Euphytica (2012) 183:19–26
DOI 10.1007/s10681-011-0436-8

Introgression of whitefly (*Aleurotrachelus socialis*) resistance gene from F_1 inter-specific hybrids into commercial cassava

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Received: 13 May 2010 / Accepted: 8 April 2011 / Published online: 24 April 2011

Abstract The most widespread disease of economic importance of cassava is caused by whitefly vector, both as a single strain or combination of strains. A B_1P_2 family was generated from the crosses of an inter-specific F_1 hybrid (CW 198-11) as a female parent with a commercial cassava cultivar (MTAI-8) as male parent at CIAT headquarters and evaluated in a high-pressure zone for whiteflies in Colombia. 227 genotypes were scored using a scale ranging from 1 (no leaf damage) to 6 (considerable leaf necrosis and defoliation, sooty mould on mid and lower leaves and young stems). The rest were considered promising. The most promising resistance was for damage ratings below 2 for 17.8% of the genotypes. The availability of the pest resistance genotypes, will serve as a means to combat the problem of CMD in Africa provided that resistance to *A. socialis* is also effective against *B. tabaci* with different virus strains that is capable of been introduced.

Keywords *Aleurotrachelus socialis* · Introgression · *Manihot esculenta* ssp flabellifolia

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Introduction

Cassava (*M. esculenta* Crantz) is an important source of cheap food in all Sub Saharan Africa (Horton et al. 1984; Dahniya 1994). The crop is widely grown by resource-poor farmers who consume the fresh or processed roots and generate income from the sale of the products. Cassava is a hardy crop and can thrive in the poor soils usually found in the marginal areas of the world.

Cassava leaves contain 5.1 to 6.9% protein on dry matter basis (Onwueme 1978; Gomez and Valdivieso 1985). In Zaire, cassava leaves are a basic vegetable, being the cheapest and richest source of protein. Cassava leaves are also widely consumed as vegetable in other countries in Africa (Lutaladio and Ezumah 1981; Dahniya 1994).

The most widespread cassava disease of economic importance in Africa is cassava mosaic disease (CMD, Akano et al. 2002; Balyejusa Kizito et al. 2005; Ogbe et al. 2006). Since the introduction of cassava to the West and East Africa in the 1850 s,

CMD epidemics had caused severe losses (Balyejusa Kizito et al. 2005). In the 1990 s a major setback was suffered in cassava production due to this disease in Sub Saharan Africa (Zhou et al. 1997; Okogbenin et al. 1998; Otim-Nape et al. 2000; Balyejusa Kizito et al. 2005; Ogbe et al. 2006). Cassava yields are severely reduced by pests and diseases that are worsened by the fact that it is a long season crop, which exposes it to infestation or infection by a host of pests and pathogens in all growing areas (Egesi et al. 2007). CMD is caused by at least four geminiviruses of the genus *Begomovirus* (Family Geminiviridae) and is transmitted by the whitefly (*B. tabaci*: Russell 1978; Thresh et al. 1994; Wool et al. 1994; Bellotti and Arias 2001; Akano et al. 2002; Ariyo et al. 2002, 2004; Ogbe et al. 2006).

Whiteflies are considered one of the world's major agricultural pests, attacking a wide range of crop hosts and causing considerable crop loss. As direct feeding pest and virus vector, whiteflies cause major damage in agro-ecosystems based on cassava (Bellotti and Arias 2001). Whiteflies, especially in the Neotropics, cause direct damage to cassava by feeding on the phloem of the leaves (Carabali et al. 2010). This causes symptoms such as chlorosis and leaf fall, which result in considerable reduction in root yield if prolonged feeding occurs. Yield losses resulting from *Aleurotrachelus socialis* and *Aleurotrachelus aepim* activity (Vargas and Bellotti 1981; Farias 1994; Bellotti et al. 1999; Carabali et al. 2010) are common in Colombia and Brazil. However, African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV) and the new Ugandan virus strain of EACMV (EACMV–Ug_2) is a major growing threat (Ogbe et al. 2006; Dixon et al. 2008).

Cassava cultivars often lack economically important characters such as resistance to pests (Nassar and Dorea 1982; Nassar and Grattapaglia 1986). This can be attributed to the mode of evolution of the species and modifications of the allogamy system of the plant (Nassar and O'Hair 1985). Lost genes can be restored to the gene pool of the cultigen by interspecific hybridisation with wild relatives which possess these genes (Nassar and Grattapaglia 1986; Carabali et al. 2010). The objective of this study was to introgress genes from wild progenitors of cassava for increased whitefly resistance into commercial cassava.

Materials and methods

Population development of whitefly resistance

An inter-specific F_1 hybrid CW 198–11 was earlier developed at CIAT, Cali, Colombia by genetic crosses of OW230–1 (FLA 441–5 with protein content of 10.45%) and CW30–65, an inter-specific hybrid between an improved cassava variety SG427–87 and an accession of *M. esculenta* ssp *flabellifolia*. The inter-specific crosses were 'back-crossed', to MTAI–8 to generate a BC_1 (B_1P_2) family with 227 individuals. The wild maternal grand parent of B_1P_2 has, in addition to high protein content in the roots, high dry matter and resistance to various cassava diseases (African cassava mosaic disease, cassava bacterial blight, cassava anthracnose disease) and pests (hornworm, whiteflies). The male parent (MTAI–8) is a successful elite Thailand cultivar with high dry matter content, good roots formation, and cream coloured roots from the breeding programme at the Thailand Agricultural Research Centre. Parents for crossing were planted in the crossing block at CIAT, Palmira in single rows of 1 m between plants and 2 m between rows, to facilitate movement during crosses. Genotypes were monitored daily for onset of flowering. At the onset of flowering, plants were inspected every morning for flowers about to open, and such flowers were enclosed with transparent bags, to prevent contamination from stray pollen on opening. Pollen were collected in plastic bottles (perforated), from MTAI–8 male parents. At around 11.00 am when flowers open, the transparent bags were removed, and pollen from the MTAI–8 parent dusted on the stigma of CW 198–11 after the emasculation of the immature female flowers, then tagged with a label containing the pedigree, number of female flowers pollinated, and date of pollination. The transparent bag used to prevent contamination from insects was removed after pollination to allow the fruit to develop freely. Four weeks after pollination, fruits were covered with bags made of gauze to collect the fruits that explode explain at maturity (Jennings and Iglesias 2002). Seeds were collected from the field after 60 days. They were cleaned, and viable seeds identified and germinated in vitro.

Geographical location of the experimental site and evaluations

Stem cuttings were used to establish this replicated experiment. The field trial was conducted in CIAT–Palmira, in 2006, at Palmira in Valle del Cauca Department (elevation 965 m, 3°49′N, 76°36′W), located in the mid altitude tropics of Colombia. The site has bimodal rainfall, although there are yearly variations, with peaks usually between March to June and October to December. The soil in Palmira is a fertile alluvial clay loam. Meteorological data at the location during experimentation are presented in Table 1. Field plot layout was a complete block design with three replicates of 12 blocks, involving 227 genotypes of the B_1P_2 population. The total area of the trial was 5989 m^2, comprising of eight plants per genotype with three replicates, with border plants at the hedges. Planting was on ridges at a spacing of 0.7×1.4 m^2. The plants were not fertilized or sprayed with insecticide, but weeded when necessary. Traits evaluated were pest infestation, infection, yield and quality traits. Data were collected on the seven internal plants and means calculated.

Harvesting was carried out at 10 months after planting (MAP). Seven plants were harvested and their storage roots were weighed to determine yield. Sub-samples of roots of various sizes, depending on the genotype yield, were taken on genotype basis for dry matter content determination. DMC assessment was done by pill and oven dried 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:

Table 1 Meteorological data at Palmira in 2006 and 2007

Climatic factors	Palmira	
	2006	2007
Precipitation (mm)	104.5	82.85
Evaporation (mm)	135.73	135.08
Radiation (MJ m^{-2})	17.68	16.86
Maximum temperature (°C)	30.14	30.23
Minimum temperature (°C)	19.32	18.94
Mean relative humidity (%)	76.79	76.72
Mean wind velocity (m/s)	56.58	58.96

$$\%\mathrm{DMC} = \frac{\mathrm{Weightoftheovendriedsample}}{\mathrm{Weight\ of\ the\ fresh\ sample}} \times 100$$

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

Data analysis

Agrobase (2000); SAS Institute Inc (2002) and Sigmaplot 10.0 statistical programmes were used for data analysis. Since roots per plant, root weight and fresh and dry root yield data were not normally distributed, data were transformed by the square root method using the formula: $y = \mathrm{sqrt}(x + 0.5)$. Percent dry matter content was transformed by the square root method using the formula: $y = \mathrm{sqrt}(x)$, where y is the resulting transformation and x the data point (Kang 1994; Ojulong 2006).

The SAS correlation (proc corr.), univariate (proc univariate) and regression (proc reg) procedures were used to estimate correlation and regression coefficients between different parameters. Pest evaluation, yield and yield components were subjected to simple ANOVA. Agrobase (2000) was used for estimating broad-sense heritability. Sigmaplot 10.0 was used to plot the histogram of different damage grades.

Methods for distinguishing resistant and susceptible cultivars in the field

Field screening of the B_1P_2 family for resistance to whiteflies was done at CIAT headquarters where the natural whitefly population is high and damage levels are significant so as to distinguish susceptible cultivars.

The evaluation was done during the dry period of the growing season when the population build up for the whiteflies is very high. *Aleurotrachelus socialis* adult and nymph feeding damage is most noticeable on the young, tender apical leaves of the cassava plant. Feeding induces a yellow-to-green mottled appearance and twisted or curled leaves, eventually resulting in chlorosis and defoliation. Resistant and

susceptible check were not used at this stage of the introgression bearing in mind that the genetic load is still high and at the advance multi-location trials the check genotypes will be incorporated. Field evaluations of the B_1P_2 used a population scale combined with a leaf-damage scale (Table 2). The scoring was done on individual plant per genotype and was repeated in the three replications at six months after planting during the dry spell. Evaluations were done in three different parts of the cassava plant, the severities (scoring of 1–6) were measured at the upper leaves surface part (DSup), at the middle part of the cassava stem with leaves (DMed), and at the lower portion of the cassava stem with leaves (DBajo). The incidence and severity of the population of *A. socalis* of adult population on the leaf surface (UAdl), eggs number on the leaf surface (UEgg) nymph population on the leaf surface (Unphl), pupa population on the leaf surface (UPul), nymph population on the middle part of the plant (MNp2), pupa population on the middle part of the plant (MPul2), pupa population on the lower part of the plant (APul3), superior part severity damage (Sup), middle severity damage (Med), and lower part severity damage (Bajo).

Table 2 Population and damage scales for evaluating B_1P_2 population for resistance to whitefly *Aleurotrachelus socialis*

[a]Population scale

1 = no whitefly stages present

2 = 1–200 individuals per cassava leaf

3 = 201–500 individuals per cassava leaf

4 = 501–2000 individuals per cassava leaf

5 = 2001–4000 individuals per cassava leaf

6 = > 4000 individuals per cassava leaf

[a]Damage scale

1 = no leaf damage

2 = young leaves still green but slightly flaccid

3 = some twisting of young leaves, slight leaf curling

4 = apical leaves curled and twisted; yellow-green mottled appearance

5 = same as 4, but with sooty mold and yellowing of leaves

6 = considerable leaf necrosis and defoliation, sooty mold on mid and lower leaves and young stems

[a] Scale adapted from Bellotti and Arias (2001)

Results

Of the 227 genotypes evaluated, 13.3% were considered susceptible with damage ratings above 3.5 (Fig. 1). The remaining 86.7%, with damage ratings below 3.5, were considered promising. The most promising resistance was for damage ratings below 2.0 for 17.8% of the accessions.

A relatively high number of roots per plant was obtained (average 5.53), with genotype B_1P_{2-251} having the highest number of 16.50. The average commercial sized storage roots were 1.20 with genotype B_1P_{2-190} having the highest number of 9.00 commercial sized roots. Highest root weight was recorded for genotype B_1P_{2-2}. Recorded dry matter content ranged from 10.83 in B_1P_{2-218} to 50.51 in B_1P_{2-109}. Highest fresh root yield was recorded in B_1P_{2-2} (58.59 ton ha^{-1}) while highest dry root yield was recorded in B_1P_{2-2} (22.31 ton ha^{-1}) (Table 3).

In the evaluations, genotypes B_1P_{2-10}, B_1P_{2-79}, B_1P_{2-312}, B_1P_{2-98}, B_1P_{2-176}, B_1P_{2-168}, B_1P_{2-176}, B_1P_{2-246}, B_1P_{2-248}, B_1P_{2-25}, B_1P_{2-89}, B_1P_{2-311} and B_1P_{2-64} consistently expressed the highest level of resistance across replications. A number of genotypes expressed moderate to high levels of resistance. Low severity of *A. socialis* was recorded in this field despite the high pest pressure at the location of screening. Surface damage was low in all the developmental stages of the pest with a skewness of 0.55 for adult *A. socialis*, 0.12 for eggs, −0.37 for

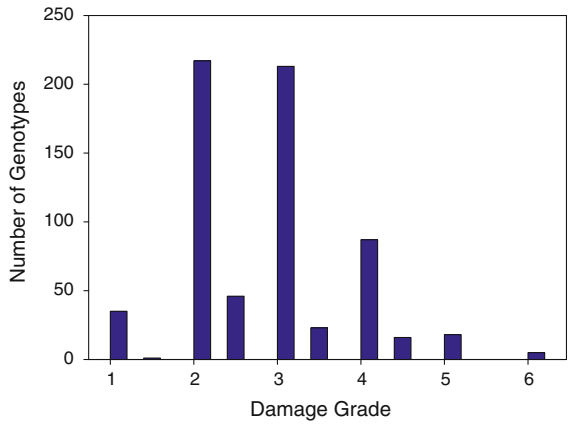

Fig. 1 The distribution of the damage of whiteflies on a cassava backcross population evaluated at CIAT for resistance to whiteflies [(damage scores are based on 1 (no damage) to 6 (severe damage) rating scale]

Table 3 Range of values for agronomic traits of 227 progenies of a cassava backcross population in CIAT, Palmira in May, 2007

Variables	Minimum	Maximum	Average	SD[a]
Rtplt[b]	0.16	16.50	5.53	2.47
ComRt[c]	0.00	9.00	1.20	1.44
Rtwt[d]	0.03	1.20	0.20	0.08
FRY[e]	0.26	58.59	8.97	5.91
DRY[f]	0.09	22.31	3.50	2.27
HI[g]	0.01	0.88	0.33	0.13
DMC[h]	10.83	50.51	39.34	4.14

[a] Standard deviation

[b] Roots per plant

[c] Commercial roots

[d] Root weight (ton ha^{-1})

[e] Fresh root yield (ton ha^{-1})

[f] Dry root yield (ton ha^{-1})

[g] Harvest index (0–1)

[h] Dry matter content (%)

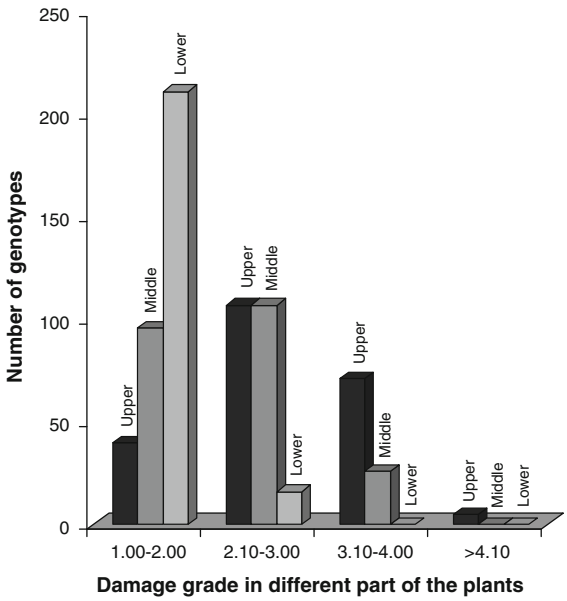

Fig. 2 Frequency distribution of different degrees of damage done to different parts of the plants by *Aleurotrachelus socialis*

nymphs, and 0.24 for pupa (data not shown). There was a skewness of 0.52 for superior, 0.97 for middle, and 1.16 for the lower part for severity (Fig. 2). The distribution of severity of *A. socialis* was asymmetrical with a long tail to the right, and a concentration of frequencies around the low damage grade level of *A. socialis*.

General linear model analysis showed mean squares for genotypes to be highly significant for all yield and pest characteristics evaluated (Table 4). High broad-sense heritability was estimated for yield, yield related traits and pest severity damage.

On genotype basis, there was no significant correlation between the yield and yield related traits evaluated with the grade of damage that was done to the genotypes by *A. socialis* in all the parts of the plants evaluated (DSup, DMed and DBajo), but DSup was positively correlated ($P \leq 0.0001$) with DMed and DBajo (Table 5). There was highly significant correlations ($P \leq 0.0001$) between the yield and yield related traits measured. There was positive correlation between the pest severity damage on the superior part of the plant ($P \leq 0.0001$) with adult incidence, egg number, nymph incidence, and pupa incidence (Table 6).

Discussion

Whitefly-borne geminiviruses (CMG) occur in all main cassava-growing areas of Africa where it has been ranked as the most important vector-borne disease of any food crop (Geddes 1990), and has become the object of extensive research (Thresh et al. 1994; Bellotti and Arias 2001; Fregene et al. 2000; Akano et al. 2002; Legg and Fauquet 2004; Tomkins et al. 2004; Ogbe et al. 2006; Okogbenin et al. 2007; Dixon et al. 2008). The discovery and use of new resistance genes from wild relatives have steadily increased in different crops (Carabali et al. 2010). Breeders continue to isolate and introgress genes from wild relatives for resistance to pests and diseases of economically important crops (Hajjar and Hodgkin 2007). Tropical manioc selection (TMS) cassava cultivars, developed by the International Institute for Tropical Agriculture (IITA) using crosses with *Manihot glaziovii* Műll. Arg., in combating cassava mosaic disease is one of the major breakthroughs recorded thus far, which have contributed to a 40% yield increment in Nigeria (Nweke 2004).

Results from this study show the potential of gene introgression for pest resistance. From an earlier report by Bellotti and Arias (2001) who screened

Table 4 General linear model (GLM) analysis of yield and severity grade of whitefly *Aleurotrachelus socialis* evaluated at CIAT, Palmira, Colombia in 2007

Source of variance	df[a]	Mean square				
		Rtplant[b]	RtWt[c]	HI[d]	FRY[e]	Severity
Block	11	12.81**	0.78 ns	0.02**	47.81 ns	6.94**
Rep[f]	2	3.84 ns	0.76 ns	0.014 ns	56.46 ns	0.09 ns
Genotype	223	11.31**	1.10**	0.04**	67.29**	1.09**
Error	424	3.10	0.24	0.005	14.70	0.41
Cv[g]		31.93	42.98	22.53	42.98	23.41
H[h]		0.70	0.56	0.85	0.76	0.30

[a] Degree of freedom

[b] Root per plant

[c] Root weight (kg)

[d] Harvest index

[e] Fresh root yield (t/ha)

[f] Replication

[g] Coefficient of variation

[h] Broad-sense heritability

** $P \leq 0.0001$

Table 5 Phenotypic correlation for yield related traits and whitefly damage grade in the B_1P_2 family evaluated at CIAT, Palmira, Colombia 2007

	Variables					
	Rtplt[a]	RtWt	HI	FRY	DSup	DMed
RtWt[b]	0.75**					
HI[c]	0.61**	0.66**				
FRY[d]	0.75**	1.00**	0.66**			
DSup[e]	0.08	0.04	0.14	0.04		
DMed[f]	0.06	0.02	0.09	0.02	0.69**	
DBajo[g]	0.05	0.002	0.01	0.002	0.24**	0.14

[a] Root per plant

[b] Root weight (kg)

[c] Harvest index

[d] Fresh root yield (t/ha)

[e] Superior severity (1–6)

[f] Middle severity (1–6)

[g] Below severity (1–6)

** $P \leq 0.0001$

5363 clones of cassava from the CIAT gene bank, 73% of the clones were susceptible, but from the current study only 13.3% of the genotypes were susceptible, which is an indication that gene introgression has taken place, bearing in mind that the same high pest pressure field of CIAT headquarters was used for the screening, though in different year but the pest pressure was still high in this location during the year of screening this population. The distribution of *A. socialis* damage grade was asymmetrical with a long tail to the right, with a concentration of frequency around high resistance severity.

The range of broad-sense heritability recorded in this study was relatively high compared to those documented from others working on cassava (Ceballos et al. 2004; Okogbenin 2004; Ojulong et al. 2008) and going by the definition of heritability given by Kang (1994), the broad-sense heritability recorded for *A. socialis* resistance was high (0.30) which also pointed to the fact that introgression has taken place.

Fresh root yield averaged 8.97t/ha across the 227 genotypes ranging from 0.26t/ha to 58.59t/ha. No significant correlation between yield and pest severity suggested that yield was not affected by the severity of the *A. socialis* in this high pest pressure zone of Colombia. There was a correlation between pest incidence and severity in the population of B_1P_2 but it had no effect on the yield, which could have been as a result of multiple introgression from the wild progenitor of *M. esculenta* spp *flabellifolia* where previous reports recorded yield losses ranging from

Table 6 Correlation between incidence and severity of the population of whitefly *Aleurotrachelus socialis* on B_1P_2 family evaluated in CIAT, Palmira, Colombia in May 2007

	UAdl[a]	UEgg	Unph1	UPul1	MNp2	MPul2	APul3	Sup	Med
UEgg[b]	0.76***								
Unph1[c]	0.34***	0.54***							
UPul1[d]	0.04	0.16*	0.05						
MNp2[e]	0.02	0.09	0.32***	−0.57***					
MPul2[f]	0.36***	0.33***	0.13**	0.26***	−0.004				
APul3 [g]	−0.02	0.05	0.36***	−0.23***	0.48***	0.04			
Sup[h]	0.18***	0.36***	0.37***	0.47***	−0.01	0.37***	0.10		
Med[i]	0.13*	0.27***	0.16***	0.54***	−0.21***	0.47***	−0.07	0.69***	
Bajo[j]	0.08	0.18***	0.24***	0.01 ns	0.23***	−0.007	0.36***	0.24***	0.14***

[a] Adult population on the leaf surface

[b] Eggs number on the leaf surface

[c] Nymph population on the leaf surface

[d] Pulpa population on the leaf surface

[e] Nymph population on the middle part of the plant

[f] Pulpa population on the middle part of the plant

[g] Pulpa population on the lower part of the plant

[h] Superior part severity damage

[i] Middle severity damage

[j] Lower part severity damage

* $P \leq 0.05$, *** $P \leq 0.0001$

5, 42 and 79% respectively from the commercial cassava varieties (Vargas and Bellotti 1981; Farias 1994; Bellotti et al. 1999). This work has confirmed introgression of resistance to the *A. socialis* in the B_1P_2 family. Special emphasis will be placed on those genotypes that have shown the highest resistance to *A. socialis* and they will be re-evaluated to determine their final status and final selection will be done to identify parents for breeding purposes.

Acknowledgments This is part of a PhD thesis of the first author who was supported by a pre-doctoral fellowship granted to CIAT from Rockefeller Foundation.

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