Bringing wild alleles back into the primary gene pool: introgression of high protein content from *Manihot esculenta* ssp *flabellifolia* into cassava (M. *esculenta* Crantz)

Akinbo, O[1,2], Labuschagne, M.T[2], Ospina, C[1], Marin, J[1], Gutierrez, J[1] and Fregene, M[1]
1 Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
2 Department of Plant Science, University of the Free State, Bloemfontein, South Africa E-mail: akinbooa@yahoo.co.uk

INTRODUCTION

Cassava has enormous potential to reduce hunger and malnutrition for millions of people that live on cassava as food security crop. Wild relatives of cassava have become a source of improving the crop by introgressing useful genes from it. One of the bottlenecks of cassava is its low protein in the root of the existing cassava germplasm. In general, breeding programmes seek to improve crop productivity, widen the genetic base and maintain its adaptation. The potential for genetic improvement of cassava has been demonstrated and progress has been made in increasing yield potential and stability (Kawano, 1998) Unfortunately, the genetic variability for the protein trait is relatively small in *M esculenta* and therefore, inter-specific crosses with other *Manihot species* are necessary to introgress useful alleles from them (Ceballos *et al.*, 2004). Wild relatives of cassava are known sources of high root protein content (Nassar, 2000). 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). The objective of this work was to introgress genes from wild progenitors of cassava for increased root protein and dry matter content to commercial cassava.

MATERIALS AND METHODS

An inter-specific F_1 hybrid CW 198 - 11 was earlier developed at CIAT, Cali, Colombia (CIAT, 2002) by genetic crosses of OW 230-1 (FLA 441 – 5 with protein content of 10.45%) and CW 30-65, an inter-specific hybrid between an improved cassava variety SG427-87 and an accession of *M esculenta* ssp *flabellifolia*. The inter-specific cross was 'backcrossed', in the sense of another cross to cassava, to MTAI - 8 to generate a BC_1 family (B1P2) with 227 individuals. The pedigree of these parents is illustrated in Figure 1. Embryo axes of sexual seeds from the B_1P_2 family were cultured *in vitro* and micro -propagated to produce 6-8 plantlets. Matured stem cuttings from the plants harvested at CORPOICA were used to establish a preliminary yield trial experiment made up of 227 genotypes, a complete block design with three replicates of 12 blocks, eight plants per row. The field trial was conducted in CIAT – Palmira in Valle del Cauca department, located in the mid altitude tropics of Colombia in 2006. The site has bimodal rainfall, although there are yearly variations, with peaks usually between March - June and October – December. Yield and quality traits were evaluated on the seven middle plants and means were calculated.

Samples of roots from several plants of a single genotype were taken for dry matter content determination. Dry matter content (DMC) assessment was done by peeling of the back of the fresh tuber and oven dried at 60oC for 48 hours 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 = [(wt of the oven dried sample + wt of the petri dish) – wt of the petri dish] x 100/ [(wt of the fresh sample + wt of the petri dish) – wt of the petri dish]

The dry root yield was calculated as follows: %DMC x fresh root yield. The aerial part (stems and leaves) of the plants were also 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

All the protein samples were analysed at the plant tissue analytical laboratory at CIAT. Nitrogen determination was based on a modification of the Kjeldahl method (Skalar, 1995). Evaluation for PPD was done at seven days after harvest. Immediately after harvest, 10-15cm sections were taken from each randomly picked root. The colour of the root pulp was assessed as described by Iglesias *et al.* (1997) with modification. Sigmaplot 10.0 (2007) statistical programme was used for frequency distributions of phenotypic classes.

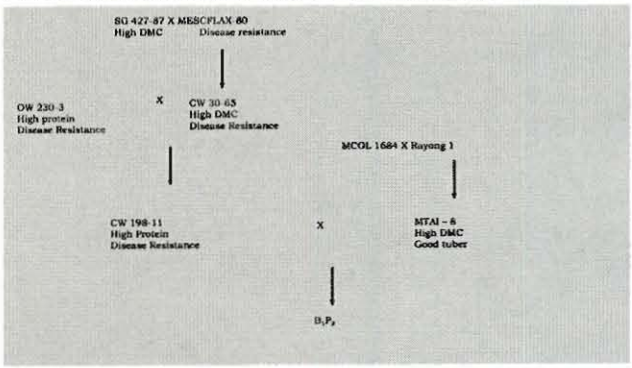

Fig. 1. Pedigree of the crosses from wild relatives to BC_1 generation

Table 1: Simple statistics of agronomic variables evaluated on 227 genotypes of a backcross population in CIAT, Palmira in May, 2007

Variables	Minimum	Maximum	Average	SD[a]	LSD[b]
FRY[c] (t/ha)	0.26	58.59	8.97	5.91	5.42
DRY[d] (t/ha)	0.09	22.31	3.50	2.27	2.08
HI[e] (0-1)	0.008	0.88	0.33	0.13	0.11
DMC[f] (%)	10.83	50.51	39.34	4.14	5.29
PPD[g] (%)	0.00	72.57	13.92	14.86	2.53
PC[h] (%)	0.77	9.61	2.71	1.06	11.44
RtCol[i] (%)	1.00	7.00	1.74	1.31	1.28

[a]Standard deviation; [b]Least square deviation; [c]Fresh root yield (t/ha); [d]Dry root yield (t/ha); [e]Harvest index (0-1); [f]Dry matter content (%); [g]Post-harvest deterioration (%); [h]Protein content (%); [i]Root colour (1-8)

RESULTS AND DISCUSSION

An earlier evaluation of root protein content in 579 wild Manihot esculenta ssp flabellifolia were conducted roots were "milked" from each of the genotypes and evaluated for crude protein, dry matter content, crude fiber, ash, amylose and storage root production (data not shown). Result from the BC_1 showed a relatively high number of roots per plant, high number of commercial sized storage root, high root weight, high fresh root yield, high dry matter content and dry root yield were obtained (Table 1).

There were high correlation among the yield quality traits (P<0.0001). Post harvest deterioration and protein content was negatively correlated with other yield quality traits. The frequency distribution of the dry matter content, protein content and post harvest deterioration had a skewness values of –0.92, 0.96 and 1.81 respectively. All of these traits showed normal distribution (Figure 2). The root colour ranges from 1 to 8.

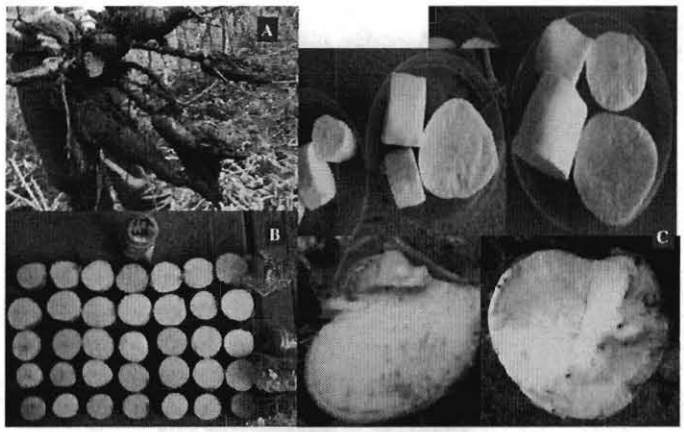

Figure 2: Showing commercial tuber size (A), no physiological deterioration on the roots after 7 days (B), Variability with respect to colour intensity in the roots (C), from BC_1 family

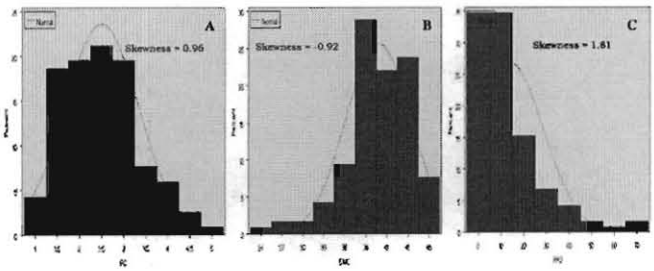

Figure 3: Percentage protein content (A), dry matter content (B) and post harvest physiological deterioration (C) distribution in B_1P_2 family

CONCLUSIONS AND ONGOING WORK

- wide genetic variability was observed, indicating that there is potential for improving these traits
- B_1P_2 family are being evaluated in the second year

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