a highly significant location effect for all the traits.

DISCUSSION

Despite the world-wide importance of cassava, cassava

Table 5. Combined general linear model (GLM) sum of squares of yield parameters and quality traits in a cassava backcross population at CIAT, Colombia in 2006/2007 and Quilichao in 2007/2008 season.

Source of variation	Sum of squares						
	df ^a	Rtplt ^b	HI ^c	FRY ^d	DRY ^e	DMC ^f	PC ^g
Genotype	208	233.42	3.57	23342.65	2033.66	5834.09	245.43
Year	1	37.79	0.83	3779.48	173.63	173.16	26.09
Replication	2	12.80	0.13	1280.96	31.52	361.43	6.59
Year*Geno	76	69.61	0.52	6961.80	572.23	1138.21	63.34
Year*Geno*Rep	24	28.41	0.13	2841.76	191.84	275.99	22.20
Error	552	733.15	7.67	73315.62	5752.30	11113.45	618.36

^aDegree of freedom; ^bRoots per plant; ^cHarvest index (0-1); ^dRoot weight (kg); ^eFresh root yield (ton ha⁻¹); ^fDry root yield (ton ha⁻¹); ^gDry matter content (%); ^hProtein content (%).

Table 6. Combined general linear model (GLM) table of protein and yield parameters evaluated in three environments in 2006-2008 in CIAT and CIAT and Quilichao, Colombia.

Source of variation	Mean squares							
	df ^a	RtPlt ^b	ComRt ^c	FRY ^d	DRY ^e	HI ^f	DMC ^g	PC ^h
Genotype (G)	220	0.14 ^{ns}	0.19 ^{**}	1.68 ^{ns}	0.45 ^{ns}	0.01 ^{**}	0.20 ^{**}	0.16 [*]
Environment (E)	2	3.73 ^{***}	2.18 ^{****}	41.93 ^{***}	11.62 ^{**}	0.36 ^{****}	2.77 ^{****}	1.70 ^{***}
Rep (R)	2	0.65 ^{ns}	0.11 [*]	7.52 ^{ns}	1.04 ^{ns}	0.01 [*]	3.47 ^{****}	0.13 ^{ns}
E x G	303	0.09 ^{ns}	0.10 [*]	1.10 ^{ns}	0.30 ^{ns}	0.002 ^{ns}	0.10 [*]	0.05 ^{ns}
R x G	334	0.09 ^{ns}	0.09 [*]	1.09 ^{ns}	0.28 ^{ns}	0.002 ^{ns}	0.09 [*]	0.03 ^{ns}
E x R	4	0.46 ^{ns}	0.75 ^{***}	5.32 ^{ns}	2.00 ^{**}	0.02 ^{ns}	0.58 ^{**}	0.28 [*]
E x R x G	137	0.09 ^{ns}	0.10 [*]	1.04 ^{ns}	0.24 ^{ns}	0.001 ^{ns}	0.09 [*]	0.03 ^{ns}
Error	1045	0.07	0.02	0.88	0.23	0.001	0.02	0.036
CV ⁱ		13.28	13.65	14.87	14.72	3.53	2.65	12.64

^aDegree of freedom; ^bRoots per plant; ^cCommercial Root; ^dFresh root yield (tonha⁻¹); ^eDry root yield (tonha⁻¹); ^fHarvest index (0-1); ^gDry matter content (%); ^hProtein content (%); ⁱCoefficient of variance; ns=not significant at P<0.05; * P<0.05; ** P<0.01; ***P<0.001; **** P<0.0001.

cultivars have low protein content (Anonymous, 1968; Nassar and Dorea, 1982). Efforts have been made in the past to introgress root protein trait from wild progenitors but failed during the backcross phase (Asiedu et al., 1992). The low protein content in the roots of cassava can be attributed to the selection methods adopted by the cassava breeders where emphasis has not been placed on protein content as a part of the selection criteria (CIAT, 2004). Recently, storage root proteins have proved to be an increasingly important target for cassava breeders and geneticists who are now using marker-assisted selection and genetic engineering because of the role of protein in determining the nutritional quality of storage roots (Zhang et al., 2003).

The two parents (CW 198 - 11 and MTAI 8) that were used to generate this B₁P₂ population had large differences in their protein and dry matter content values of 11.20 (CW 198 - 11) and 2.30 (MTAI 8) for protein content, 33.24% (CW 198 - 11) and 44.96% (MTAI 8) for dry matter content. In the wild relative *M. esculenta* ssp *flabellifolia* (OW 230), the dry matter content and protein content were 46.12 and 10.50% respectively. The use of

wild progenitors is part of the development at the CIAT breeding programme partly reported by Ojulong et al. (2008) of a new selection scheme, where the root protein content is a priority.

The results of this study differ from those reported by Ceballos et al. (2006) from another population with highest protein content of 7.20% in an unreplicated trial of a wide range of local neo-tropical varieties and higher than the value reported by Chávez et al. (2005) with the highest protein content of 8.72% of the same materials in an un-replicated trial. Efforts are ongoing in the National Root Crops Research centres in Africa where materials with high protein content introduced from Latin America are being utilised in the breeding activities to combine both protein and beta carotene (C. Egesi, NRCRI, Umudike, Nigeria, personal communication). Selection based on one breeding goal for the target environment is being implemented in the Nigerian cassava breeding community to meet a specific nutritional and agro-ecological and industrial goal (Nigeria Presidential initiative on Cassava).

Results from simple statistics showed that the potential

percentage dry matter content in this introgression (50.51%) was higher than the past documentations (Ojulong et al., 2008; Ceballos et al., 2006; Jaramillo et al., 2005; Iglesias., 1994; Magoon et al., 1973). Ceballos et al. (2006) reported negative correlation between dry matter and protein contents in the roots, suggesting that clones with higher protein content tended to have lower levels of dry matter content. This is contrary to what was found in this study, where it was not significant.

Simple correlation analysis in this population showed that all traits (commercial roots, roots per plant, harvest index, root weight, and fresh root yield) contributed to economic yield, which is by implication, an improvement over the previous studies reported by Kawano et al. (1998) and Ojulong et al. (2008) that association was detected between dry matter content and fresh root yield at the early stage, this might be as a partial result of other genes affecting this stage of introgression.

The use of Genotype by environment interactions is a means by which clones are tested for a wide adaptation to a range of environments with higher yield (Ngeve et al., 2003; Aina et al., 2007; Egesi et al., 2007; Okechukwu and Dixon, 2009). The contribution of genotype sum of squares to total sum of squares in yield and quality traits was significant, which indicated a large genetic component. This is in agreement with a report by Ceballos et al. (2006) which provides strong evidence to support the hypothesis of a genetic origin of protein content in the cassava root. The possibilities of further increasing the protein content in the root are therefore encouraging (Steel and Torrie 1960; Dudley, 1974; Gomez and Gomez, 1984; CIAT, 2003; Ceballos et al., 2006).

High broad-sense heritability was reported for fresh root yield, dry root yield, dry matter content, root weight, harvest index, roots per plant, commercial roots and protein content, which is in agreement with the findings of Pérez et al. (2002), Okogbenin (2004), Ceballos et al. (2004) and Ojulong et al. (2008).

There have been wide variation ranges of other qualitative traits from both wild and landraces of cassava roots (Asiedu et al., 1992; Sánchez et al., 2009; Rolland-Sabaté et al., 2012). However, studies have been carried out on other root quality traits of cassava at CIAT, the International Institute of Tropical Agriculture (IITA), National Roots Crops Research Institute (NRCRI), and other root crops research institutes around the world (Egesi, personal communication; Nuwamanya et al., 2009; Njoku et al., 2011). Other efforts have also been made in the use of genetical engineering to incorporate root quality traits into cassava to improve the health of cassava root consumers around the world (Sayre et al., 2011).

Results from this study are indeed very promising. Perhaps the most relevant benefit from these introgressions would be in improving the nutritional status of millions of people who depend heavily on cassava as a food security crop. The novelty of this finding is in the

addition of higher protein content with higher dry matter content in the roots of cassava. The genotypes with the combination of these two traits have been pre-selected for further backcrossing to other cassava genotypes with other root quality traits so as to pyramid these genes from various sources and select the best for the benefit of the end users (farmers and consumers). These pre-selected genotypes for the second backcross are presently being evaluated in root crops research institutes in Nigeria, Ghana, Uganda and Tanzania.

Conflict of Interest

The authors have not declared any conflict of interest.

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