Keywords: Community field, cassava, farmers, project WASDU, improved variety

Introduction

Cassava occupies the second row of food crops in Côte d'Ivoire, after yam. It annual production is estimated to 2.34 millions tons in 2007 (FAO, 2009). Cassava is essentially cultivated for its tuberous roots that are transformed in a hand-crafted and industrial way in foutou, attiéké, gari, flour, starch, etc.. Nevertheless, cassava crop remains traditional type with a frequent recourse to cultivars which are not very productive and are sensitive to pests and diseases.

With the growth of the population, the agroclimatic changes and the good marketable value of by-products of cassava, the request of material plant is in net progression. Therefore, the satisfaction of such request necessitates the diffusion of improved varieties more performance. The present works were carried out from 2001 to 2002 in farming environment, thanks to the financial contribution of CNRA (Centre national de recherche agronomique) and project /WASDU (West Africa for seed development unites) as well as the collaboration of NGO, OIC (Opportunities Industrialization Center) Côte d'Ivoire and ANADER (Agence nationale d'appui au développement rural). They aimed, through the evaluation of 4 improved varieties of cassava, at permitting adoption that technology by farmers.

Material and Methods

Plant material. Four improved varieties, e.g., IM84, IM89, IM93 and TMS4(2)1425 (table 1), and a local cultivar (control) were used in that study. The control variety varied from field to another. Those improved varieties were already tested in several regions of Côte d'Ivoire (N'Zué and *al.*, 2001) in which, they are priority in course of vulgarization.

Table 1: Characteristics of cassava improved varieties used

Variety	Origin	Resistance to mosaic	Yield (t/ha)	Rate of dry matter (%)	Utili zation
IM84	IDESSA (CI)	moyenne	30	35	polyvalente
IM89	IDESSA (CI)	moyenne	28	39	**attiéké, ...**
IM93	IDESSA(CI)	bonne	28	38	**attiéké, ...**
TMS4(2)1425	IITA	bonne	30	36	polyvalente

CI : Côte d'Ivoire
IDESSA : Institut des savanes (Côte d'Ivoire)
IITA : International Institute of tropical agriculture

Presentation of the experimentation region. The tests were conducted in four villages, Kouakro (region of Bouaké), Niambrun (region of Béoumi), Tiengala (region of Katiola), Ouokoukro (region of M'Bahiakro). All those regions are localized in the soudano-guinensis zone and in the Centre of Côte d'Ivoire. The rainfall is limited to two rainy seasons (April to July and September to October) and to two dry seasons (August and November to March). The annual average rainfall is comprised between 1100 and 1200 mm (ANAM, 1987; quoted by Ndabalishye, 1995).

Used methods

Experimental fields. In every village, a community field of 4000 m² was used. Groups of 7 to 100 farmers led the different tests of which set up was done in August and September 2001. Every field was divided in 4 blocks of 1000 m² on which the five varieties were affected. A given variety occupied, in a random way, a plot of 200 m². That one contained 10 rows of 20 cuttings with a planting density of 10 000 per hectare (1m x 1m).

The useful plot concerned 6 rows of 16 plants each. Field of Niambrun was alone plowed by a tractor. A month after planting, the feet lacking were replaced. The maintenance of fields is limited to weeding that did not be uniform everywhere. The harvest was carried out 12 months after planting. All the operations, from set up to harvest, were realized by farmers.

Measures and observations. The observations and measures carried out concerned : rate of sprouting, planting density at harvest, incidence of pests and diseases (mosaic, mites, mealybugs, rodents, oxen), yield, cooking and taste. The reactions of farmers on agronomic and sensory traits (vegetative growth, difficulty of harvest, cooking and taste, etc.) were also recorded.

For cooking and taste, a sample of 4 tuberous roots was removed in every bit. The roots were peeled, cut and cooked in water. After a moment time judged sufficient, 7 to 30 farmers proceeded to tasting. The damages of mealybugs and of animals were rare. The incidence of african cassava mosaic was weak (less than 5%) on improved varieties but strong on the control cultivars (more than 50%).

Statistic analysis. The collected data were subjected to an analysis of variance by GLM (General Linear Models). The classification of the averages of the treatments was realized according to Duncan's method at the level of 5 %.

Results and discussion

Analysis of variance according to the localities

Locality of Kouakro (region of Bouaké)

Rates of sprouting: Globally, the rates of sprouting were high, with a general average of 90 %. They varied of 81 % to 94 %. The improved varieties IM89, TMS4(2)1425, and IM93 recorded the better rate of sprouting (table 2). The maintenance of plots was quick for the improved varieties that had an abundant vegetable cover (limiting weeds density), and slow for the local control that had an erected harbor.

Density of plants: Average densities obtained at harvest were satisfactory, with a general average of 9318 plants per hectare. They varied from 8932 plants per hectare to 9557 plants per hectare. The weakest density was obtained by TMS4(2)1425 with 8932 plants per hectare (table 2). The harvest was easy for all the varieties, except the control Agba wakaoclè.

Incidence of mites: The mites symptoms were observed on all the varieties. Their incidence oscillated between 86 % and 97 %. The variety IM84 recorded the highest rate (97%) (table 2).

Average weights and yields. The production average by plants was weak taken as a whole. The averages varied from 0.66 kg to 1 kg. The control (Agba wakaoclè) gave the weakest average production (0.66 kg).

The yields oscillated between 6.21 t/ha (control) and 9.47 t/ha (IM84) but the difference was not significant between varieties. The weak yields could be due to the irregular weeding, poor fertility of soil and the presence of the couch grass (*Imperata cylindrica*). The improved varieties were all superior to the local cultivar (table 2).

Table 2: Characteristics of 5 varieties of cassava evaluated on farm at Kouakro (region of Bouaké)

Variety	Rate of sprouting (%)	Incidence of mites (%)	Density (plantes/ha)	Pmoyp (kg/plante)	Yield (t/ha)
	81 b	94 a	947 9 ab	0.66 a	6.21 a
IM84	86 ab	97 a	9427 ab	1.00 a	9.47 a
IM89	94 a	91 a	9557 a	0.77 a	7.40 a
IM93	93 a	86 a	9193 ab	0.73 a	6.67 a
TMS4(2)1425	94 a	92 a	8932 b	0.78 a	7.41 a
General mean	90	92	9318	0.79	7.43
Coef. variation (%)	6.70	9.46	3.73	37.59	38.56

T: control ; Density: number of plants at the harvest
Pmoyp: production average by plants 12 months after plantation.
In each column, the averages, follow by the same letter, are not significantly different between them

Cooking and taste after cooking: Cooking was good for varieties, Agba wakaoclè, IM84, TMS4(2)1425 , IM89, and moderate for IM93. They also gave a soft taste, except variety IM89, that provided a lightly bitter taste. The choice of farmers concerned the varieties IM84 and TMS4(2)1425 for their high yield, their good cooking and their soft taste.

Locality of Niambrun (region of Béoumi)

Rate of sprouting: The rate of sprouting was high and varied from 90 % to 96 %. The varieties IM89 and TMS4(2)1425 with 96 %, recorded the strongest rate of sprouting. The general average was 92 %. There was no significant difference between varieties (table 3).

Density of plants: Observed densities at harvest were satisfactory, except the local control that obtained a weak density, 6510 plants per hectare. They varied from 6510 to 9323 plants/ha (table 3). The harvest was not easy because of the texture of soil that was essentially clayey. It was painful for the varieties IM89 and Im93.

Incidence of mites: The mites were present on all of the varieties evaluated. The strongest incidences were noted on the varieties, Adama (78 %), TMS4(2)1425 (67 %) and IM93 (63 %). The varieties IM84 (9 %) and IM89 (15 %) were the less sensitive (table 3).

Average weights and yields: The average obtained weights oscillated between 1,74 kg and 3,80 kg by plants. The weak weights were furnished by the varieties IM93 (1,74 kg) and IM89 (2,16 kg).

The average recorded yields were raised and varied of 14,18 t hectares to 33,82 t hectares with a significant difference between the varieties. This good production would be had to the mechanical labour, to the fertile ground and to the uniform weeding. The better yields were obtained by the varieties IM84 (33,82 t hectares) and TMS4(2)1425 (30,55 t hectares). The average yield the weakest one was produced by IM93 (14,18 t hectares) (table 3).

Table 3 : Characteristics of 5 varieties of cassava evaluated on farm at Niambrun (region of Béoumi)

Variety	Rate of sprouting (%)	Incidence of mites (%)	Density (plantes/ha)	Pmoyp (kg/plante)	Yield (t/ha)
Adama (T)	90 a	78 a	6510 b	2.73 bc	17.66 b
IM84	90 a	9 b	8945 a	3.80 a	33.80 a
IM89	96 a	15 b	9323 a	2.16 cd	20.19 b
IM93	92 a	63 a	8125 a	1.74 d	14.18 b
TMS4(2)1425	96 a	67 a	8867 a	3.44 ab	30.55 a
General mean	92	46	8419	2.88	24.33
Coef. variation (%)	6.66	42.55	10.51	17.57	17.49

T: control ; Density: number of plants at the harvest
Pmoyp: production average by plants 12 months after plantation.
In each column, the averages, follow by the same letter, are not significantly different between them

Cooking and taste: All the varieties had a good cooking and a soft taste, to except IM93 that had a moderate cooking and IM89 that had a somewhat bitter taste.

At the end of harvest, the criteria of high yield, good cooking, soft taste, size of roots, easy harvest and weak level weeds, guided the choice of farmers. They chose TMS4(2)1425 and Im84.

Locality of Tiengala (region of Katiola)

Rate of sprouting. With a general average, in the order of 92 %, all the varieties had a good sprouting. Rates varied from 89 % (TMS4(2)1425) to 93 % (IM84), without significant difference between varieties (table 4).

Density of plants: Average densities observed at harvest oscillated between 8854 plants and 9557 plants/ha (table 4). The harvest was easy for the improved varieties. On the other hand, it was difficult for the local control despite the texture of soil that was at dominance sandy.

Incidence of mites: The incidence of mites oscillated between 14 % and 25 % without significant difference between varieties. The more weak (14 %) rate was obtained by the varieties TMS4(2)1425 and IM84 (table 4).

Average weights and yields. The average weights varied from 0.78 kg to 1.38 kg per plant without significant difference between varieties. With 0.78 kg, the control Bonoua provided the weakest weight.

Yields were situated between 7.07 t/ha and 13.23 t/ha. The highest yield was obtained by IM84 (13.23 t/ha) while the local control (Bonoua) furnished the weakest yield (7.07 t/ha) (table 4). In the whole, yields were weak. The low fertility level of soil, the irregular weeding of field and the presence of couch grass (*Imperata cylindrica*) could explain this low production.

Cooking and taste: At level of cooking, the varieties Bonoua, IM84 and TMS4(2)1425 presented a good cooking, while IM89 and IM93 gave an moderate cooking. For the taste, the varieties Bonoua, IM93, TMS4(2)1425 and IM84 had a soft taste, while IM89 presented a light bitter taste.

The preference of farmers went to two varieties, IM89 and IM84, for the good production, the good cooking, the size of tuberous roots and for the brown colour of skin.

Table 4 : Characteristics of 5 varieties of cassava evaluated on farm at Tiengala (region of Katiola)

Variety	Rate of sprouting (%)	Incidence of mites (%)	Density (plantes/ha)	Pmoyp (kg/plante)	Yield (t/ha)
Bonoua (T)	92 a	22 a	9089 a	0.78 b	7.07 b
IM84	93 a	14 a	9557 a	1.38 a	13.23 a
IM89	92 a	25 a	9521 a	1.18 ab	11.20 ab
IM93	92 a	25 a	8854 a	1.22 ab	10.76 ab
TMS4(2)1425	89 a	14 a	9234 a	1.21 ab	11.44 ab
General mean	92	20	9251	1.15	10.74
Coef. variation (%)	3.47	96.68	5.73	28.52	29.91

Locality of Ouokoukro (region of M'Bahiakro)

Rate of sprouting: The rate of sprouting was high for the local control Zoglo (92 %) and IM89 (90 %). The variety TMS4(2)1425 , with 59 %, obtained the weakest rate of growing (table 5).

Density of plants. The general average was of 7194 plants/ha. The variety TMS4(2)1425 obtained the most weak density (5938 plants/ha) while IM89 obtained the highest density (8047 plants/ha) (table 5). The improved varieties IM89 and TMS4(2)1425 presented an easy harvest, while IM84, IM93 and Zoglo were difficult to harvesting.

Incidence of the mites: The mites symptoms were rare and their incidence oscillated between 0% and 9%. The improved varieties IM84 (0%), (TMS4(2) 1425 (0%) and IM89 (3%) were the less sensitive (table 5).

Average weights and yields: The average weights by plant varied from 0.73 kg (IM93) to 2.75 kg (IM84) (table 5). All the yields were in weak with a general average of 12.87 t/ha. The variety IM84 obtained the highest yield with 18.90 t/ha while IM93 recorded the lowest yield (5.10 t/ha). The low production level could be explained by the weak density of plants at the harvest (table 5) and by the irregular weeding.

Table 5 : Characteristics of 5 varieties of cassava evaluated on farm at Ouokoukro (region of M'Bahiakro)

Variety	Rate of sprouting (%)	Incidence of mites (%)	Density (plantes/ha)	Pmoyp (kg/plante)	Yield (t/ha)
Zoglo (T)	92 a	9 a	7604 a	1.86 b	14.33 bc
IM84	76 b	0 c	6840 ab	2.75 a	18.90 a
IM89	90 ab	3 bc	8047 a	1.90 b	15.37 b
IM93	78 ab	6 ab	6823 ab	0.73 c	5.10 d
TMS4(2)1425	59 c	0 c	5938 b	1.93 b	11.48 c
Genral mean	79	4	7194	1.77	12.87
Coef. variation (%)	11.07	64.91	10.85	11.64	14.17

T: control ; Density: number of plants at the harvest
Pmoyp: production average by plants 12 months after plantation.
In each column, the averages, follow by the same letter, are not significantly different between them

Cooking and taste. All the varieties had a good cooking, except IM93 that presented an moderate cooking. They were also soft, except the variety IM89 that was lightly bitter.

Referring to the criteria, of yield, size of the roots, of cooking and taste, farmers chose two varieties, TMS4(2)1425 and IM89.

Global analysis

Analysis according to improved varieties used

The rate of sprouting varieties, oscillated between 84 and 93 % without statistical difference. For the density of plants at harvest, averages were good. The presence of mites was often marked on the varieties IM93 (45%) and TMS4(2)1425 (43%). Yields varied between 8.85 t/ha (IM93) and 18.85 t/ha (IM84) (table 6).

The interaction between localities and varieties was significant for all the studied parameters, except density. That justifies the statistic analysis done by locality (cf. paragraph 2.1.)

Analysis according to the localities of tests

The tests conducted in the 4 regions recorded rates of sprouting satisfactory. Those varied from 76 % to 93 %, without significant difference between regions (table 7). The densities at harvest were high at Kouakro, Tiengala and Niambrun. At the level of mites, the incidence was more important at Kouakro than in the other sites. The locality of Niambrun obtained the best yield with 25.76 t/ha. This superiority would be due to regular weeding, good fertility level of soil and mechanical ploughing.

Table 6 : Aggregate characteristics of varieties tested (without controls) in the regions of Bouaké, Béoumi, Katiola and M'Bahiakro

Variety	Rate of Sprouting (%)	Incidence of mites (%)	Density (plantes/ha)	Pmoyp (kg/plante)	Yield (t/ha)
IM84	86 bc	30 b	8816 ab	2.20 a	18.85 a
IM89	93 a	33 b	9098 a	1.46 c	13.09 c
IM93	88 ab	45 a	8257 c	1.07 d	8.85 d
TMS4(2)1425	84 c	43 a	8572 bc	1.83 b	15.75 b
Locality x varety (Pr>F)	0.001	0.000	0.469	0.000	0.000
General mean	88	38	8688	1.63	14.11
Coef. variation (%)	7.17	32.03	7.18	20.56	23.16

Density: number of plants at the harvest
Pmoyp: production average by plants 12 months after plantation.
In each column, the averages, follow by the same letter, are not significantly different between them

Table 7 : Aggregate characteristics of the four regions in which tests of cassava were conducted

Variety	Rate of sprouting (%)	Incidence of mites (%)	Density (plantes/ha)	Pmoyp (kg/plante)	Yield (t/ha)
Kouakro	92 a	92 a	9277 a	0.82 d	7.73 c
Niambrun	93 a	38 b	8828 a	2.91 a	25.76 a
Tiengala	92 a	20 c	9291 a	1.25 c	11.66 b
Ouokoukro	76 a	2 d	7067 b	1.74 b	12.43 b
Locality x variety (Pr>F)	0.001	0.000	0.469	0.000	0.000
General mean	88	38	8688	1.63	14.11

Density: number of plants at the harvest
Pmoyp: production average by plants 12 months after plantation.
In each column, the averages, follow by the same letter, are not significantly different between them

Conclusion

In final, new varieties evaluated, except IM93, were more productive than cultivars controls used. Their yields varied from 7 to 34 t/ha against 6 to 18 t/ha for controls. Referring to criteria (high yield, size of tuberous roots, easy harvest, good cooking and soft taste, cover dense vegetable and, to a least degree, the brown colour of skin), the groups of farmers chose three improved varieties, IM84, TMS4(2)1425 and IM89.

In the second phase of the project, the 3 improved varieties, chosen by the farmers, had to be multiplied in demonstration plots. A training of farmers for the multiplication of quick technique by ratooning was foreseen. By that approach, more farmers will be able to cultivate those improved varieties.

Besides, the root and tuber crops programme of CNRA envisages to follow the same transfer process for another improved clones of cassava from, CNRA and IITA, proved very performance at the end of evaluation in an experimental station (yield, oscillating between 28 and 40 t/ha, rate of dry matter, varying from 35 to 44 %, etc.).

References quoted

FAO. 2009.
Http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Ndabalishye (I). 1995. Agriculture vivrière ouest-africaine à travers le cas de la Côte d'Ivoire. Monographie. Institut des Savanes, Bouaké, Côte d'Ivoire. 47-51.

N'Zué, B. , Zohouri, G.P. et Kouadio, K. 2001. Introduction de nouvelles variétés de cassava en milieu paysan. *In* Variétés améliorées de cassava en milieu paysan en Afrique de l'Ouest. Actes d'un atelier régional sur le cassava. IITA, Cacavali, Togo. 11-12 septembre 2001. 42-51.

Sweet potato for adapting to climate change in the mixed crop-livestock systems of East Africa

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Abstract

Sub-Saharan Africa (SSA) is predicted to experience considerable negative impacts of climate change. The IPCC Fourth Assessment emphasizes that adaptation strategies are essential. Addressing adaptation in the context of small-scale, semi-subsistence agriculture raises special challenges. An important constraint is that data demands are high, because site-specific bio-physical and economic data are required. The development of relatively simple methods for ex ante evaluation of adaptation at the household and system levels is therefore needed. We test a new approach to ex ante impact assessment that produces site-specific results that can also be aggregated for regional analysis. The methodology uses the kinds of data that are more often available in resource-poor countries. The stochastic approach integrates socio-economic and bio-physical data on farmers' land allocation, production, input and output use. Characteristics of the agricultural system regarding resources and productivity are analyzed and compared for both current and projected climate. Possible adaptation strategies are then assessed for their capability to reduce the adverse effects of climate change. We apply the methodology to two study areas in Kenya (Vihiga and Machakos-Makueni districts). Drought and heat tolerant varieties of sweet potato are currently being promoted in Kenya and in general sweet potatoes are known as a reliable food security crop giving good yields in marginal climatic and soil conditions. After characterizing the current system with actual climate data, the effects of a perturbed climate are analyzed and a variety of sweet potato technologies are tested for their ability to overcome or reduce the negative impacts of climate change. Despite the limitations, the new approach offers a flexible framework for evaluating adaptation strategies using scarce data of resource-poor countries in SSA and other parts of the world. It allows a rapid integrative analysis for timely advice to policymakers and for exploration of technology and policy options.

Keywords: adaptation, climate change, sweet potato, East Africa, impact assessment

Introduction

The changing climate is exacerbating existing vulnerabilities of the poorest people who depend on semi-subsistence agriculture for their survival (Slingo et al., 2005; IPCC, 2007). Sub-Saharan Africa (SSA) in particular is predicted to experience considerable negative impacts of climate change (e.g., Thornton et al., 2006). The IPCC Fourth Assessment emphasizes that adaptation strategies are essential and these must be developed within the broader economic development policy context (IPCC, 2007). Addressing adaptation in the context of small-scale, semi-subsistence agriculture in SSA raises special challenges that cannot be addressed adequately by the approaches taken thus far in most studies (Adger, 2003). Most of the existing research has focused on impacts of climate change and adaptation to climate change in the agricultures of industrialized countries. In the relatively few studies conducted in Africa, agricultural research has either focused on individual crops (e.g., Jones and Thornton, 2003), has used aggregated data and models (e.g., Winters et al., 1999, Mendelsohn et al., 2000), or used statistical analysis too general to be useful for site-specific adaptation strategies (e.g., Kurukula-suriya and Mendelsohn, 2006). One of the important constraints to carrying out this type of research is that the data demands are high, because site-specific bio-physical and economic data are required, typically obtained from costly multi-year farm-level surveys. The development and application of relatively simple and reliable methods for ex ante evaluation of adaptation strategies at the household and system levels are needed to provide timely assessments of the potential impacts in the context of climate change.

Drought and heat tolerant varieties of sweet potato are currently being promoted in Kenya and in general sweet potatoes are known as a reliable food security crop giving good yields in marginal climatic and soil conditions (Diop, 1998, Bovell-Benjamin, 2007, Andrade et al., 2009). In this paper, we use the proposed methodology to ex ante

evaluate whether sweet potato technologies can overcome or reduce the negative effects of climate change in two study areas in Kenya.

Methods

This paper applies a new approach to ex ante impact assessment that produces locally useful, site-specific results that can also be aggregated for regional policy analysis. The Tradeoff Analysis Minimum Data model (TOA-MD) has been used for the analysis of technology adoption and payments for environmental services (Antle and Validivia, 2006, Antle and Stoorvogel, 2008, Immerzeel et al., 2008, Claessens et al., 2009) but can also be set up and interpreted for climate change applications. Antle et al. (2010) provide an overview of the methodology, and present a validation of the MD approach against more complex, spatially-explicit models of semi-subsistence agricultural systems. The analysis presented here is based on an extension of the technology adoption model to include calculation of poverty rates associated with adoption. Technical details are provided in Antle (2010). The methodology makes use of the kinds of data that are more often available, especially in resource-poor countries. The stochastic approach uses and integrates available socio-economic and bio-physical data on farmers' land use allocation, production and input and output use. Spatially heterogeneous characteristics of the agricultural system regarding resources and productivity are analyzed and compared for both current climate conditions and predicted climate changes. A variety of possible adaptation strategies is then assessed for their capability to overcome or reduce the adverse effects of climate change. A static expected profit maximization model is used to characterize the opportunity cost of adaptation (Antle and Valdivia, 2006). The model represents the impact of climate change as the "compensating variation", i.e., the loss in income that producers experience relative to the base climate scenario.

We apply the methodology to the mixed crop-livestock systems of Vihiga district in western Kenya and Machakos-Makueni districts in eastern province (Figure 1). After characterizing the current agricultural system with actual climate data, the effects of a perturbed climate on bio-physical and economic indicators are analyzed and a variety of adaptation strategies (agricultural technologies) are tested (Table 1). The data for Vihiga originate from the PROSAM project, 'systems prototyping and impact assessment for sustainable alternatives in mixed farming systems in high-potential areas of east Africa' (Waithaka et al., 2005), that aimed to assess natural resource management interventions that promote sustainability of prototype farming systems. Farm data were collected in 2000 and 2002 (Waithaka et al., 2005, Salasya, 2005). For Machakos and Makueni we used farm survey data for 120 households in six villages obtained from studies conducted in the NUTMON project (de Jager et al. 2001, Gachimbi et al. 2005) (Table 1).

To simulate the potential effects of climate change on crop yields, crop growth simulation models as currently implemented in version 4.0 of the Decision Support System for Agrotechnology Transfer (DSSAT, Jones et al., 2003; ICASA, 2007) were used for maize and beans (Thornton et al., 2009a). The yields used are the mean simulated yields for four combinations of the two GCMs and SRES scenarios. For both crops and both study areas, a declining yield trend is projected, caused by an increased temperature without adequate rainfall (Thornton et al., 2010) (Table 1). The introduction and adoption of an improved (heat/drought tolerant) maize variety, bringing yields back to 95% of the base level, is tested as an adaptation strategy in both study areas ('imz' in Table 1). For sweet potato, Napier and the 'mixed crops', no crop growth simulation models are currently available. For Napier and 'mixed', a 20% yield decline was estimated. The vegetables in Machakos-Makueni are irrigated and no yield reduction was assumed under climate change. We tested a 20% milk yield reduction for both study areas, caused by a combination of declined yields (less on farm produced fodder available for feed) and heat stress affecting the animals. Introducing dual-purpose sweet potato (DPSP) as an adaptation strategy increases both quantity and quality (mainly crude protein content) of on farm produced animal feed and can substantially improve milk yields and farm incomes (Claessens et al., 2009). In combination with an anticipated genetic improvement through both natural and artificial selection as adaptation strategy (Seré et al., 2008; Thornton et al., 2009b), we tested bringing up the milk yield back to 100% and 120% of the base production for both study areas ('dpsp100' and 'dpsp120' respectively in Table 1).

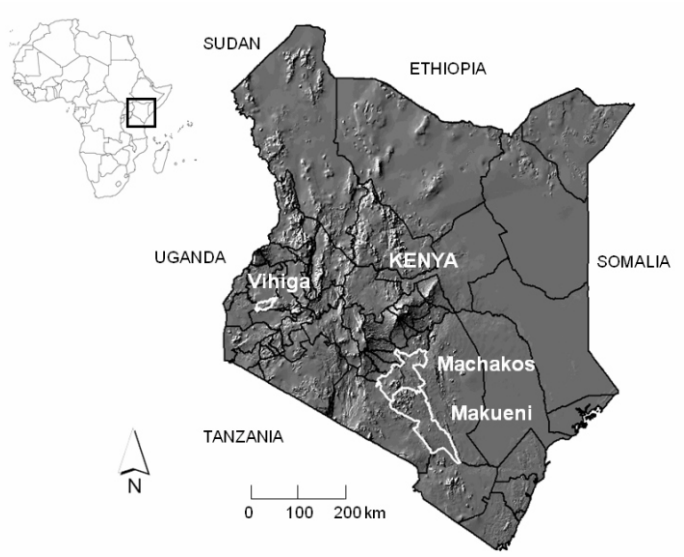

Figure 1. Location of the study areas.

Table 1. Summary of data used in the TOA-MD analysis. Characterization of base system from survey data (means with standard deviation between brackets). Alternative systems are expressed as % productivity changes.

Vihiga	**Base System**			**Alternative Systems***					
	System 1			*System 2*	*System 3*				
Activities	Area	Crop Yield	Net Returns	CC	imz	dpsplw	dpsp	dpsp100	dpsp120
	Ha/season/farm	Kg/ha/season	KSh/ha	% of base yield					
Maize-Beans	0.24 (0.21)	1512 (1319)	15360 (16726)	68	95	68	68	68	68
Napier Grass	0.16 (0.19)	34450 (23643)	22366 (23545)	80	80	80	80	80	80
Mixed	0.23 (0.21)	4031 (1701)	27551 (15971)	80	80	80	80	80	80
Sweet potato	0.05 (0.04)	4006 (2092)	11587 (8352)	100	100	100	100	100	100
DPSProots	-	8000 (3675)	27618 (3676)	-	-	37.5	100	100	100
DPSP vines	-	14800 (8036)	21018 (8036)	-	-	70	100	100	100
		Liters/season/farm							
Milk	-	3211 (2473)	52317 (51723)	80	80	80	80	100	120
Machakos	**Base System**			**Alternative Systems***					
- Makueni	*System 1*			*System 2*	*System 3*				
Activities	Area	Crop Yield	Net Returns	CC	imz	dpsplw	dpsp	dpsp100	dpsp120
	Ha/season/farm	Kg/ha/season	KSh/ha	% of base yield					
Mixed	0.95 (1.39)	1187 (1631)	7085 (13313)	80	80	80	80	80	80
Maize	0.78 (0.79)	1597 (1624)	12704 (16996)	75	95	75	75	75	75
Beans	0.44 (0.59)	1390 (1374)	24658 (17942)	75	75	75	75	75	75
Vegetables	0.75 (1.00)	4121 (3369)	40718 (139490)	100	100	100	100	100	100
Napier Grass	1.49 (3.10)	12318 (14435)	11310 (18146)	80	80	80	80	80	80
DPSP roots	-	7100 (4501)	27618 (3676)	-	-	42	100	100	100
DPSP vines	-	12600 (9013)	21018 (8036)	-	-	83	100	100	100
		Liters/season/farm							
Milk	-	1784 (1992)	39238 (48208)	80	80	80	80	100	120

*CC = climate change, imz = improved maize, dpsp = dual purpose sweet potato, dpsplw = low yielding dpsp, dpsp100 = dpsp with 100% of base milk yield under CC, dpsp120 = dpsp with 120% of base milk yield under CC.

Results and discussion

Aggregated results of the TOA-MD analysis for both study areas are shown in Figure 2. The interpretation of the curves is as follows: The point where a curve crosses the x axis tells the percentage of farms that gain from the scenario, conversely 100 minus that value is the percentage of farms that loses from the scenario. Accordingly, the points on a curve to the left of where it crosses the x axis show the percentage of farms with gains (i.e., negative losses) greater than the amount shown on the vertical axis. Conversely, points to the right of where a curve crosses the x axis show the percentage of farms with losses less than or equal to the amount on the vertical axis. Figure 2 shows that climate change is projected to have a negative economic impact on 79% of the farmers in Vihiga and on 68% in Machakos-Makueni.

By testing different adaptation strategies with the TOA-MD model (Table 1), we can simulate the aggregate economic impact on each of the study areas. The introduction of an improved maize variety as adaptation strategy (bringing back yields to 95% of the base level), has very limited effect in both study areas. Substituting half of the mixed system with low yielding DPSP has almost no effect in Vihiga (it is worse than improved maize), but does have already a profound effect in Machakos, reducing the percentage of farmers that are negatively affected from 68 to 51% and offsetting the negative effects of climate change on the aggregate level. Increasing the average yield of DPSP to the observed levels but keeping the loss in milk yield at 20%, has a positive effect in both study areas (negatively affected farmers from 79 to 63% in Vihiga and from 68 to 34% in Machakos-Makueni). In general the Vihiga study area still has negative impacts of climate change while Machakos-Makueni gains with this adaptation strategy. By increasing milk yields to 100 and 120% of the base level, Machakos-Makueni has almost no additional gain, whereas in Vihiga this offsets the negative effects of climate change or even brings down the percentage of negatively affected farmers to 40%. Bringing in a low yielding DPSP in the cropping system of Machakos-Makueni is simulated to be sufficient to offset the negative effects of climate change whereas improved livestock breeds that can perform under increased heat stress are and additional requirement for Vihiga.

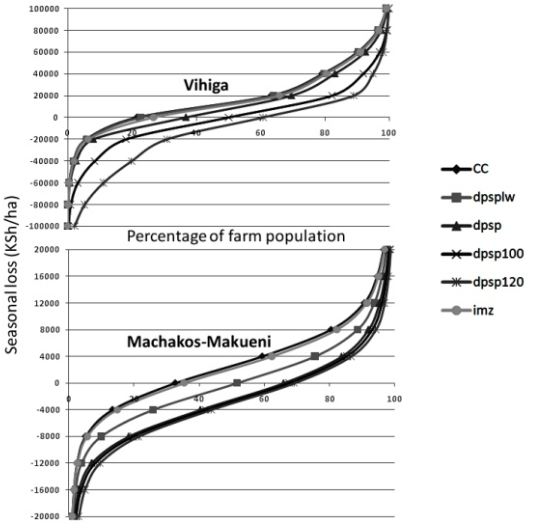

Figure 2. Economic impact of climate change and simulated adaptation strategies on farmers in Vihiga and Machakos-Makueni, Kenya. Notation of legend as in Table 1.

Conclusions

The development and application of relatively simple and reliable methods for assessing the impacts of climate change and adaptation strategies at the agricultural system and/or household level are needed to provide timely recommendations on the potential impacts of alternative technologies and policies. In this paper the TOA-MD methodology was proposed to evaluate the impacts of climate change and the economic viability of adaptation strategies using limited data that are often available in resource-poor countries. The method was applied to the mixed crop-livestock systems of Vihiga and Machakos-Makueni districts in Kenya. With a combination of simulated and estimated changes in crop and livestock productivity, the economic impacts of climate change to 2050 were analyzed. Climate change is projected to have a negative economic impact on 79% of the farmers in Vihiga and on 68% in Machakos-Makueni. Different adaptation strategies were tested by changing crop and livestock productivity under climate change. For Machakos-Makueni, the introduction of a low yielding variety of DPSP can already offset the negative economic impacts of climate change. For Vihiga, high yielding DPSP and improved livestock breeds adapted to increased temperatures are needed to counter the effects of climate change and improve farmers' livelihoods. As in all scenario studies using models, and especially in

the context of climate change, various assumptions and uncertainties are associated with using the proposed approach and results should be interpreted with caution. But despite the limitations of the methodology, this study yielded insights into the way different adaptation strategies could assist in improving the livelihoods of smallholder farmers operating in the mixed crop-livestock systems in East Africa. The TOA-MD approach offers a rapid integrative analysis for exploring options and timely advice to farmers and policymakers.

References

Adger, W. N., Huq, S., Brown, K., Conway, D., Hulme, M. 2003. Adaptation to climate change in the developing world. Progress in Development Studies, 3(3):179-195.

Andrade, M., Barker, I., Cole, D., Dapaah, H., Elliott, H., Fuentes, S., Grüneberg, W., Kapinga, R., Kroschel, J., Labarta, R., Lemaga, B., Loechl, C., Low, J., Lynam , J., Mwanga, R., Ortiz, O., Oswald, A., Thiele, G., 2009. Unleashing the potential of sweetpotato in Sub-Saharan Africa: Current challenges and way forward. International Potato Center (CIP), Lima, Peru. Working Paper 2009-1. 197 p.

Antle, J.M. 2010. Parsimonious Technology Impact Assessment. American Journal of Agricultural Economics, submitted.

Antle, J.M., Stoorvogel, J.J., 2008. Agricultural carbon sequestration, poverty and sustainability. Environment and Development Economics 13, 327-352.

Antle, J.M., Valdivia, R.O., 2006. Modelling the supply of ecosystem services from agriculture: a minimum-data approach. The Australian Journal of Agricultural and Resource Economics 50: 115.

Bovell-Benjamin, A., 2007. Sweet potato: a review of its past, present, and future role in human nutrition. Advances in Food and Nutrition Research 52

Claessens, L., Stoorvogel, J.J., Antle, J.M. 2009. Ex ante assessment of dual-purpose sweet potato in the crop-livestock system of western Kenya: A minimum-data approach. Agricultural Systems, 99(1):13-22.

de Jager, A., D. Onduru, M. S. van Wijk, J. Vlaming, Gachini, G. N., 2001. Assessing sustainability of low-external-input farm management systems with the nutrient monitoring approach, a case study in Kenya, Agricultural Systems 69(1-2), 99-118.

Diop, A., 1998. Storage and processing of roots and tubers in the tropics. In "Food and Agriculture Organization of the United Nations, Agro-Industries and Post-Harvest Management Service" (D.J.B. Calverley, ed.), pp. 3850. Agricultural Support Systems Division. Food and Agriculture Organization, Rome, Italy.

Gachimbi, L. N., H. van Keulen, E. G. Thuranira, A. M. Karuku, A. Jager, S. Nguluu, B. M. Ikombo, J. M. Kinama, J. K. Itabari, Nandwa, S. M., 2005. Nutrient balances at farm level in Machakos (Kenya), using a participatory nutrient monitoring (NUTMON) approach, Land Use Policy 22(1), 13-22.

ICASA, 2007. The International Consortium for Agricultural Systems Applications website. Online at www.icasa.net.

IPCC (Intergovernmental Panel on Climate Change) 2007. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Immerzeel, W., J. Stoorvogel, Antle, J., 2008. Can payments for ecosystem services secure the water tower of Tibet? Agricultural Systems 96(1-3), 52-63.

Jones, P.G., Thornton, P.K. 2003. The potential impacts of climate change on maize production in Africa and Latin America in 2055. Global Environmental Change, 13(1):51-59.

Jones, P. G., Thornton, P. K., Heinke, J., 2009. Software to generate characteristic daily weather data at any resolution using downscaled climate model data from the IPCC's Fourth Assessment. BMZ Project Discussion Paper, ILRI, Nairobi.

Kurukulasuriya, P., Mendelsohn, R. 2006. Crop Selection: Adapting Climate Change in Africa. CEEPA Publication 26. http://www.ceepa.co.za/docs/CDPNo26.pdf.

Mendelsohn, R., Morrison, W., Schlesinger, M.E., Andronova, N.G. 2000. Country-Specific Market Impacts of Climate Change. Climatic Change, 45(3-4):553-569.

Salasya, B.D.S., 2005. Crop production and soil nutrient management: an economic analysis of households in western and central Kenya. PhD Thesis, Wageningen

University, Wageningen, The Netherlands.

Thornton, P.K., Jones, P.G., Owiyo, T., Kruska, R.L., Herrero, M., Kristjanson, P., Notenbaert, A., Bekele, N., Omolo, A., with contributions from Orindi, V., Ochieng, A., Otiende, B., Bhadwal, S., Anantram, K., Nair, S., Kumar, V., Kelkar, U., 2006. Mapping climate vulnerability and poverty in Africa. Report to the Department for International Development, ILRI, Nairobi, Kenya, May 2006, 200 pp. http://www.dfid.gov.uk/research/mapping-climate.pdf.

Thornton, P.K., Jones, P.G., Alagarswamy, G., Andresen, J. 2009a. Spatial variation of crop yield response to climate change in East Africa. Global Environmental Change, 19(1):54-65.

Thornton, P.K., van de Steeg, J., Notenbaert, A., Herrero, M., 2009b. The impacts of climate change on livestock and livestock systems in developing countries: A review of what we know and what we need to know. Agricultural Systems 101, 113-127.

Thornton, P.K., Jones, P.G., Alagarswamy, G., Andresen, J., Herrero, M., 2010. Adapting to climate change: Agricultural system and household impacts in East Africa. Agricultural Systems 103, 73-82.

Seré, C., van der Zijpp, A., Persley, G., Rege, E., 2008. Dynamics of livestock production systems, drivers of change and prospects for animal genetic resources. Animal Genetic Resources Information 42, 3-27.

Slingo,J. M., Challinor, A. J., Hoskins, B. J., Wheeler, T. R. 2005. Food crops in a changing climate. Philosophical Transactions of the Royal Society B, 360(1463):1983-1989.

Waithaka, M.M., Thornton, P.K., Herrero, M., Shepherd, K.D., Stoorvogel, J.J., Salasya, B., Ndiwa, N., Bekele, N., Croucher, M., Karanja, S., Booltink, H., 2005. System prototyping and impact assessment for sustainable alternatives in mixed farming systems in high-potential areas of Eastern Africa. Final Program Report to the Ecoregional Fund to Support Methodological Initiatives.

Winters, P., Murgai, R., Sadoulet, E., de Janvry, A., Frisvold, G. 1998. Economic and Welfare Impacts of Climate Change on Developing Countries. Environmental & Resource Economics, 12(1):1-24.

Discovery of Single Nucleotide Polymorphisms (SNPs) as a tool for marker-assisted breeding of cassava, *Manihot esculenta*

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Abstract
Discovery of single nucleotide polymorphisms, (SNPs) as a tool in marker-assisted breeding of cassava, *Manihot esculenta* was studied. The principal objective of this study is to discover single nucleotide polymorphisms (SNPs) in some panels of elite lines (ELs) of cassava and in some mapping populations to be used as a tool for marker-assisted breeding in cassava (*Manihot esculenta*). As a nucleotide base constitutes genetic information for inheritance, SNPs provide the ultimate form of molecular genetic markers. Small insertion or deletion occurrences (indel for insertion/deletion) are other factors that bring about genetic mutations. These mutations may be detected as SNPs as the insertion or deletion of nucleotides changes the sequence read. The rapid advance of the sequencing technology and the steadily declining cost of sequencing prompted the development of a gamut of SNP discovery and genotyping technologies. A panel of ELs and three mapping populations (TME3xTME117, TME3x30555 and TME14x96/1089A) were used. Six full gene sequences (both nuclear and chloroplast genes), involved in starch, sucrose, and cyanogenic glucoside biosynthesis, and three promoter sequences were used for designing primers. Single nucleotide polymorphism analysis of a total of 9,993 base pairs of sequence revealed 184 SNP sites and 43 InDels, giving rise to frequencies of 1 SNP per 54 bp and 1 SNP per 232 bp respectively. Exonic and intronic regions were identified to be 106 and 78 respectively. Synonymous and non-synonymous changes were identified using Codon-based Test of Neutrality. The frequency of SNPs was much higher than that of the InDels. The polymorphisms observed were due to the effects of transitions (C/T or A/G), transversions (C/G, A/T, A/C or G/T), hetero zygosities and the occurrence of InDels within the sequenced samples. A total of 126 polymorphisms (68.5%) were transitions (C/T or A/G) and 58 polymorphisms (31.5%) were transversions (C/G, A/T, A/C, or G/T). Analysis of preference using Z-test of selection for estimation of synonymous substitutions per synonymous sites (dS) and nonsynonymous substitutions per nonsynonymous sites (dN) was also done on TME14 x 96/1089A. The dN (306.7) > dS (125.5) was noted showing that positive selection is operating within the genes used. Parentage of F1 hybrids of TME14X96/1089A was verified using SNPs generated from four genes out of the aforementioned number of genes used in this study and these highly informative primers not only differentiated the parent genotypes but also confirmed the parentage of their true F1 hybrids.

Keywords: SNPs *marker*, InDels, cassava, genotyping

Introduction

Cassava, *Manihot esculenta* Crantz is a root crop that belongs to the family of Euphorbiaceae. All Manihot species, including cultivated cassava, that have been studied so far have a chromosome number of n = 36 and show regular bivalent pairing at meiosis (Jennings and Iglesias, 2002). Cassava is the only one out of 98 species in its family that is widely cultivated for food production. Cassava uniformly is 2n = 36 in nature.

A growing number of molecular markers are becoming available for cassava research community in the past few years. Currently, the most common type of molecular markers accessible to the cassava research community is predominantly simple sequence repeats (SSR) (Mba *et al.* 2001; Okogbenin *et al*. 2006). Lately, the increasing number of available expressed sequence tags (ESTs) (Anderson *et al*. 2004; Lokko *et al.* 2007; Sakurai *et al.* 2007) and the completion of the cassava genome sequencing is poised to spur the discovery and utilization of single nucleotide polymorphism (SNP) markers that are suitable for high throughput genotyping platforms. The use of SNP markers is becoming more and more relevant for cassava as the amounts of publicly available genomic and cDNA sequences continue to grow at an accelerated pace.

Single nucleotide polymorphism (SNPs) markers are the most abundant and common form of DNA polymorphism in a given genome. They are expressed as a single base mutation in DNA or an individual nucleotide base difference between two DNA sequences. There are three different categories of SNPs such as transitions (C/T or G/A), transversions (C/G, A/T/ C/A or T/G) and small insertions and deletions (InDels) (Duran *et*

al., 2009). The abundance of SNP in the genome and their suitability for automated discovery and genotyping make SNPs, markers of choice in the post-genomic era (Oraguzie *et al.,* 2007). Therefore, the objective of this study was to discover SNPs in cassava by using standard Sanger sequencing methods.

Materials and Methods

Plant Materials

Forty nine (49) accessions of cassava DNA samples regarded as the elite lines (ELs) and three mapping populations (TME3 x TME117, TME3 x 30555 and TME14 X 96/1089A), were used for discovery of SNPs. Nine (9) different pairs of genes were used to amplify the above-mentioned cassava accessions of panel of ELs. Partial sequence of the Ty3/Gypsy-like retrotransposon sequence was amplified in the panel of segregating populations and unrelated individuals.

Target Genes and Primers

Partial and full sequences of a number of genes were retrieved from public databases and utilized to design primers for re-sequencing of the above samples. We have utilized about six full gene sequences (both nuclear and chloroplast genes), involved in starch, sucrose, and cyanogenic glucoside biosynthesis, and three promoter sequences (e.g. A superoxide dismutase) of *Manihot esculenta* for designing primers.

In addition, partial sequence of the Ty3/Gypsy-like retrotransposon sequence was amplified in the above panel of segregating populations and individuals. Explicitly shown below is a simplified schematic representation of the steps involved in single nucleotide polymorphisms (SNPs).

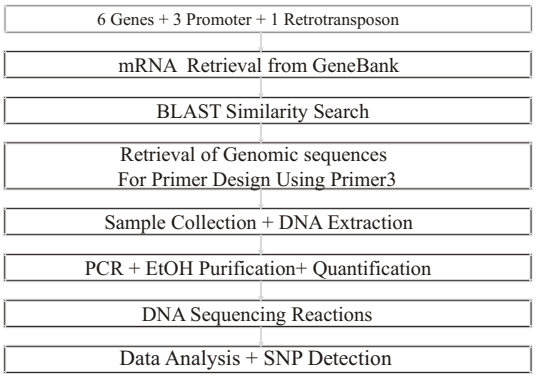

Figure 1. Schematic representation of steps involved in data analysis and SNP detection

Results

For the forty nine (49) genotypes and mapping populations used for this study of SNP discovery, SNP analysis of a total of 9,993 base pairs of sequence revealed 184 SNP sites and 43 InDels, giving rise to frequencies of 1 SNP per 54 bp and 1 SNP per 232 bp respectively. Exonic and intronic regions were identified to be 106 and 78 respectively. A total of 126 polymorphisms (68.5%) were transitions (C/T or A/G) and 58 polymorphisms (31.5%) were transversions (C/G, A/T, A/C, or G/T). The frequency of SNPs was much higher than that of the InDels. The polymorphisms observed were due to the effects of transitions transversions, heterozygosities and the occurrence of InDels within the sequenced samples. Highest number of SNPs was detected in Ty3/Gypsy gene while the lowest number of SNPs was detected in SBE gene and none in GBSS gene. Highest number of InDels occurrence was recorded in SOD gene while the lowest was recorded in CYP79D2 gene and none in C4F-C6R, SBE and Linamar respectively. It was observed that transitions contributed the highest number of SNPs in this study.

The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. (Parameters = codon positions included were 1st+2nd+3rd+Noncoding; pairwise deletion option). There were a total of 878 positions in the final dataset.

Estimates of numbers of nonsynonymous (dN) and synonymous (dS) per their respective sites from averaging over all sequence pairs was done. All results were based on pairwise analysis of 121 sequences of 1M mapping populations using the modified Nei-Gojobori (assumed transition/transversion bias = 2) method in

MEGA4 (Zhang *et al.,* 1998., Tamura *et al.,* 2007). There were a total of 277 positions in the final dataset. The results showed that dN (306.7) > dS (125.5). In addition, verification of hybrids generated from TME1X96/1089A was confirmed via the application of SNPs. For instance, at position 393 of CYP79D1, the parents had T and C respectively while the progeny had C/T indicating inheritance of both paternal and maternal genetic traits by the progeny.

Occurrence of Heterozygosity and Homozygosity in the sequences

Occurrence of heterozygosity and homozygosity within the aligned sequences contribute to the effects of single nucleotide polymorphisms (SNPs). In Fig. 1a, illustration of occurrence of heterozygosities within the aligned sequenced genotypes is indicated by arrows. Here, A/G (**R**) occurs at 338 positions of consensus sequences. Heterozygosities contribute gigantically to the SNP occurrence, especially in cassava which is highly heterozygous in nature. Peaks of chromatograms normally exhibit a certain degree of reduction when there is occurrence of heterozygosities at a particular consensus position. Apart from occurrence of A/G as a heterozygote, there are other forms of heterozygosities and their ambiguity codes which include A/T (**W**), G/T (**K**), C/T (**Y**) (Fig. 1b and 1c), among others and they contribute significantly to single nucleotide polymorphisms (SNPs). However, in the case of homozygosity, it could be either of the two taking place in one locus chromosome and not the two together as observed in heterozygosity. Here, adequate and stringent measures are always put into consideration to avoid mistaking contaminations as heterozygosities.

Figure 1a ➡

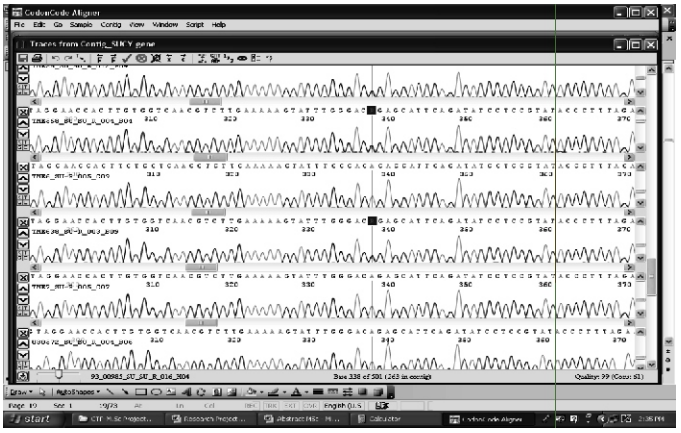

Figure 1b ➡

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A01_D21089A_644930   AAAACTTCAAAAAAGGGCWTCCTACAAGAAAGCTAGYAAGAAC
A02_PROG9_644938     AAAACTTCAAAAAAGGGCTTCCTACAAGAAAGCTAGTAAGAAC
A03_PROG17_644946    AAAACTTCAAAAAAGGGCTTCCTACAAGAAAGCTAGTAAGAAC
B01_D2TME14_644931   AAAACTTCAAAAAAGGGCWTCCTACAAGAAAGCTAGYAAGAAC
B02_PROG10_644939    AAAACTTCAAAAAAGGGCWTCCTACAAGAAAGCTAGTAAGAAC
B03_PROG18_644947    AAAACTTCAAAAAAGGGCTTCCTACAAGAAAGCTAGTAAGAAC
C01_PROG3_644932     AAAACTTCAAAAAAGGGCWTCCTACAAGAAAGCTAGYAAGAAC
C02_PROG11_644940    AAAACTTCAAAAAAGGGCWTCCTACAAGAAAGCTAGYAAGAAC
C03_PROG19_644948    AAAACTTCAAAAAAGGGCWTCCTACAAGAAAGCTAGTAAGAAC
D01_PROG4_644933     AAAACTTCAAAAAAGGGCATCCTACAAGAAAGCTAGCAAGAAC
D02_PROG12_644941    AAAACTTCAAAAAAGGGCWTCCTACAAGAAAGCTAGTAAGAAC
```

Figure 1c ➡

```
                          310        320        330
                     ....|....|....|....|....|....|
A01_D21089A_644930   CTTGACGACGATKGTGGTGCCATACAATGA
A02_PROG9_644938     CTTGACGACGATKGTGGTGCCATACAATGA
A03_PROG17_644946    CTTGACGACGATKGTGGTGCCATACAATGA
B01_D2TME14_644931   CTTGACGACGATKGTGGTGCCATACAATGA
B02_PROG10_644939    CTTGACGACGATGGTGGTGCCATACAATGA
B03_PROG18_644947    CTTGACGACGATKGTGGTGCCATACAATGA
C01_PROG3_644932     CTTGACGACGATKGTGGTGCCATACAATGA
C02_PROG11_644940    CTTGACGACGATKGTGGTGCCATACAATGA
C03_PROG19_644948    CTTGACGACGATTGTGGTGCCATACAATGA
D01_PROG4_644933     CTTGACGACGATKGTGGTGCCATACAATGA
D02_PROG12_644941    CTTGACGACGATKGTGGTGCCATACAATGA
D03_PROG20_644949    CTTGACGACGATTGTGGTGCCATACAATGA
E01_PROG5_644934     CTTGACGACGATGGTGGTGCCATACAATGA
```

Figure 1. a) Depicting the existence of heterozygosity within the aligned sequences in the CodonCode Aligner A/G occurred at the same position (heterozygosity) and is represented by an ambiguity code, R. It is part of SNPs.
(b) and (c) represent graphic view of sequences aligned in BioEdit, showing heterozygosities. R=A/G, K= G/T, W=A/T, Y=C/T and these are called ambiguity codes.

Exon and Intron Structure

Exon detections within the reference sequences of the six (6) genes that had their primer design based on the mRNA sequences derived from NCBI database, was initiated for the necessity of getting the coding and non-coding regions of the referred genes fully identified. This in turn will undoubtedly assist in prediction of the regions of occurrence of synonymous and non-synonymous mutations within the coding regions of the genes, among other reasons. To actually identify the number of exons inherent in each gene, application of Spidey NCBI (Http://www.ncbi.nlm.nih.gov/spidey/) was made. The mRNA reference sequences were aligned together with their respective PCR amplified sequencing reaction products. Table 1 shows the total number of exons detected in each gene or promoter which also facilitated the revelation of the number of coding and non-coding regions in the fragments. Provision of mRNA coordinates with respect to their genomic coordinates, identities of the reference sequences, number of mismatches and gaps were provided for each primer.

Also, reference sequences of all the fragments were used to carry out full BLAST analysis in the *Manihot esculenta* Phytozome for confirmation of the exons identified using NCBI Spidey. Reference sequences derived from genomic sequences could not be used for exon identification in NCBI Spidey but NCBI Phytozome was used.

Table 1: List of exons, genomic coordinates, mRNA coordinates, length, Identity, mismatches and gaps

Gene Used	No of Exon	Genomic Coordinates	mRNA Coordinates	Length	Identity	Mismatches	Gaps
GBSS	1	1-64	681-744	64	100.0%	0	0
	2	169-269	745-845	101	100.0%	0	
	3	374-489	846-961	116	100.0%	0	
SOD	1	1-518	530-1046	517	96.3%	19	5
SUCY	1	1-119	1261-1379	119	99.2%	1	0
	2	216-382	1380-1546	167	100.0%	0	
	3	467-691	1547-1771	225	99.1%	2	
bgIA	1	2-320	661-974	314	93.3%	21	5
SBE	1	1-622	924-1545	622	99.8%	1	0
ACCO3	1	1-325	663-987	325	96.6%	11	0
CYP79D1	1	1-680	103-782	680	99.0%	7	0
CYP79D2	1	1-806	69-874	806	99.6%	3	0
Linamar	1	92-442	836-1186	351	90.3%	34	2
C4F/C6R	1	1-237	71-307	237	95.8%	10	0
Ty3/Gypsy	1	1-742	171-912	742	86.8%	98	0

SBE=Starch branching enzyme., ACCO3=M.esculenta 1-amino-cyclopropane-1-carboxylic acid oxidase., bgIA= M.esculenta bgIA gene., GBSS=granule-bound starch synthase., M. esculenta Linamar=Linamarase-beta-glucosidase., CYP79D1=M. esculenta N-hydroxylating cytochrome P450 CYP79D1., M. esculenta N-hydroxylating cytochrome P450 CYP79D2., SOD=superoxide dismutase., SUCY=Sucrose synthase., Ty3/Gypsy=like retrotransposon.

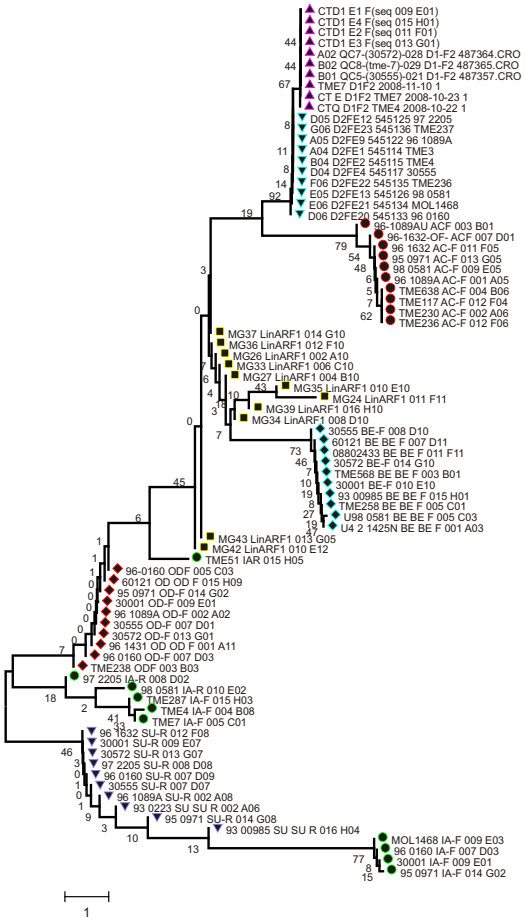

Figure 2: Inference of some ELs using the Neighbor-Joining method (Saitou *et al*.,1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. There were a total of 878 positions in the final databaset.

Conclusion

The principal objective of this study was to discover single nucleotide polymorphisms (SNPs) in a panel of elite lines of cassava and in two mapping populations to be used as a tool for marker-assisted breeding in cassava (*Manihot esculenta*). Single nucleotide polymorphism analysis of a total of 9,993 base pairs of sequence revealed 184 SNP sites and 43 InDels, giving rise to frequencies of 1 SNP per 54 bp and 1 SNP per 232 bp respectively. Exonic and intronic regions were identified to be 106 and 78 respectively. Highest number of SNPs was recorded in Ty3/Gypsy and non in GBSS. Synonymous and non-synonymous ones were identified using Codon-based Test of Neutrality. The frequency of SNPs was much higher than that of the InDels. The polymorphisms observed were due to the effects of transitions (C/T or A/G), transversions (C/G, A/T, A/C or G/T), heterozygosities and the occurrence of InDels within the sequenced samples. A total of 126 polymorphisms (68.5%) were transitions (C/T or A/G) and 58 polymorphisms (31.5%) were transversions (C/G, A/T, A/C, or G/T). Transitions contributed immensely to SNPs occurrence in this study. Analysis of preference using Z-test of selection for estimation of synonymous substitutions per synonymous sites (dS) and nonsynonymous substitutions per nonsynonymous sites (dN) was also done on TME14 x 96/1089A. The dN (306.7) which is > dS (125.5) implied existence of positive selection in the genes. Validation of discovered SNPs is axiomatically necessary for their usefulness and applications for future genomic studies. The discovered SNPs will be assessed for potential use as markers. It is still crucial to dramatically bring about a total decrease in the false discovery rate of SNPs. Discovered SNPs will be assessed for their potential as molecular marker.

References

Anderson, J. V., Delseny, M., Fregene, M. A., Jorge, V., Mba, C., Lopez, C., Restrepo, S., Soto, S., Piegu, B., Verdier, V., Cooke, R., Tohme, J., Horvath, D. P. (2004). An EST resource for cassava and other species of Euphorbiaceae. *Plant Molecular Biology* 56:527539.

Duran, C., Nikki, A., David, E., Jacqueline, B. (2009**).** Molecular Genetic Markers: Discovery, Applications, Data Storage and Visualisation, Current Bio-informatics, *4:* 16-27.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Jennings, D.L. and Iglesias, C. (2002). Breeding for Crop Improvement. In: Cassava:Biology, Production and Utilization, eds. Hillocks, R.J., Thresh, J.M. and Bellotti, A.C., CAB

International, pp 149-166.

Lokko, Y., Anderson, J .V., Rudd, S., Raji, A., Horvath, D., Mikel, M.A., Kim, R., Liu, L., Hernandez, A., Dixon, A.G., Ingelbrecht, I. L. (2007). Characterization of an 18,166 EST dataset for cassava (*Manihot esculenta* Crantz) enriched for drought-responsive genes. *Plant Cell Reporter* 26:16051618.

Mba, R. E. C., Stephenson, P., Edwards, K., Melzer, S., Nkumbira, J., Gullberg, U., Apel, K., Gale, M., Tohme, J., Fregene, M. (2001). Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava. *Theoretical and Applied Genetics* 102:2131.

Okogbenin, E., Marin, J., Fregene, M. (2006). An SSR-based molecular genetic map of cassava. *Euphytica* 147:433440.

Oraguzie, N. (2007). Association mapping in plants. Springer.

Saitou N & Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Sakurai, T., Plata, G., Rodriguez-Zapata, F., Seki, M., Salcedo, A., Toyoda, A., Ishiwata, A., Tohme, J., Sakaki, Y., Shinozaki. K., Ishitani, M. (2007). Sequencing analysis of 20,000 full-length cDNA clones from cassava reveals lineage specific expansions in gene families related to stress response. *BMC Plant Biology* 7:66.

Tamura, K., Nei, M. & Kumar. S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Tamura, K., Dudley, J., Nei, M & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596-1599.

Zhang, J., Rosenberg, H.F & Nei, M. (1998). Positive Darwinian selection after gene duplication in primate ribonuclease genes. *Proceedings of the National Academy of Sciences* (*USA*) 95:3708-3713.

Isolation and characterization of resistant gene analogs in cassava, wild *Manihot* species, and Castor Bean (*Ricinus communis)*

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Abstract

Qualitative pathogen resistance in both dicots and monocots have been attributed to the action of those resistance (R) genes encoding nucleotide binding site leucine rich repeat (NBS-LRR) proteins. Utilization of host plant disease resistance (R) genes in breeding programs has been documented to be the most efficient strategy for coping with the threat of the plant diseases. This study aimed at the isolation and characterization of candidate R genes, from the genomic DNA and constitutively expressed cDNA in cassava (*Manihot esculenta*), *M. glaziovii*, *M. epruinosa*, *M. tripartita, M. brachyandra* and castor bean (*Ricinus communis*) using degenerate primers based on the conserved motif (P- loop and GLPL) of the NBS domain. A total of 649 R sequences were identified, of which 261 putative NBS- LRR gene-like sequences were characterized and functionally annotated. Analysis of motif structure and R gene phylogeny demonstrated that RGA's of cassava shared conserved motifs found in other plant disease resistance genes such as *Arabidopsis thaliana* disease resistance protein, *Manihot* (RCa10.2) NBS type resistance protein gene, *Manihot esculenta* NBS-LRR resistance protein (RGH1 gene), *Prunus avium* clone , and predicted NBS-LRR genes in *Ricinus communis*. The molecular evolutionary forces, analysed using Ka/Ks ratio, reflected purifying selection on NBS and LRR regions of the discovered RGA's in these species. Single nucleotide polymorphisms (SNPs) predicted through re-sequencing of amplicons from the parental genotypes of the two segregating mapping population of 1M (TME 3 x TMS 30555) and 23M (TME 3 x TME 117) will be examined for association with disease resistance traits such as cassava mosaic disease. This study will give a comprehensive analysis of homologs of disease resistance genes in cassava and other members of the family *Euphorbiaceae*. Ultimately, SNPs that

are found to be associated with resistance traits will be converted into simple assays for marker-assisted introgression and pyramiding of R genes in cassava. Genetic information derived from this experiment is expected to facilitate the identification of gene-targeted marker for molecular breeding and provide an insight into the organization and evolution of NBS-LRR genes in cassava.

Keywords Cassava - Resistance gene analog (RGA) - Nucleotide binding site (NBS) - Plant disease resistance

Introduction

Cassava (*Manihot esculenta* subsp. *esculenta* Crantz) is the principal or second most important source of calories for more than 500 million people (Cock 1985; Best and Henry 1992). Cassava mosaic disease, caused by Gemini viruses of the genus Begomovirus (Family Geminiviridae) and transmitted by a white fly vector, *Bemisia tabaci* (Gennadius) is endemic in sub Saharan Africa, India and Sri Lanka (Tomkins *et al*, 2004). Host plant resistance to CMD is the best option to combat these problems as they offer a low-cost, effective and long-term solution.Utilization of host plant disease resistance (R) genes in breeding programs has been documented to be the most efficient strategy for coping with the threat of the plant diseases. Establishment of durable resistance requires the isolation of R genes, as it quickens the process of stacking R gene into elite cultivars through the marker assisted breeding. This study reports on isolation, characterization of RGAs from PCR based motif targeted genomic DNA and RNA fingerprinting for cloning and sequencing constitutively expressed genes from cassava (*Manihot esculenta*), *M. glaziovii*, *M. epruinosa*, *M. tripartita, M. brachyandra* and castor bean (*Ricinus communis*) using degenerate primers based on the conserved motif (P- loop and GLPL) of the NBS domain. The NBS profiling strategy was envisaged in cassava to assess the efficiency of NBS profiling method to provide polymorphic markers in segregating progeny, to develop markers close to major resistance genes for MAS and also determine the overall genomic organization of some NBS/LRR like sequences in Cassava.

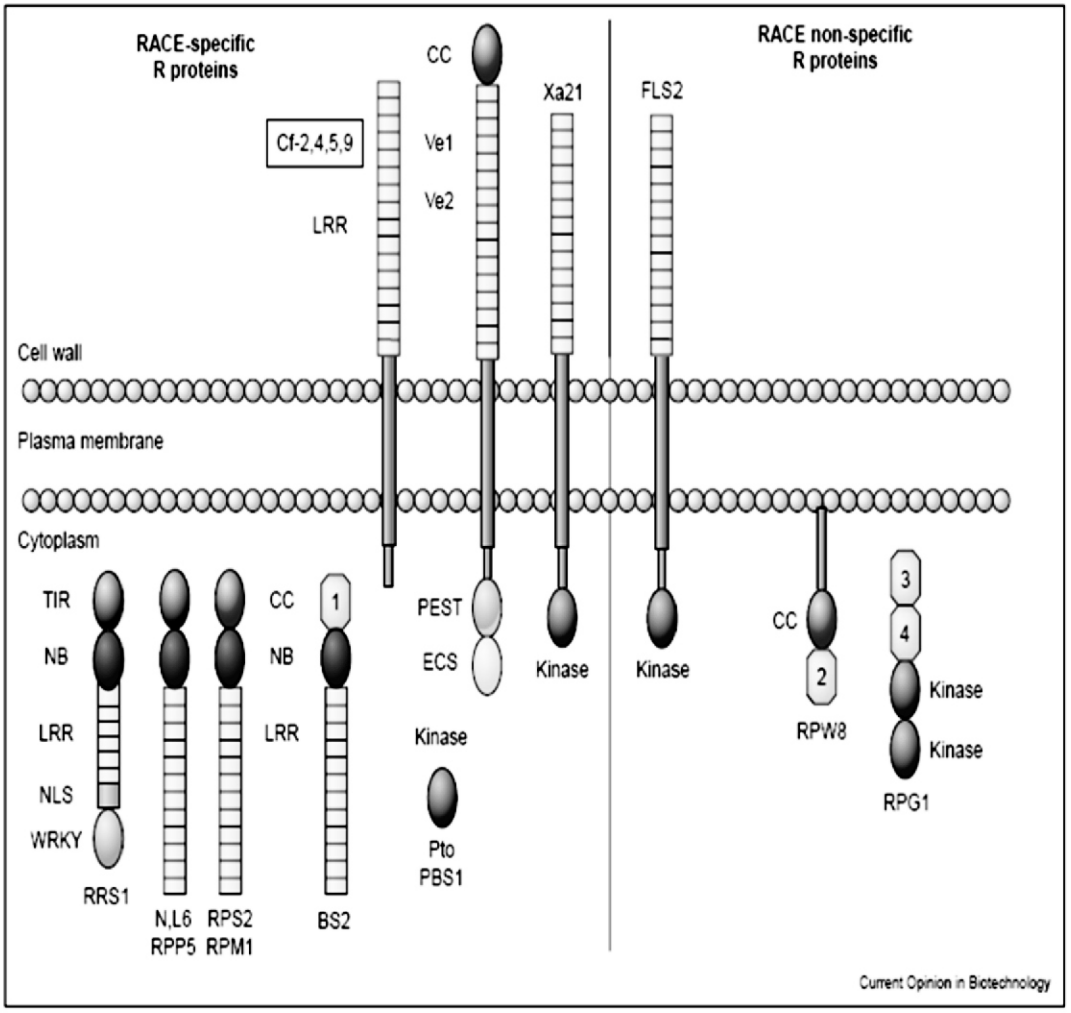
SNPs identified with candidate R genes provide a valuable resource for mapping cross derived populations and further germplasm analysis using association genetics. They provide means to identify genefor-gene mechanisms for multiple host pathogeninteractions and ultimately to obtain durable field based resistance. Single nucleotide polymorphisms (SNPs) predicted through re-sequencing of amplicons from the parental genotypes of the two segregating mapping population of 1M (TME 3 x TMS 30555) and 23M (TME 3 x TME 117) will be examined for association with disease resistance traits such as cassava mosaic disease. Ultimately, SNPs that are found to be associated with resistance traits will be converted into simple assays for marker-assisted introgression and pyramiding of R genes in cassava.

Background

Identification of the molecular basis of major resistance determinants to different pathogens is very essential for improving the selection of favorable alleles during cultivar development. Development of cultivars resistant to the disease is considered to be the effective strategy for infection control. More than 90 plant disease resistance genes have been cloned and sequence characterized in PRGdb (http://www.prgdb.org) (Sanseverino *et al*. 2010). R genes are clustered together in the genome and belong to families of tightly linked genes. They have conserved domains in the protein sequences

(Fig. 1), and are commonly characterized by the presence of Nucleotide Binding site (NBS) and an LRR domain in the corresponding protein products (Hammond Kosack and Jones 1997). The NBS LRR gene family is further subdivided into two, based on the motifs at the N terminal to the NBS

(Cannon *et al*., 2002). The Toll- Interleukin receptor-like regions (TIR) subfamily (TIR-NBS-LRR, TNL) contains approximately 200 amino acid residues at the N terminal with high similarity to Drosophila Toll and mammalian Interleukin receptor like regions (Meyers *et al*., 1999; Cannon *et al*., 2002). In the non-TIR subfamily (non- TIR-NBS-LRR), the N-terminal region consists of either a coiled (CC) or leucine zipper (LZ) structure both of which facilitate protein-protein interactions (Baker *et al*., 1997; Cannon *et al*., 2002). There is a clear distinction between monocotyledons and dicotyledons with respect to the presence of TNL- type R gene families.



Schematic representation of the predicted domains of R proteins which confer either race-specific or race non-specific resistance. Further details for each named R protein are given in Table 2. The two Ve proteins differ slightly in protein structure. Ve1 contains a putative CC domain but no PEST sequence in the C terminus, whereas Ve2 lacks the CC domain at the N terminus but contains a C-terminal PEST sequence. Four protein domains are indicated (grey; labelled 1–4) that lack significant homology to known proteins. BS2, bacterial speck resistance 2; Cf-2,4,5,9, resistance to *Cladosporium fulvum* races 2, 4, 5 and 9; ECS, endocytosis signal; L6, flax rust resistance 6; NLS, nuclear localisation sequences; PEST, Pro-Glu-Ser-Thr-like sequence; PBS1, resistance to *Pseudomonas* bacterial speck expressing avrPphB; Pto, *P. syringae* pv. tomato

Figure 1. Schematic representation of the predicted domains of R proteins (Adapted from Hamm ond-Kosack and Parker, 2003).

Materials and Methods

Plant materials used for DNA isolation were obtained from the cassava breeding program, International Institute of Tropical Agriculture (IITA), Nigeria. These include several African land races of cassava, *M. esculenta* (5), four wild Manihot species, namely, *M. brachyandra, M . epruinosa, M. glaziovii, M. tripartita*, and castor bean (*Ricinus communis*).

Degenerate primer design

Primers were designed from the NBS coding sequences obtained by data base searches with preference to Arabidopsis genome. Two pairs of degenerate primes were developed in this study. The first pair were targeting various domains of the At RGA alignment (Meyers et al. 809-34). Three forward primers, and 7 reverse primers were designed flanking the P-loop and GLPL motifs (Fig. 2). All pairwise 21 combinations were tested

for patterns of amplifications. Primer pair NL-50-F (GGN GGN STN GGN AAR CAN CAN CTN) and TNL-470-R (CAT GCA TGY GAD ATN AGN GAY TT) was selected for further assay. The second pair of degenerate primers (Gedil *et al*. 2001) was used to P-loop and GLPL motifs of NBS domain - RGA-F (GGI GGI GTI GGI AAI ACI AC); RGA-R (IAG IGC IAG IGG IAG ICC).

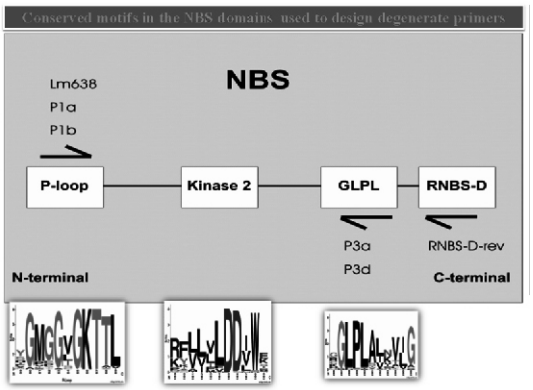


Figure 2. Target domain/motif of NBS-LRR disease resistance gene for degenerate primer design

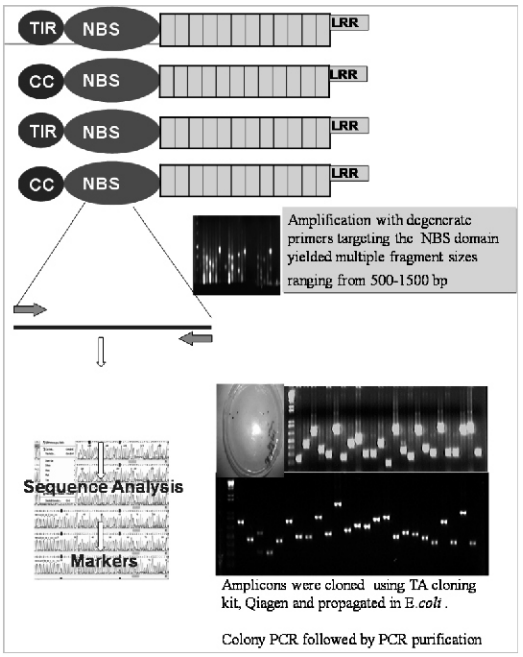


Figure 3. Overview of the Materials and Methods

Cloning and sequencing of PCR products

The amplified products were separated by electrophoresis on 1.5% agarose gel and stained with ethidium bromide for visualization. The amplicon size ranged from 200 900 bp. Each DNA , cDNA fragment were cloned into p-Drive vector (Qiagen) system, and propagated in *E. coli* grown on Luria Bertani (LB) medium supplemented with 100 µg/ml ampicillin using standard procedures. The positive transformants were selected by blue /white screening. Large white colonies were picked to amplify the inserted sequences using universal primers T7 and SP6 (Fig. 3).

Amplification of performed in volumes of 25 µl reaction containing 2.5 µl, 10 mM Tris-Hcl (pH 8.3), 1.5 µl, 5.0 mM MgCl2, 1.6 µl, 2.5 mM dNTPs, 1.0 µl, 5 mM of each primer, 1.0 µl of DMSO, and 10 µl of DNA, and 0.2U *Taq* DNA polymerase (Sigma Aldrich, St. Louis, Mo.), and 0.2 mM each of T7 and SP6 universal primers. PCR was programmed in Bio Rad DNA engine, Peltier Thermal Cycler with initial denaturation at 94C for 2 min followed by 30 cycles of denaturation (15s at 93C), and annealing (45 sec for 50C) and extension 30 s at 72C followed by a final extension step 10 min at 72C. Products were visualized on 1% agarose gel for presence of insert. Clones with insert were purified with ethanol precipitation and used as a template for sequencing.

Sequencing of the selected clones

Cycle sequencing of the purified clones was carried out using the ABI PRISM Dye terminator Kit and products were run on ABI 3130 DNA analyzer (Applied Biosystems, Foster City, USA).

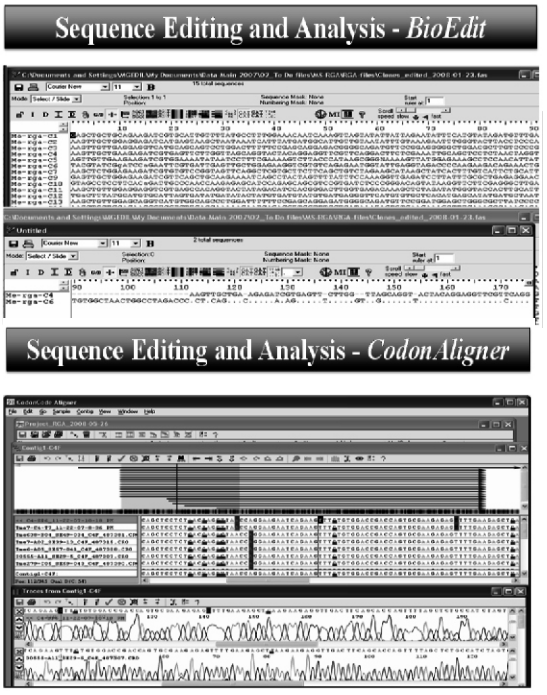


Figure 4. Screenshot of sequence editing with BioEdit and Codon Aligner

Sequence analysis

Raw sequences were edited in Codoncode Aligner (Codon Code Aligner Version 3.5.7. copyright 2002-2010, CodonCode Corporation, LI-COR, Inc.) for base calling error and other artifacts (Fig. 4). Primer sequences were trimmed from the sequences before they were used for similarity search. The sequences were exported and analyzed in Bioedit.

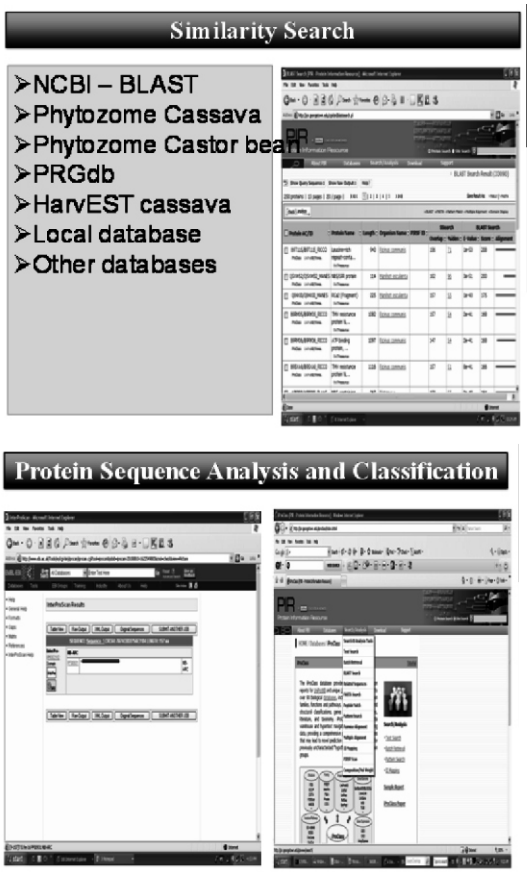


Figure 5. RG sequences were compared with those in NCBI (*http://www.ncbi.nlm.nih.gov/blast/Blast.cgi*), Phytozome (*http://www.phytozome.net.org),* PRGdb (*http://www.prgdb.org,*), EMBL and GenBank databases and analyzed using the BLASTn algorithm (Altschul *et al*. 1997). Protein structure was by (*http://www.ebi.ac.uk/interpro/*), Pfam(*www.sanger.ac.uk/Software/Pfam/search.shtml*).

RG sequences were compared with those in NCBI (Fig. 5), PRGdb (*http://www.prgdb.org,*), EMBL and GenBank databases and analyzed using the BLASTn algorithm (Altschul *et al*. 1997)

Analysis of protein motif structure

The structure of few RGAs was deduced based on the information BLASTP search of NCBI (*http://www.ncbi.nlm.nih.gov/blast/Blast.cgi*) and the protein structure was confirmed by the Pfam (Fig. 5) (*www.sanger.ac.uk/Software/Pfam/search.shtml*). Multiple Expectation Maximisation for Motif Elicitation (MEME) was used to detect conserved motifs between sequences with NBS domains (*http://www.meme.ncbr.net)*

Sequences that are matching known NBS-LRR accessions were aligned by using ClustalW built in MEGA (Nei and Kumar, 2000). Nucleotide sequence analysis and translation to the corresponding amino acid sequence was performed using CodonCode Aligner (Codon Code Aligner Version 3.5.7. copyright 2002-2010, CodonCode Corporation contains licensed copyrighted material from LI-COR, Inc.) or ORF finder (*http://www.bioinformatics.org/sms/orf_find).* Phylogenetic trees were constructed by the neighbour joining method (Saitou and Nei 1987) with 1000 bootstrap iterations to provide an estimate of confidence for each branch point and the final tree. Distances for phylogenetic analyses were conducted using the MEGA software (version 4.0) by using nucleotide differences parameter.

Results

- BLAST based sequence comparison of all RGA clones from Cassava, Wild *Manihot* species, and Castor Bean. Sequences of the cloned PCR products were compared with those in EMBL and GenBank databases using the BLASTn algorithm (Altschul *et al*. 1997).
- Sequences that were matching R genes were named as NBS-LRR accessions. Identified NBS sequences from the genomic DNA and expressed genes of Cassava, Wild *Manihot* species, and Castor Bean.
- A total of 795 genomic clones and 549 cDNA clones altogether 1344 clones were sequenced.
- After sequence editing and assembly 649 genomic and cDNA clones were identified of which 261 putative NBS- LRR gene-like sequences were characterized, subjected to similarity analysis based on nucleotide differences using the software MEGA (Nei

and Kumar, 2000).

A combination of analysis used to group the sequences

- 521 grouped in to 41 classes with 3-99 members
- 126 were not grouped
- About half contained open reading frame (ORF)
- Different NBS-LRR specific motifs found in the sequences
- Domain/motif characterization and search for species specific features to be performed
- Phylogenetic analysis together with published R gene sequences
- Sequences will be submitted to the GenBank

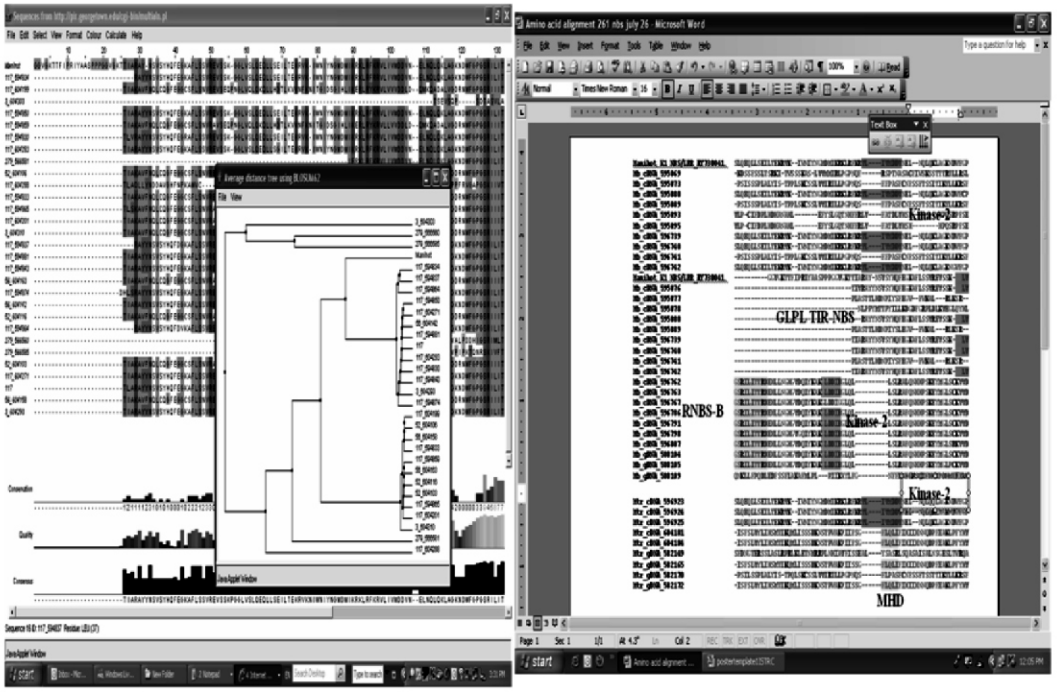


Figure 6 (a) Comparison of the amino acid sequences of the cassava RGA's with Manihot K1 NBS/LRR_AY730041. Dendrogram produced by aligning deduced amino acid sequences of *Manihot* RGAs with predicted genes. (b) Alignment analysis was performed using the Clustal W program (Eur. Bioinformatics Inst., Cambridge, UK). Four conserved motifs have been highlighted according to Meyers *et al*.(1999). The positions of aspartic acid (D) and tryptophan (W) residues characteristic of TIR and non-TIR sequences in the kinase-2, respectively are seen

Alignment of the putative amino acid sequences of RGAs from cassava and the nucleotide binding site of the resistance genes from Manihot **K1 NBS/LRR_AY730041** using CLUSTAL W (Fig 6a). Residues in bold are part of internal conserved motifs as determined by Meyers et al. (1999). The final residue in the kinase-a motif, which can be used to predict the presence of the Toll/Interleukin-1 receptor-like domain, is indicated (Fig 6b).

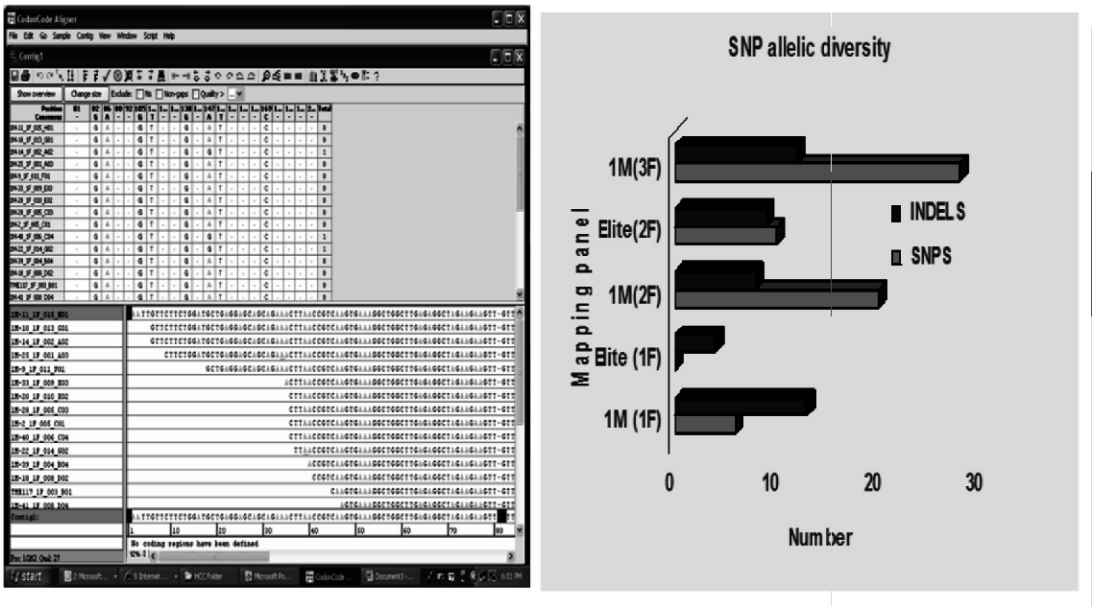


Figure 7 (a) Screen shot of sequence from Codon code, identifying the SNPs in the Mapping panel (b) A total of 107 Cassava genomic DNA templates were subjected to *in vitro* SNP discovery through re-sequencing from parental genotypes and progeny of the F_1 1M (45), 23M (43) mapping population, and the Elite lines (19) using the four pairs of primers 1F & R, 2F & R, 3F & R, 14F & R. DNA from 27 genotypes of IM mapping population and 20 Elite lines was utilized as template for PCR amplification, by 3 pairs of primers

Identification of SNPs

Resequencing of selected RGA region and identification of SNP polymorphism was carried out using the template DNA of two segregating mapping population of 1M (TME 3 x TMS 30555) and 23M (TME 3 x TME 117). PCR amplified genomic amplicons were sequenced and DNA sequences were aligned in Codoncode (Fig 7a). SNPs were validated across the mapping panel in the amplified regions in the Codoncode. A total of 107 Cassava genomic DNA templates are to be subjected to *in vitro* SNP discovery through re-sequencing from parental genotypes and progeny of the F_1 1M (45), 23M (43) mapping population, and the Elite lines (19) using the four pairs of primers 1F & R, 2F & R, 3F & R, 14F & R. So far re-sequencing of 1M population has been done with primer 1F (25 lines), 2F (15) and 3F (19). Elite lines which have been re-sequenced with primer are 1F (12), and 2F (20). DNA from 27 genotypes of IM mapping population and 20 Elite lines was utilized as template for PCR amplification, by 3 pairs of primers designed from published RGA. There were 64 SNPs and 46 indels in total, from 47 cassava genotypes in 3,108 bp (Fig. 7b).

Further confirmatory assay will be performed to establish the exact number of SNPs and Indels and to determine the rate of occurrence.

Strategies for R gene isolation in cassava, wild manihot and its closely related Ricinus species

An approach by degenerate PCR primer, cloning and the bioinformatics discovery method (Fig. 8) resulted in the identification of 649 R sequences were identified, of which 261 putative NBS- LRR gene-like sequences were characterized. Resequencing of selected RGA region and identification of SNP polymorphism revealed 64 SNPs and 46 indels in total, from 47 cassava genotypes in 3,108 bp. Representative genomic sequences types will be deposited as accessions in GenBank.

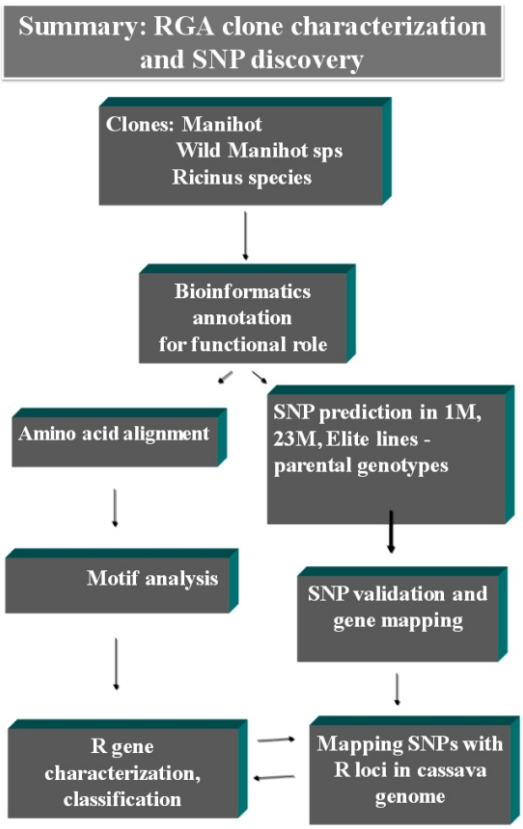


Figure 8. Schematic representation of RGA clone characterization and SNP discovery of *Manihot*, wild *Manihot* and *Ricinus* R genes. The bioinformatics annotation leads to sequence characterization, and *in vitro* SNP discovery and genetic mapping

Conclusion

In view of the effectiveness of host plant resistance to viruses, this comparative genomic approach provides an alternative means of marker discovery. Large number of RGAs have been identified in cassava, wild Manihot species and castor bean. NBS domain is highly conserved in these Euphorbeaceae species and other plant disease resistance genes such as Arabidopsis thaliana disease resistance protein. Development of markers associated with resistance to viral diseases is underway.

Genetic information derived from this experiment is expected to facilitate the identification of gene-targeted marker for molecular breeding and provide an insight into the organization and evolution of NBS-LRR genes in cassava.

Also, the data will provide an insight into the organization and structure of R gene clusters in cassava

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25: 2389-3402

Baker, B., Zambryski, P., Staskawicz, B., and Dinesh-Kumar, S.P. 1997. Signalling in plant-microbe interactions. Science 276:726-733

Best, R., and Henry, G.1992. Cassava: towards the year 2000. *In* International network for cassava genetic resources. Report of the first meeting of the International Network for Cassava Genetics International Crop Network Series number 10. International plant Genetic resources institute (IPGRI) 10:3-11

Cannon, S. B., Zhu, H., Baumgarten, A. M., Spangler, R., May, G., Cook, D. R. and Young, N. D. 2002. Diversity, distribution, and ancient taxonomic relationships within the TIR and non-TIR NBS-LRR resistance gene subfamilies. Journal of Molecular Evolution 54, 548562.

Cock, J.H. 1985. Cassava: new potential for a neglected crop. Westview press, Boulder Colorado USA.

Gedil, M.A., Slabaugh, M.B., Berry, S., Johnson, R., Michelmore, R., Miller, J., Gulya, T., and Knapp, S.J. (2001) "Candidate disease resistance genes in sunflower cloned using conserved nucleotide-binding site motifs: genetic mapping and linkage to the downy mildew resistance gene Pl1", Genome, 44, 205-212

Hammond-Kosack, K.E., Jones, J.D. 1997. Plant disease resistance genes. Ann Rev Plant Physiol Plant Mol Biol 48:575607

Hammond-Kosack, K.E., and Parker, J.E. 2003. Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. Curr Opin Biotechnol 14(2): 177-193

Meyers, B.C., Dickerman, A.W., Michelmore, R.W., Sivaramakrishnan, S., Sobral, B.W., Young, N.D.1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J 20:317332

Meyers, B.C., Kozik, A., Griego, A., Kuang, H. and Michelmore ,R.W. 2003. Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. Plant Cell 15:809-834

Nei , M., and Kumar, S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, Oxford.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425

Sanseverino , W., Roma, G., Simon, M., Faino, L., Melito, S., Stupka, E., Frusciante, L., Ercolano, M. R. 2010. PRGdb: a bioinformatics platform for plant resistance gene analysis. Nucl Acid Res 38:D814-D821

Tomkins, J., Fregene, M., Main, D., Kim, H., Wing, R., and Tohme, J. 2004. Bacterial artificial chromosome (BAC) library resource for positional cloning of pest and disease resistance genes in cassava (*Manihot esculenta* Crantz). Plant Molecular Biology 56: 555561

Meyers, B. C., et al. "Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis." Plant Cell 15.4 (2003): 809-34.